



Enhanced
DIGITAL
VERSION
Included

SPERLING

PEDIATRIC ENDOCRINOLOGY

Mark A. Sperling

Joseph A. Majzoub

Ram K. Menon

Constantine A. Stratakis

**FIFTH
EDITION**



Any screen. Any time. Anywhere.

Activate the eBook version
of this title at no additional charge.



Expert Consult eBooks give you the power to browse and find content, view enhanced images, share notes and highlights—both online and offline.

Unlock your eBook today.

- 1 Visit expertconsult.inkling.com/redeem
- 2 Scratch off your code
- 3 Type code into “Enter Code” box
- 4 Click “Redeem”
- 5 Log in or Sign up
- 6 Go to “My Library”

It's that easy!

Scan this QR code to redeem your eBook through your mobile device:



Place Peel Off
Sticker Here

For technical assistance:
email expertconsult.help@elsevier.com
call 1-800-401-9962 (inside the US)
call +1-314-447-8200 (outside the US)

ELSEVIER

Use of the current edition of the electronic version of this book (eBook) is subject to the terms of the nontransferable, limited license granted on expertconsult.inkling.com. Access to the eBook is limited to the first individual who redeems the PIN, located on the inside cover of this book, at expertconsult.inkling.com and may not be transferred to another party by resale, lending, or other means.

**SPERLING
PEDIATRIC
ENDOCRINOLOGY**

This page intentionally left blank

FIFTH EDITION

SPERLING PEDIATRIC ENDOCRINOLOGY

EDITOR

Mark A. Sperling, MD

ASSOCIATE EDITORS

Joseph A. Majzoub, MD

Ram K. Menon, MD, FRCP

Constantine A. Stratakis, MD, D(MED)Sc, PhD(hc)



Elsevier
1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

PEDIATRIC ENDOCRINOLOGY, FIFTH EDITION

ISBN: 978-0-323-62520-3

Copyright © 2021 by Elsevier, Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions. This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notice

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds or experiments described herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made. To the fullest extent of the law, no responsibility is assumed by Elsevier, authors, editors or contributors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Previous editions copyrighted 2014, 2008, 2002, 1996.

Library of Congress Control Number 2020936250

Content Strategist: Sarah Barth
Content Development Specialist: Meghan Andress
Publishing Services Manager: Shereen Jameel
Project Manager: Rukmani Krishnan
Design Direction: Ryan Cook

Printed in China

Last digit is the print number: 9 8 7 6 5 4 3 2 1



*To our wives and families who provide meaning, joy
and continuity to our lives and whose support was integral to the completion of this book.*

Foreword to the First Edition

The aim of the editor and contributors to this volume is to establish an effective bridge between the surging progress in biomedical science and the clinical practice of pediatric endocrinology. Half a century ago the biochemical elucidation of the structure and subsequent synthesis of steroid hormones provided the basis for a revolution in the diagnosis and treatment of a number of endocrine and nonendocrine disorders. That era was soon followed by a succession of fundamental discoveries: structure of peptide hormones, identification of releasing hormones from the brain, rapid and precise assay methods, and synthesis of peptide hormones by molecular biological techniques, to name but a few.

In no field has laboratory science been more effectively translated into clinical progress than in pediatric endocrinology.

A glance at the roster of contributors to this volume may well provide insight into why; many who are responsible for the dramatic advances in the laboratory also pursue active clinical careers.

This volume includes many new sections that were not presented in previous texts devoted to clinical pediatric endocrinology. It will serve as a valuable reference for family physicians, internists, pediatricians, and other health professionals, covering as it does the gamut of information from basic molecular biology to practical considerations in the diagnosis and treatment of pediatric endocrine disorders.

Solomon A. Kaplan, MD 1924-2017

Preface

PREFACE TO THE 5TH EDITION

In his foreword to the first edition in 1996, Solomon A. Kaplan, MD (1924-2017) stated “The aim of the editor and contributors to this volume is to establish an effective bridge between the surging progress in biomedical science and the clinical practice of pediatric endocrinology.” This has been the guiding principle of each edition and remarkably, the surging pace of progress in biomedical sciences remains unabated as does its application to pediatric endocrinology, justifying this updated and expanded fifth edition. Now, some 25 years later, a quarter century after the first edition, the vista of pediatric endocrinology is much expanded and vastly changed, especially by the application of molecular genetic techniques to the dissection of the etiology and potential remedy of congenital endocrine disorders. Although the disorder(s) manifest in childhood, adolescence, or even in adults, many have their origin in congenital or acquired genetic alterations, some possibly via environmentally induced epigenetic modifications.

To accommodate these changes, we have expanded the editorial team by including three outstanding physician-scientists as associate editors; each has made significant contributions to pediatric endocrinology in original research, leadership, and mentorship. We also introduced two new chapters dealing with maternal and fetal endocrinology in parturition and gender medicine, and included an expanded section on precision medicine in the chapter on “Molecular Endocrinology, Endocrine Genetics, and Precision Medicine.” Several of the chapters have entirely new authors, acknowledged leaders in their field, or new authors with expertise complementing established leader(s) in

the field; each chapter has been appropriately and often extensively updated, although it is possible that in this fast-moving era, the newest discoveries may have been published after finalized manuscripts were submitted. There also may be inadvertent errors that slipped by our careful attention; we apologize and thank those readers who brought them to our notice.

Nevertheless, we marvel at the vastly increased knowledge since the prior edition, and the changes in diagnostic and therapeutic armamentarium that have become available. For example, in childhood diabetes mellitus, closed-loop systems and continuous glucose monitoring are increasingly the norm in highly developed countries; glucagon-like peptide 1 agonists are approved for children with type 2 diabetes mellitus (T2DM); and gastric bypass surgeries are increasingly applied to adolescents with obesity and T2DM. Novelty abounds in each chapter, so we affirm that there is sufficient new material for both the serious student and/or the established endocrine practitioner/investigator to make this a “must have” book.

I have always considered that the editorship of this book is a great privilege, a labor of love well worth the enormous effort involved. On this occasion, I express my sincere thanks to the associate editors, Drs Majzoub, Menon, and Stratakis, who equally shared the work of editing and contributing. I extend sincere and warm-hearted thanks to all contributors; each of your contributions is highly respected and highly valued.

Mark A. Sperling
New York, New York
Spring 2020

Acknowledgments

An enterprise of this nature requires the contribution and support of numerous individuals. I gratefully acknowledge the contributions of each of the authors, whose friendship and collegiality were—and continue to be—indispensable to bring this work to publication. Constructive critique was provided by colleagues, postdoctoral trainees, residents, and visiting scholars

to our departmental programs in endocrinology. A special thanks to Ms. Meghan Andress of Elsevier, whose experience, diligence, and patience enabled this project to move to timely conclusion. We express our thanks to Elsevier for giving us the privilege of editing this book.

Contributors

Heide Aungst, MA

Division of Human Genetics
Center for Pediatric Genomics
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio
United States

Philippe Backeljauw, MD

Professor of Clinical Pediatrics
Division of Pediatric Endocrinology
Cincinnati Children's Hospital Medical Center
University of Cincinnati College of Medicine
Cincinnati, Ohio
United States

Jeffrey Baron, MD

Senior Investigator
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland
United States

Tadej Battelino, MD, PhD

Professor of Pediatrics
Department of Pediatric Endocrinology, Diabetes &
Metabolism
University Children's Hospital, University Medical Center
Faculty of Medicine
University of Ljubljana
Ljubljana
Slovenia

Andrew J. Bauer, MD

Director, The Thyroid Center
Division of Endocrinology and Diabetes
The Children's Hospital of Philadelphia, Philadelphia
Associate Professor
Department of Pediatrics
Perelman School of Medicine
The University of Pennsylvania
Philadelphia, Pennsylvania
United States

David T. Breault, MD, PhD

Associate Professor of Pediatrics
Harvard Medical School
Associate Chief
Division of Endocrinology
Boston Children's Hospital
Boston, Massachusetts
United States

Yee-Ming Chan, MD, PhD

Associate Physician
Department of Pediatrics, Division of Endocrinology
Boston Children's Hospital
Assistant Professor
Department of Pediatrics
Harvard Medical School

Boston, Massachusetts
United States

Steven D. Chernausek, MD

Professor of Pediatrics
Department of Pediatrics
University of Oklahoma Health Sciences Center
Oklahoma City, Oklahoma
United States

David W. Cooke, MD

Associate Professor
Department of Pediatrics
Johns Hopkins University School of Medicine
Baltimore, Maryland
United States

Sarah C. Couch, MS, PhD, RDN

Professor
Department of Rehabilitation, Exercise, and Nutrition Sciences
University of Cincinnati
Cincinnati, Ohio
United States

Stephen R. Daniels, MD, PhD

Professor and Chair
Department of Pediatrics
University of Colorado School of Medicine
Pediatrician-in-Chief and L. Joseph Butterfield Chair in
Pediatrics
Children's Hospital Colorado
Aurora, Colorado
United States

Mehul T. Dattani, MD, FRCP, FRCPCH

Professor of Paediatric Endocrinology
Genetics and Genomic Medicine Programme
UCL Great Ormond Street Institute of Child Health
Consultant and Head of Clinical Service in Paediatric
Endocrinology
Great Ormond Street Hospital for Children
Consultant in Adolescent Endocrinology
UCL Hospitals
London
United Kingdom

Diva D. De Leon, MD, MSCE

Professor of Pediatrics
Perelman School of Medicine at the University of Pennsylvania
Chief, Division of Endocrinology and Diabetes
Director, Congenital Hyperinsulinism Center
The Children's Hospital of Philadelphia
Philadelphia, Pennsylvania
United States

Johnny Deladoey, MD, PhD

Associate Professor
Department of Pediatrics
CHU Ste-Justine/U de Montreal
Montreal, Quebec
Canada

Leo Dunkel, MD, PhD

Professor of Paediatric Endocrinology
William Harvey Research Institute
Barts and The London School of Medicine and Dentistry
Queen Mary University of London
London
United Kingdom

Brian J. Feldman, MD, PhD

Associate Professor of Pediatrics
Walter L. Miller Distinguished Chair in Pediatric
Endocrinology
University of California
San Francisco, California
United States

Lauren Fishbein, MD, PhD

Assistant Professor
Department of Medicine; Division of Endocrinology,
Metabolism and Diabetes
University of Colorado School of Medicine
Aurora, Colorado
United States

Christa E. Flück, MD

Professor
Division of Pediatric Endocrinology, Diabetology and
Metabolism
Departments of Pediatrics and BioMedical Research
University of Bern
Bern
Switzerland

Claus Højbjerg Gravholt, MD, PhD, Dr Med Sci

Professor
Departments of Endocrinology and Molecular Medicine
(MOMA)
Aarhus University Hospital
Aarhus, Denmark

Siri Atma W. Greeley, MD, PhD

Associate Professor of Pediatrics and Medicine
Section of Pediatric and Adult Endocrinology, Diabetes and
Metabolism
Kovler Diabetes Center
University of Chicago
Chicago, Illinois
United States

Adda Grimberg, MD

Professor
Department of Pediatrics
Perelman School of Medicine, University of Pennsylvania
Philadelphia, Pennsylvania
Scientific Director
Diagnostic and Research Growth Center
Division of Endocrinology and Diabetes
Children's Hospital of Philadelphia
Philadelphia, Pennsylvania
United States

Michael J. Haller, MD

Professor of Pediatrics
Chief, Pediatric Endocrinology
University of Florida
Gainesville, Florida
United States

Joan C. Han, MD

Associate Professor
Departments of Pediatrics and Physiology
University of Tennessee Health Science Center, Memphis
Director
Pediatric Obesity Program
Le Bonheur Children's Hospital
Memphis, Tennessee
United States

Helen N. Jones, PhD

Associate Professor and Head of Research
Center for Fetal and Placental Research
Cincinnati Children's Hospital Medical Center
Department of Surgery
School of Medicine
University of Cincinnati
Cincinnati, Ohio
United States

Alexander A.L. Jorge, MD

Associate Professor of Medicine at University of Sao Paulo
Principal Investigator of the Genetic Endocrinology Unit
(LIM25)
Division of Endocrinology and Metabolism
Hospital das Clinicas de Sao Paulo (HC-FMUSP)
Sao Paulo, Brazil

Paul Kruszka, MD, MPH, FACMG

Clinical Geneticist
National Human Genome Research Institute
National Institutes of Health
Bethesda, Maryland
United States

Bassil Kublaoui, MD, PhD

Associate Professor
Department of Pediatrics
University of Pennsylvania
Philadelphia, Pennsylvania
United States

Peter A. Lee, MD, PhD

Professor
Department of Pediatrics
Penn State College of Medicine
Penn State Hershey Medical Center
Hershey, Pennsylvania
United States

Michael A. Levine, MD

Chief Emeritus
Division of Endocrinology and Diabetes
The Children's Hospital of Philadelphia
Philadelphia, Pennsylvania
Professor Emeritus of Pediatrics and Medicine
Perelman School of Medicine of the University of Pennsylvania
Philadelphia, Pennsylvania
United States

David Maahs, MD, PhD

Associate Director, Stanford Diabetes Research Center
The Kirkwood Distinguished Packard Fellow
Professor of Pediatrics and, by courtesy of Health Research and
Policy (Epidemiology)
Chief of Pediatric Endocrinology
Lucile Packard Children's Hospital
Stanford University, School of Medicine
Stanford, California
United States

Joseph A. Majzoub, MD

Thomas Morgan Rotch Professor of Pediatrics, Professor of
Medicine
Harvard Medical School
Chief Emeritus, Division of Endocrinology
Department of Pediatrics
Boston Children's Hospital
Boston, Massachusetts
United States

Tani Malhotra, MD

Fellow
Department of Maternal Fetal Medicine
Metrohealth Medical Center/University Hospitals Combined
Program
Cleveland, Ohio
United States

Mary K. McCauley, MD

Pediatric Endocrinology Fellow
Section of Pediatric and Adult Endocrinology, Diabetes and
Metabolism
Kovler Diabetes Center
University of Chicago
Chicago, Illinois
United States

Ram K. Menon, MBBS, MD, FRCP

Professor of Pediatrics
Professor of Molecular and Integrative Physiology
Director, Pediatric Endocrinology, CS Mott Children's Hospital
Michigan Medicine
University of Michigan
Ann Arbor, Michigan
United States

Sam Mesiano, PhD

William H. Weir, Professor of Reproductive Biology
Department of Reproductive Biology,
Case Western Reserve University
Department of Obstetrics and Gynecology
University Hospitals of Cleveland
Cleveland, Ohio
United States

Walter L. Miller, MD

Distinguished Professor of Pediatrics, Emeritus
Emeritus Chief of Endocrinology
University of California
San Francisco, California
United States

Louis J. Muglia, MD, PhD

President and CEO
Burroughs Wellcome Fund
Research Triangle Park, NC
Adjunct Professor
Department of Pediatrics
Cincinnati Children's Hospital Medical Center
University of Cincinnati College of Medicine
Cincinnati, Ohio
United States

Jon Nakamoto, MD, PhD

Regional Medical Director
Quest Diagnostics Nichols Institute
San Juan Capistrano, California
United States

Bimota Nambam, MBBS, MD

Attending Physician
Department of Medicine
Division of Endocrinology
Johns Hopkins All Childrens Hospital
St. Petersburg, Florida
United States

Mark R. Palmert, MD, PhD

Associate Chair of Pediatrics
Hospital for Sick Children
Professor of Pediatrics and Physiology
University of Toronto
Toronto, Ontario
Canada

Louis H. Philipson, MD, PhD

Professor
Departments of Medicine and Pediatrics
University of Chicago
Chicago, Illinois
United States

Moshe Phillip, MD

Director
The Jesse Z and Sara Lea Shafer Institute for Endocrinology and
Diabetes
National Center for Childhood Diabetes
Schneider Children's Medical Center of Israel
Petah Tikva
Israel
Vice Dean for Research and Development
Sackler Faculty of Medicine
Tel Aviv University
Tel Aviv
Israel

Sally Radovick, MD

Professor and Henry Rutgers Term
Chair
Department of Pediatrics
Senior Associate Dean for Clinical and Translational Research
Robert Wood Johnson Medical School
New Brunswick, New Jersey
United States

Scott Rivkees, MD

Professor
Department of Pediatrics
University of Florida
Gainesville, Florida
United States

Allen W. Root, MD

Adjunct Professor of Pediatrics
Johns Hopkins School of Medicine
Professor of Pediatrics Emeritus
University of South Florida
Morsani College of Medicine
Johns Hopkins All Children's Hospital
St. Petersburg, Florida
United States

Robert L. Rosenfield, MD

Professor Emeritus
Departments of Pediatrics and Medicine
The University of Chicago Pritzker School of Medicine
Chicago, Illinois
United States
Adjunct Professor
Department of Pediatrics
University of California
San Francisco, California
United States

Stephen M. Rosenthal, MD

Professor
Department of Pediatrics
Medical Director, Child and Adolescent Gender Center
Emeritus Program Director
Department of Pediatric Endocrinology
University of California San Francisco
San Francisco, California
United States

Desmond Schatz, MD

Professor
Department of Pediatrics
University of Florida
Gainesville, Florida
United States

Mark A. Sperling, MD

Emeritus Professor and Chair
Department of Pediatrics
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania
Professorial Lecturer, Pediatric Endocrinology
Icahn School of Medicine at Mt Sinai, New York
United States

Abhinash Srivatsa, MBBS

Attending Physician
Division of Endocrinology
Department of Pediatrics
Boston Children's Hospital
Harvard Medical School
Boston, Massachusetts
United States

Charles A. Stanley, MD

Senior Endocrinologist
Endocrinology Division
Children's Hospital of Philadelphia
Professor of Pediatrics (Emeritus)
University of Pennsylvania Perelman School of Medicine
Philadelphia, Pennsylvania
United States

Constantine A. Stratakis, MD, D(med)Sci, PhD (hc)

Senior Investigator
Section of Endocrinology, Pediatrics and Genetics
Eunice Kennedy Shriver National Institute of Child Health and
Human Development, National Institutes of Health
Bethesda, Maryland
United States

William V. Tamborlane, MD

Professor of Pediatrics
Chief of Pediatric Endocrinology
Yale University School of Medicine
New Haven, Connecticut
United States

Paul Thornton, MB, BCh, MRCPI

Medical Director
Congenital Hyperinsulinism Center
Cook Children's Medical Center

Fort Worth, Texas
United States

Massimo Trucco, MD

Director and Professor
Institute of Cellular Therapeutics
Allegheny Health Network, Pittsburgh
Professor
Department of Biological Sciences
Carnegie Mellon University
Pittsburgh, Pennsylvania
United States

Guy Van Vliet, MD

Professor
Department of Pediatrics
University of Montreal
Montreal, Quebec
Canada

Julia Elisabeth von Oettingen, MD, PhD, MMSc

Assistant Professor
Department of Pediatrics
McGill University Health Centre
Montreal, Quebec
Canada

Steven G. Waguespack, MD

Professor
Department of Endocrine Neoplasia and Hormonal Disorders
and Department of Pediatrics
The University of Texas MD Anderson Cancer Center
Houston, Texas
United States

Ram Weiss, MD, PhD

Professor
The Bruce Rappaport Faculty of Medicine, Technion
Department of Pediatrics
Ruth Children's Hospital
Rambam Medical Center
Haifa
Israel

William E. Winter, MD

Professor
Departments of Pathology, Immunology & Laboratory
Medicine, Pediatrics, and Molecular Genetics &
Microbiology
University of Florida
Gainesville, Florida
United States

Amy B. Wisniewski, PhD

Research Professor of Psychology
Oklahoma State University
Stillwater, Oklahoma
United States

Selma Feldman Witchel, MD

Professor of Pediatrics
Director, Pediatric Endocrinology Fellowship Program
UPMC Children's Hospital of Pittsburgh
University of Pittsburgh
Pittsburgh, Pennsylvania
United States

Joseph I. Wolfsdorf, MB, BCh

Attending Physician
Division of Endocrinology
Boston Children's Hospital Chair in Endocrinology
Professor of Pediatrics
Harvard Medical School
Boston, Massachusetts
United States

Contents

SECTION I Principles and Methods of Pediatric Endocrinology, 1

- 1 Overview and Principles of Pediatric Endocrinology, 1**
Mark A. Sperling
- 2 Molecular Endocrinology, Endocrine Genetics, and Precision Medicine, 9**
Ram K. Menon, Massimo Trucco, Joseph A. Majzoub, Constantine A. Stratakis
- 3 Receptor Transduction Pathways Mediating Hormone Action, 30**
Bassil Kublaoui, Michael A. Levine
- 4 Endocrinology Laboratory Testing, 86**
Jon Nakamoto

SECTION II Endocrine Disorders in the Neonate, 106

- 5 Fetal-Maternal Endocrinology and Parturition, 106**
Sam Mesiano, Helen N. Jones, Heide Aungst, Tani Malhotra, Louis J. Muglia
- 6 Ambiguous Genitalia, 123**
Selma Feldman Witchel, Peter A. Lee
- 7 Hypoglycemia in the Newborn and Infant, 175**
Diva D. De Leon, Paul Thornton, Charles A. Stanley, Mark A. Sperling
- 8 Disorders of the Thyroid in the Newborn and Infant, 202**
Johnny Deladoey, Julia Elisabeth von Oettingen, Guy Van Vliet
- 9 Disorders of Mineral Metabolism: Normal Homeostasis, 220**
Allen W. Root
- 10 Monogenic Diabetes Mellitus: Neonatal Diabetes and Maturity-Onset Diabetes of the Young, 279**
Siri Atma W. Greeley, Mary K. McCauley*, Louis H. Philipson, Mark A. Sperling*
**Authors contributed equally to this chapter*

SECTION III Childhood and Adolescent Endocrinology, 299

- 11 Disorders of Childhood Growth, 299**
Alexander A.L. Jorge, Adda Grimberg, Mehul T. Dattani, Jeffrey Baron
- 12 Disorders of the Posterior Pituitary, 357**
Abhinash Srivatsa, Joseph A. Majzoub

- 13 Thyroid Disorders in Children and Adolescents, 395**
Scott Rivkees, Andrew J. Bauer
- 14 The Adrenal Cortex and Its Disorders, 425**
Walter L. Miller, Christa E. Flück, David T. Breault, Brian J. Feldman
- 15 Pheochromocytoma/Paraganglioma, Medullary Thyroid Carcinoma, and Hereditary Endocrine Neoplasia Syndromes, 491**
Steven G. Waguespack, Lauren Fishbein
- 16 Puberty in the Female and Its Disorders, 528**
Robert L. Rosenfield, David W. Cooke, Sally Radovick
- 17 Turner Syndrome, 627**
Philippe Backeljauw, Steven D. Chernausek, Claus Højbjerg Gravholt, Paul Kruszka
- 18 Puberty and Its Disorders in the Male, 661**
Mark R. Palmert, Yee-Ming Chan, Leo Dunkel
- 19 Care of Transgender/Gender Nonconforming Youth, 695**
Stephen M. Rosenthal, Amy B. Wisniewski
- 20 Disorders of Mineral Metabolism II. Abnormalities of Mineral Homeostasis in the Newborn, Infant, Child, and Adolescent, 705**
Allen W. Root, Michael A. Levine
- 21 Diabetes Mellitus, 814**
Mark A. Sperling, Joseph I. Wolfsdorf, Ram K. Menon, William V. Tamborlane, David Maahs, Tadej Battelino, Moshe Phillip
- 22 Autoimmune Polyglandular Syndromes, 884**
Bimota Nambam, Michael J. Haller, William E. Winter, Desmond Schatz
- 23 Hypoglycemia in the Toddler and Child, 904**
Joseph I. Wolfsdorf, Charles A. Stanley
- 24 Obesity, Metabolic Syndrome and Disorders of Energy Balance, 939**
Joan C. Han, Ram Weiss
- 25 Lipid Disorders in Children and Adolescents, 1004**
Stephen R. Daniels, Sarah C. Couch

Index, 1022

Interactive questions for self-assessment can be found in the e-book; see inside front cover for details.

This page intentionally left blank

1 Overview and Principles of Pediatric Endocrinology

Mark A. Sperling

CHAPTER OUTLINE

HISTORICAL BACKGROUND	1
IMPACT OF HORMONAL ASSAYS AND MOLECULAR BIOLOGY	2
UNIQUE ASPECTS OF PEDIATRIC ENDOCRINOLOGY	4
Fetal Origins of Adult Disease	4
Acquisition of Patterns of Hormone Secretion and Action	4
Adaptations in Endocrine Function at Birth	5
EVALUATING ENDOCRINE DISORDERS IN INFANCY AND CHILDHOOD	6
CONCLUDING REMARKS	6
References	6

HISTORICAL BACKGROUND

Endocrinology is a discipline of science that seeks to understand how chemical signals secreted by cells regulate the function of distant (endocrine) or local (paracrine) tissues, or even their own function (autocrine), to integrate vital processes of life, such as growth, reproduction, and metabolism (Fig. 1.1). Classical endocrinology derived from careful clinical observation such as, for example, the gigantism associated with pituitary tumors, or the characteristic bodily changes now known as *Cushing disease*, which is also associated with pituitary tumors; histology indicated the former was likely the result of a product made by “acidophilic cells,” whereas the latter was associated with the expansion of “basophilic cells.” The products of these acidophilic or basophilic cells had to traverse the bloodstream to reach their distant and often multiple targets. Hence these were internal (“endocrine”) secretions, unlike the chemical substances secreted into ducts leading to a target tissue, for example, salivary glands into the cheeks; pancreatic enzymes into the duodenum (“exocrine”). Cushing disease was associated with hypertrophy of the adrenal cortex and certain tumors of adrenocortical tissues mimicked the features of Cushing disease.¹ Therefore it was readily postulated that the pituitary secretes a substance that affects the adrenal glands and function; this substance was named *adrenocorticotrophic hormone (ACTH)* and it was deduced that the features of Cushing disease/syndrome were the result of a product or products from the adrenal gland. The destruction of adrenal tissue by tuberculosis or tumor was identified by Thomas Addison in 1855 and treatment of this entity with adrenal extracts, resulting in marked improvement, was first undertaken by William Osler in 1896.² However, the purification of these “internal secretions” began in earnest only at the turn of the 20th century with spectacular success, as reviewed by Dr. Delbert Fisher.² In the first quarter of the 20th century, epinephrine, thyroxine, insulin, and parathyroid hormone were purified, followed by the purification of the sex steroids from the ovary and testis, as well as

the pituitary and placental gonadotropins that stimulated the gonads to secrete sex steroids. These purifications required laborious chemical methods and the elucidation or measurement of their function required costly and cumbersome bioassays. For example, the assay of the potency of insulin, discovered in 1921, required the use of rabbits; the definition of 1 international unit (IU) of insulin was assigned to be the amount of insulin that lowers the blood glucose of a healthy 2-kg rabbit, fasted for 24 hours, to 45 mg/dL or less, within 5 hours of injection. Clearly, such potency estimates reflected the relative crudeness of the purification; today’s pure recombinant human insulin possesses approximately 29 IU/mg, whereas the potency of porcine insulin in the early 1980s was around 23 IU/mg and likely less at the dawn of insulin therapy for diabetes mellitus. The bioassay of pure human insulin preparations has been abandoned; the purity of human insulin permits insulin to be now standardized as “one international unit of insulin (1 IU) is defined as the biological equivalent of 34.7 µg pure crystalline insulin.” It should be noted that 1 IU for insulin is not part of the International System of Units of the modern metric system, but is the pharmacologic International Unit as defined by the World Health Organization (WHO) Expert Committee on Biological Standardization. In addition to their cumbersome nature, the lack of sensitivity in the early bioassays prohibited the ability to measure insulin in normal blood or other biologic fluids; refinements, such as measuring the incorporation of labeled glucose into the fat pad or diaphragm of a rat, represented only an incremental improvement.³ Similarly, growth hormone (GH), isolated in 1944, was initially bioassayed by its ability to increase the width of the tibia growth plate in rats, after a defined period of injections, and by comparing the unknown relative to a dose response of known concentrations administered *in vivo*.⁴ Attempts to improve sensitivity and specificity led to the “sulfation factor-somatomedin hypothesis” (Fig. 1.2), in which it was postulated that GH leads to the generation of a second substance, derived from the liver, which mediates the growth-promoting (somatotropic) effects and hence was named *somatomedin*.^{4,5} Subsequent studies demonstrated that this somatomedin substance was identical to a factor in serum which had insulin-like properties *in vitro*, and this insulin-like effect was retained even after all insulin was “quenched” by an excess of antibodies specific for insulin. The convergence of these two pathways eventually led to the discovery of the factor now known as *insulin-like growth factor (IGF)-1* and later as well as IGF-2 and a family of binding proteins that acted as carriers of the hormones in serum but also possessed biologic properties of their own, by which the actions of the IGFs were mediated and modulated.^{5,6} Despite these early bioassay limitations, the scientific curiosity of these chemical substances that regulated functions as diverse as blood pressure (epinephrine, cortisol), water metabolism (arginine vasopressin, cortisol), growth (GH, sex steroids, thyroid hormone), glucose (insulin, cortisol), and reproduction (sex steroids, follicle-stimulating hormone [FSH], luteinizing hormone [LH]), spurred the formation of medical societies focused on

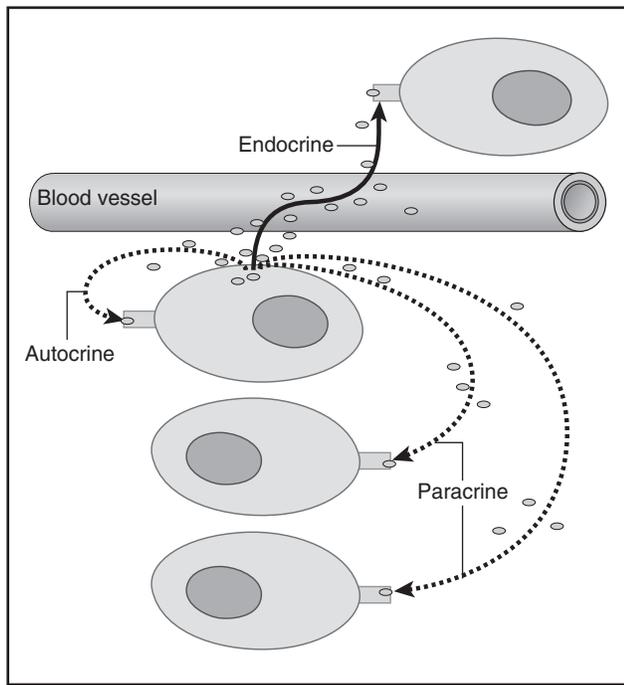


Fig. 1.1 Cellular signaling. Chemical signals synthesized and secreted by cells, may be released into the bloodstream to be distributed to target cells with specific capability to respond to the signal. These blood-borne chemicals constitute classical endocrine signals, also known as “*internal secretions*,” to distinguish them from the chemicals secreted into a duct that leads directly to another organ (e.g., pancreatic enzymes destined for the duodenum via the pancreatic duct [“*exocrine*”). However, the same cell may release the chemical that then affects nearby cells without traversing the bloodstream (these are known as *paracrine effects*) or act on a receptor on its own surface to modify the cells own functions (autocrine). (From King, T.C. (2006). *Elsevier’s Integrated Pathology*. Philadelphia: Mosby, Fig. 3.6.)

endocrine diseases. As detailed in the article by Fisher from which the following historical aspects are quoted,² the Association for the Study of Internal Secretions was established in 1918 in the United States and renamed the Endocrine Society in 1952.

Pediatric endocrinology began as a subspecialty only in the 1940s with the establishment of endocrine clinics at the Massachusetts General Hospital and at Johns Hopkins. These programs attracted postdoctoral trainees who then established their own pediatric endocrine units in the burgeoning growth of academic medical centers in the 1950s and 1960s. In the United States, the Pediatric Endocrine Society, initially named the *Lawson Wilkins Pediatric Endocrine Society (LWPES)*, was formed in 1972 and established as a subspecialty by the American Board of Pediatrics, with its first certification examination in 1978; there are more than 1000 board-certified pediatric endocrinologists today in the United States. The European Society for Pediatric Endocrinology was formed in 1966, followed by the Japanese Society for Pediatric Endocrinology in 1967 and the British Pediatric Endocrine Group in 1972, all preceding the LWPES in the United States. Several other regional pediatric endocrine groups were formed, including the Australian Pediatric Endocrine Group, the Sociedad Latino Americana de Endocrinología Pediátrica, and the Asia Pacific Pediatric Endocrine Society. All of these groups now meet jointly every 4 years at an International Pediatric Endocrine Congress.²

IMPACT OF HORMONAL ASSAYS AND MOLECULAR BIOLOGY

Two discoveries revolutionized the field of endocrinology and led to an explosion of basic, as well as clinically relevant, therapeutic knowledge in the second half of the 20th century. The first was the development of radioimmunoassay by Yalow and Berson, reported for insulin in 1960.⁷ Here was a method for measuring the low concentrations of a hormone, using as little as 10 to 50 μL of a biological fluid in an accurate, reproducible way, with precision and sensitivity adequate for *in vivo* studies in humans or other species, as well as *in vitro* studies, such as the regulation of insulin secretion by nutrients, hormones, ions, and pharmaceutical agents in whole animals, including humans, *in vivo*, or in isolated perfused pancreas, or in isolated islets. This was followed by the rapid development of assays for various hormones and an explosion of discovery, including the distinction between absolute and relative insulin deficiency as the difference between “juvenile” and “maturity onset” diabetes now known respectively as *Type 1 DM* and *Type 2 DM*, the regulation of GH secretion in normal individuals at different ages and in clinical disorders of growth, the changes in thyroid function at birth and the possibility of screening for neonatal hypothyroidism, and the changes in gonadotropins and sex hormones during the process of normal and abnormal puberty and later in aging. The discovery and purification of the hypothalamic-releasing hormones for thyroid-stimulating hormone (TSH), FSH/LH, GH, and ACTH were made possible by these precise assays using (rat) pituitary cells, perfused by protein fractions derived from the hypothalami of animals.⁸ The discovery that a hormone produced in a cell could affect the function of its neighboring cell(s), without traveling through the bloodstream (paracrine action) or even its own function (autocrine), was also enabled by the use of these sensitive and precise tools, expanding our concepts of a hormone as a chemical messenger that influences, directs, and coordinates cellular functions throughout the body (see Fig. 1.1). Similar principles enabled the identification of the receptor molecules at the cell surface or in its cytoplasm that permit the hormone signal to be transduced to a message for turning biological processes on, or off, in specific tissues.⁹ Refinements using the principles of radioimmunoassay (RIA), but without radioactivity, as well as newer techniques of liquid chromatography coupled with mass spectrometry, are the bases of modern laboratory methodologies for hormone measurement, as well as for other chemical substances, such as drugs; examples of modern application of these methods, as well as the pitfalls in performance and interpretation, are reviewed in Chapter 4. How these hormonal signals elicit a specific response after recognition and binding to a specific cell surface or cytoplasmic receptor, and the subsequent cascade of events known as the *signal transduction pathways* and their relevance to pediatric endocrinology are discussed in Chapter 3. The notion that a hormone may not be capable of eliciting a response, despite high concentrations, was implicit in the entity labeled *pseudohypoparathyroidism* by Dr. Fuller Albright,¹⁰ but receptors and their signal transduction pathways were only systematically investigated beginning in the 1970s. These systematic studies, still ongoing,⁹ continue to identify the mechanistic pathways by which a hormone, after binding to its receptor, may elicit a response in one tissue but not in another. There may be other reasons why a hormone does not elicit an appropriate tissue response despite apparent high concentrations in the circulation. For example, an abnormal sequence in a hormone may prevent its full action at the receptor, and feedback control increases the hormone’s secretion, leading to high concentrations of partially functioning hormone with only minor or moderate impairment of function. Examples of such abnormalities include disorders of

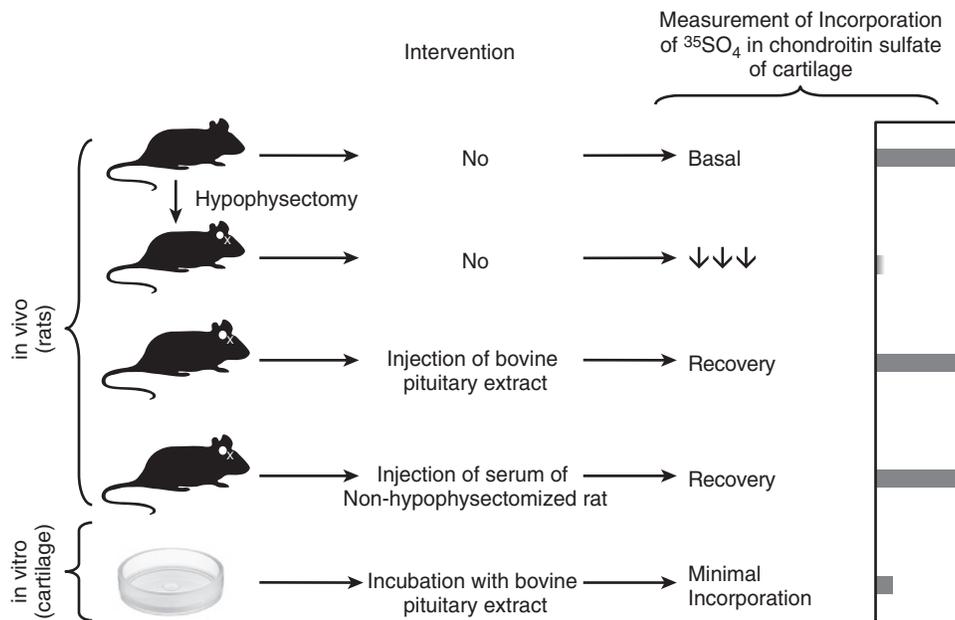


Fig. 1.2 “Sulfation factor” assay of growth hormone. The bioassay of growth hormone (GH) consisted of administering graded doses of bovine GH, with potency approximately 1.25 U/mg, via daily subcutaneous (SC) injections, for 4 days, to young, growing, prepubertal rats (approximately 31 days) that had been hypophysectomized 10 days previously. Approximately 5 animals per group and about 5 doses (0 plus 4 graded increments) were used to construct the “dose–response curve” of the increase in the width of the tibia growth plate; the unknown was 2 to 3 mL of plasma also administered SC to approximately three to five animals per test dose. The sulfation assay was an attempt to refine the technique by examining the dose–response relationship between the incorporation of $^{35}\text{SO}_4$ into chondroitin sulfate in vivo, or in vitro into uniformly prepared cartilage rings obtained from young rats. As shown in the figure, the in vivo component examined basal activity of the animal’s serum on the amount of radioactivity incorporated in vivo. Hypophysectomy almost completely abolished this ability to stimulate $^{35}\text{SO}_4$ incorporation, but injection of bovine pituitary extract or serum from a normal not hypophysectomized animal, restored this activity. However, in vitro incubation of cartilage rings with bovine pituitary extract resulted in only minimal incorporation. Thus it was proposed that GH acted on an internal organ to produce the “sulfation factor.” The sensitivity of these assays was, at best, in the 1 to 10 $\mu\text{g}/\text{mL}$ range, and precision and reproducibility were poor (Tweed, D.C., McCullagh, E.P. (1962). Assay of growth hormone-like activity in blood plasma: a comparison of two methods. *Clin Chem*, 8, 141–150; also see references 4 and 5). Today’s immunoassays permit measurement of plasma concentrations of GH with 1000-fold greater sensitivity than these early bioassays (ng/mL compared with $\mu\text{g}/\text{mL}$ previously mentioned) with high degrees of precision and reproducibility (see Chapter 4). (The author is indebted to Oscar Escobar, MD, Associate Professor, Department of Pediatrics, University of Pittsburgh School of Medicine, Division of Pediatric Endocrinology, Children’s Hospital of Pittsburgh, for creating this figure and granting permission for its use in this chapter.)

proinsulin conversion to insulin.¹¹ and OMIM #616214 Depending on the site of the abnormality in cleavage of proinsulin, both the measurement of insulin and proinsulin or its cleavage product C-peptide, may demonstrate higher than normal values that could be interpreted as “insulin resistance.” These studies also recognized that an activating mutation in a receptor will mimic the action(s) of a hormone, although the hormone concentration may be barely detectable, as exemplified by the precocious puberty in McCune-Albright syndrome or by an activating mutation of the LH receptor in males, which results in precocious puberty with high testosterone, but paradoxically very low concentrations of LH, an entity named *testotoxicosis* (see Chapter 18). In contrast, loss of function mutations cause the same clinical syndrome as hormone deficiency, even though the hormone concentration is markedly increased compared with normal, as exemplified in Laron syndrome, with poor growth and low IGF-1 concentrations, despite high circulating growth hormone concentrations (see Chapter 11). Thus the concentration of a hormone may not be directly related to its action. In summary, it is the ability to measure hormones, as well as intermediary molecules and metabolites, such as cyclic adenosine monophosphate at low concentrations (e.g., picomolar to nanomolar), in small volumes of biological fluids that enabled the rapid proliferation and understanding of endocrine regulation and function. It may be difficult for any reader who was not brought up in the era of bioassays to fully

appreciate the impact of the application of the tool of RIA and its modifications, as various competitive protein binding assays without radioactive markers, to modern endocrine concepts and practice. The theoretical basis for the competitive binding assays, pitfalls in their interpretation, and their application to studies of receptor–ligand interactions are detailed in Chapter 4.

The second revolution built on the discovery of the double helix by Crick, Watson, and Wilkins, a discovery for which they received the Nobel Prize in 1962. This discovery and its ongoing offspring propelled the ability to identify the molecular basis of cell function, the genes that regulate these processes, and the genetic mutations that underlie congenital or acquired disorders, including those of the endocrine system. Pediatric medicine is a particular beneficiary of these techniques because congenital malformations of the endocrine glands, the abnormalities of hormone signaling, as a result of defective hormone synthesis, hormone processing or receptor function to recognize and act on the hormonal signal are at the core of pediatric endocrinology, as reflected in this book. Chapters 2 and 3 provide an overview of the molecular and genetic methodologies applied in practice and research and also discusses the pitfalls in interpreting the results of genetic mutational analyses. This is a rapidly evolving field powered by the newer sequencing technologies (collectively termed *next-generation sequencing* [NGS]). These technologies enable the sequencing

of deoxyribonucleic acid (DNA) and ribonucleic acid much more quickly and cheaply than the previously used Sanger sequencing, and in concert with the ability to store and analyze huge data sets via computers have revolutionized the study of genomics and molecular biology and the direct clinical application of this knowledge. For example, whole exome sequencing and whole genome sequencing is increasingly being applied and is becoming part of established medical diagnostic practice in several situations, for example, neonates with multiple congenital malformations or metabolic disturbances in the newborn intensive care unit.^{12–14} Use of fetal DNA in maternal cell free plasma is becoming the standard for antenatal diagnosis of aneuploidy, without the need for invasive screening procedures, such as amniocentesis or chorionic villous sampling, and has been used to diagnose congenital adrenal hyperplasia in at-risk pregnancy of heterozygote carriers of this disease.^{15,16} NGS allows prompt genetic diagnosis of different mutations responsible for a specific disease or syndrome complex, for example, Neonatal Diabetes Mellitus, so that remedial treatment can be expeditiously provided,¹⁷ thereby altering what has hitherto been considered the natural history of the particular disorder. Direct therapeutic applications, such as, for example, choice of drug for maximum effectiveness, while avoiding drug interactions or excluding drug sensitivity, are also being explored. Gene therapy¹⁸ and gene editing¹⁹ for pediatric endocrine disorders are on the horizon and some show promise, but with many practical and ethical issues still to be resolved, as discussed in Chapters 2 and 3.

UNIQUE ASPECTS OF PEDIATRIC ENDOCRINOLOGY

Fetal Origins of Adult Disease

Pediatrics is all about growth and development of each aspect of human life—physical, emotional, cognitive, and sexual—from conception through birth, neonatal adaptation, infancy, childhood, puberty, and young adulthood. Changes in the mature organism continue, but the tempo is considerably slower than the rapid changes of early life. The endocrine system plays a central role in these adaptations and changes. It has long been known that environmental insults, such as viral infections (rubella, herpes, human immunodeficiency virus [HIV], cytomegalovirus, Zika) or drugs or other chemicals (thalidomide, phenytoin, alcohol), particularly in the first trimester, can result in distinct patterns of embryopathy. Only fairly recently has it been appreciated that developmental plasticity is influenced by the intrauterine nutritional environment that may predispose to the later development of diseases, such as type 2 diabetes. The notion that lack of nutrients during critical periods of development impart subsequent greater risk for development of adult diseases, such as hypertension, stroke, coronary disease, and diabetes emanated from careful epidemiologic observations of cohorts, such as those of the Dutch famine toward the end of the second world war²⁰ and a British cohort of citizens with health records from birth to death.²¹ The former documented that the effects of the famine appeared to depend on its occurrence during gestation, early gestation being the most vulnerable period, with effects on schizophrenia, depression, atherogenic lipid profile, and with an increased rate of hypertension and coronary heart disease later in life.²⁰ The latter demonstrated that a limited supply of nutrients, as reflected in intrauterine growth restriction, even in the absence of frank famine, could permanently alter the metabolism and other physiologic aspects in later adult life, including stroke and diabetes.²¹ Moreover, these effects could persist into future generations, suggesting modifications of the genome, giving rise to the concept of fetal origins of adult disease, as proposed

by Barker.²¹ As a hypothesis, the “fetal origins of adult disease” is now well supported by epidemiologic and experimental data,^{20–28} including the epigenetic modification of gene expression, via patterns of methylation or other modifications, some affecting the expression of genes regulating insulin secretion, cortisol, and other hormones.²⁹ These interactions are not restricted to early development—they may occur in the third trimester or beyond,^{30–33} but once “imprinted,” they may be passed on to succeeding generations. Thus the intrauterine and peripartum environment may permanently modify the expression of genes, including those of the endocrine system.^{30,33}

Acquisition of Patterns of Hormone Secretion and Action

At any given time, the plasma concentration of a hormone reflects its synthesis, secretion, and clearance, a concept that implies removal from the circulation in units of time. Indeed, the relationship between the production rate (PR) of a hormone (units/time), the serum or plasma concentration ($C = \text{units/mL}$), and its metabolic clearance rate (MCR) from the plasma (mL/time) is defined by the formula $PR = MCR \times C$, so knowing any two of these three variables permits one to solve the third. However, the concentration of a hormone may vary depending on such factors as time of day, stages of sleep, stage of puberty, renal or hepatic function, and the ingestion of other drugs. Hence each of these aspects represents complex interactions. For example, the secretion of ACTH and the chief product of its action, cortisol, are related in phase, the former preceding the latter and being highest in the early morning and lowest toward midnight. ACTH synthesis may have a basal rate determined by the impulses from its hypothalamic-releasing hormone, known as *corticotropin-releasing hormone (CRH)*, but both display a diurnal rhythm entrained by the light/dark cycle and relayed to the suprachiasmatic nucleus of the hypothalamus. As the fetus is not exposed to light/dark cycles and the newborn spends a majority of time asleep, it is important to know when the diurnal rhythm becomes established.^{34–37} This has importance for undertaking measurements of cortisol in the newborn to determine if the hormone is deficient, as, for example, in evaluating newborn hypoglycemia. Also, stress results in a rapid increase in the CRH-ACTH-cortisol axis and the secretion of cortisol. Such rapid adaptation is essential for the appropriate adjustments to physiologic stress resulting in the “fight or flight response.” It is known that the hypothalamic pituitary adrenal axis is established in utero and functional because inborn errors of metabolism, such as those found in congenital adrenal hyperplasia as a result of defects in enzymes responsible for cortisol synthesis, result in markedly elevated ACTH in utero, with hypertrophy of the adrenal cortex. However, after birth, there is a brief period during which adrenal secretion of cortisol is low, as reflected in the plasma levels,³⁶ and it is not known how rapidly the newborn can adjust to a stressful situation by appropriate increments in the ACTH-cortisol secretion rates.³⁷ Some hormonal cycles, such as GH secretion and FSH/LH secretion at the onset of puberty, are related to sleep rather than the light/dark cycle, as discussed for GH⁵ and separately for the gonadotropins in the relevant chapters of this book. But it is not known if these sleep-regulated patterns are operative in the newborn. Following birth, some inhibitory pathways of hormone secretion are not yet established, resulting in high hormone concentrations whose functional significance is not known. For example, inhibition of GH secretion by somatotropin release-inhibiting factor appears to develop only after birth, so that GH concentrations in serum are quite high in the newborn (averaging approximately

40 ng/mL), values that would be consistent with acromegaly in an adult.^{38,39} On the other hand, the expression of GH receptors on tissues and their linkage to postreceptor events is delayed and become fully operational only after several months, so that the effects of the high GH concentrations are muted.^{39–41} The practical implications of these findings are that GH deficiency in a full-term newborn will not be characterized by discernible small size at birth. Indeed, GH deficiency does not become manifest in a delayed growth velocity, until after 3 to 6 months when the GH–growth hormone receptor axis becomes established.^{39–41} Yet GH plays an important role in maintaining glucose homeostasis in the newborn, because GH deficiency may be associated with hypoglycemia, and this deficiency can be diagnosed without the need for stimulation tests if the GH concentration is lower than 10 ng/mL in the first week of life of a full-term newborn. Another hormone whose concentrations remain very high at birth and for several weeks thereafter is prolactin, presumably because the neurologic pathways responsible for dopamine secretion are not yet fully developed.³⁹ The physiologic implications of these adaptive processes in the secretion and action of prolactin in the newborn are not fully understood.³⁹

Adaptations in Endocrine Function at Birth

Separation of the newborn baby from its maternal blood supply after birth imposes a near-instantaneous need for adaptations in functions, such as the requirements of oxygen, maintenance of body temperature, and sources of nutrients. The endocrine system plays a vital role in several of these adaptations; three are briefly described here and are explored in more detail in relevant chapters in this book.

Maintenance of Body Temperature

The hypothalamic-pituitary-thyroid axis is intimately involved in the adaptations of body temperature regulation. The fetus, bathed in amniotic fluid and supplied by maternal blood, maintains a temperature of 37° C in utero. Delivery into an ambient temperature of approximately 20° to 25° C in a modern delivery room represents a significant fall in ambient temperature that contributes to activation of thyroid function. The concentration of TSH increases approximately 10-fold between birth and 15 to 30 minutes after cutting of the umbilical cord, to values of approximately 100 μ U/mL. Simultaneously, type 2 deiodinase is activated, converting thyroxine (T_4) to triiodothyronine (T_3) rather than the fetal pattern of type 3 deiodinase that converts T_4 to reverse T_3 . These rapid changes result in a surge of T_3 concentration, a decline in reverse T_3 concentration, and a somewhat later rise of T_4 concentration in the newborn's blood. Together, these coordinated changes in thyroid function enable T_3 to act on brown adipose tissue to activate nonshivering thermogenesis. Although TSH concentrations decline to values below 10 μ U/mL by day 2 to day 3 of life, T_3 and T_4 values remain elevated for days to weeks at concentrations that would be consistent with thyrotoxicosis in older children and adults (see Chapter 8 for details). Hence values of thyroid function reported for full-term or premature newborns must be reported as age specific; they may often be labeled erroneously as *hyperthyroid* in laboratories that only list reference values suitable for adults. Not only do premature babies have thyroid hormone-related concentrations that are lower than those of full term infants, but the acute stresses of prematurity, such as sepsis or respiratory distress syndrome, may lead to the "euthyroid sick syndrome" and further complicate the interpretation of thyroid function tests, as discussed in Chapter 8.

Glucose Homeostasis

The fetus obtains all of its glucose via placental transfer from the mother, with little, if any, endogenous glucose production until delivery. Following cord cutting, epinephrine and glucagon each rise approximately three- to five-fold, growth hormone is high at approximately 40 ng/dL (as mentioned previously), as are cortisol values, which are highest at about 2 hours after birth and, on average, remain in the range of 2.7 to 7.6 μ g/dL, mostly in the free form, in the first week of life.^{39,42–44} The coordinated effects of these four classic "counterregulatory" hormones, together with a small fall in insulin, stem the initial decline in blood glucose concentration, activate glycogen breakdown and gluconeogenesis, and initiate lipolysis with later activation of ketone body production by day 2 to day 3 of life.^{42–44} Understanding these critical adaptations is essential for the appropriate management of hypoglycemia in a newborn, as detailed in Chapter 7. All hormonal measurements must be age specific; a common problem with hypoglycemia in the newborn is the range of normal fasting insulin values from an adult reference range, generally in the range of 5 to 29 μ U/mL. A newborn with hypoglycemia who at the time of hypoglycemia has an insulin value greater than 5 μ U/mL likely has some form of genetic or acquired hyperinsulinism.

Gonadotropins and Sex Hormones

In males, testosterone concentrations on day 1 of life are high, ranging from 75 to 400 ng/dL, values that are consistent with Tanner stages 3 to 4 of male puberty. These high concentrations decline rapidly within the first few days after delivery but remain elevated at 20 to 50 ng/dL compared with males aged 1 to 10 years, in whom values are less than 10 ng/dL. A second rise in testosterone concentrations occurs between 1 week and 1 to 2 months, the mean values being approximately 200 ng/dL. Both FSH and LH also are relatively high in males at this age of life and decline to prepubertal levels only at about the end of the first year. In females, concentrations of estradiol are markedly elevated after birth and fall rapidly during the first week of life to prepubertal values, with a secondary rise occurring between 30 and 60 days followed by decline to prepubertal concentrations after 1 to 2 years. Values of FSH may range up to 14 mIU/mL in females and decline more slowly than in males, reaching prepubertal values only after 2 to 3 years. Likewise, LH values in females may be in the classical pubertal range in the first few months of life and decline to prepubertal values only after 1 to 2 years. This so called *minipuberty of infancy* is discussed in greater detail in Chapters 6, 16, and 18. A precise function for these perinatal changes in sex hormones and gonadotropins is unknown, but it has been proposed that they may have relevance to developmental patterning of male or female neural function. In addition, clinical relevance is related to the common problem of thelarche in newborn-infant females, as discussed in Chapter 16.

In summary, each of the major endocrine organs and systems undergo dramatic neonatal adaptations, so that knowledge of these endocrine adaptations following birth is essential for the appropriate evaluation of suspected abnormalities in endocrine function, and the interpretation of age- and sex-specific values of circulating hormones or other metabolic variables. A major deficit in healthcare systems is the application of standards of normal ranges for hormones or other biochemical indices derived from adult reference ranges to the newborn and infant; values must be relevant to the age- and sex-specific situation being evaluated.

EVALUATING ENDOCRINE DISORDERS IN INFANCY AND CHILDHOOD

A general principle of pediatric endocrinology is that the earlier the manifestation of either underactivity or excess hormone function, the more likely the cause is to be a genetic disorder, together with possible structural abnormalities. For example, the entity of septooptic dysplasia with underactivity of anterior and/or posterior pituitary function may be associated with typical structural abnormalities of optic nerve hypoplasia, absence of the septum pellucidum or corpus callosum, a small anterior pituitary, interruption or absence of the pituitary stalk, and an ectopic or absent posterior pituitary bright spot on magnetic resonance imaging (MRI) of the brain.^{45,46} In the majority of these patients, no genetic abnormality can be detected. However, mutations in three genes (*HESX1*, *OTX2*, and *OX2*) that play a role in the embryonic development of the pituitary gland, eyes, and forebrain, have been identified in a small subset of these patients.^{45,46} Hypothyroidism found on newborn screening is most commonly associated with an ectopic thyroid gland; total absence or goitrous hypothyroidism should suggest a defect in the genes responsible for thyroid gland formation (*TTF-1*, *TTF-2*, *PAX8*) or an “inborn error of thyroid hormone synthesis”⁴⁷ (see Chapter 8). To be sure, perinatal events and maternal illness or medication must be considered. For example, perinatal asphyxia or a difficult delivery may be associated with later evidence of hypopituitarism. Maternal ingestion of antithyroid medications would result in their transfer across the placenta and may cause transient neonatal hypothyroidism, as these agents affect the fetal thyroid in the same way as they affect the maternal thyroid gland. Similarly, transfer of immunoglobulin G antibodies that block or stimulate thyroid function will result in newborn hypothyroidism or hyperthyroidism that lasts for several weeks, until maternal antibodies are cleared from the circulation. A newborn with severe hyperthyroidism in the absence of any evidence of autoimmune disease in the mother almost certainly has an activating mutation of the TSH receptor⁴⁸ (see Chapter 8).

More subtle defects in endocrine function may appear later in childhood, but still have a genetic basis, for example, hypogonadotropic hypogonadism. This entity may be considered at birth in male infants because of underdeveloped penile and testicular size, but often does not become manifest or discovered until delayed puberty is investigated.⁴⁹ Macrosomia in an infant born to a mother with poorly controlled diabetes mellitus reflects secondary hyperinsulinism in the fetus, with hypoglycemia in the newborn when the maternal supply of glucose is curtailed. However, similar features in a baby born to a healthy young mother should immediately suggest the possibility of a genetic form of hyperinsulinism, most commonly the result of an inactivating mutation in the genes regulating the adenosine triphosphate (ATP)-regulated potassium channel (K_{ATP}).^{50–52} If the biochemical profile and clinical course are consistent with hyperinsulinism, but insulin concentrations are low, consider an activating mutation in the insulin receptor signaling pathway. Conversely, significant intrauterine growth retardation may be caused by many factors and is a major feature of neonatal diabetes mellitus, so early diagnosis via measurement of glucose may be lifesaving by providing insulin or sulfonyleurea drugs, to which some 90% of newborns with activating mutations of the *KCNJ11* gene respond with improved endogenous insulin secretion and restoration of near normal glucose metabolism⁵³ and as discussed in detail in Chapter 10. Severe intrauterine growth retardation also is a feature of inactivating mutations in insulin receptors, for example, Donohue syndrome or Rabson-Mendenhall syndrome.⁵⁴ Defects in the secretion or action of IGF-1 mimic the effects of insulin

secretion or action, both hormones and both receptors being structurally, as well as functionally related.^{55,56}

Autoimmunity, trauma, and chemo/radiotherapy for a childhood malignancy are the most common causes of acquired endocrine disorders. Thus as in all of medicine, a careful and thorough history, careful physical examination, and targeted investigation of hormonal measurements (taking into consideration age, time of day, and the value of obtaining a “free” hormone versus total hormone measurement) form the basis of the diagnostic approach. This may be followed by imaging of the suspected organ involved to diagnose the basis of the suspected endocrine dysfunction, in the particular patient being investigated. Increasingly, molecular diagnostics is becoming an integral component of this evaluation, both to establish the cause of the entity and to provide guidance for prognosis, such as multiple endocrine neoplasia 2 (see Chapter 15).

Definitive diagnosis may require stimulation tests (e.g., for growth hormone deficiency or with ACTH for suspected adrenal insufficiency) because a single random value is not sufficiently informative. Such stimulation tests are an integral and essential component of diagnosis and management of endocrine disorders at any age but particularly in the pediatric age range, where performance of the test must take into consideration its safety, easing anxiety and correct sample handling.

Finally, modern biology has provided pure synthetic compounds to replace what may be missing, such as thyroid hormone or cortisol, administration of pulsatile gonadotropin-releasing-hormone (GnRH) for syndromes of hypogonadotropic hypogonadism via programmable pumps, GnRH analogues for suppressing puberty, growth hormone for GH deficiency or other forms of approved short stature, various insulin preparations with ultrashort, short, intermediate, long-acting and ultralong acting forms for managing diabetes, and ultralong-acting somatostatin for childhood acromegaly as occurs in Carney complex,⁵⁷ or some patients with McCune-Albright syndrome.⁵⁸ Hormone replacement must also take into consideration administration via the dermal rather than the oral route to avoid “first pass” considerations in bypassing the liver, as, for example, giving sex hormones, such as estrogen to females with delayed puberty, including Turner syndrome (see Chapters 16 and 17).

CONCLUDING REMARKS

Endocrinology is the science of cellular communication that enables the biochemical integration of life’s vital processes. Pediatric endocrinology is the linchpin for these processes during the developmental epoch from fetus to mature adult. Evolving developments in molecular biology, bioinformatics, pharmacogenetics, and bioimaging will ensure that this specialty remains at the forefront of pediatric research and practice. This chapter is intended as an introduction to this important field, with greater detail to be found in the chapters that follow. Much has been learned since the prior edition of this chapter in 2014 and some of this newer knowledge is incorporated here; much remains to be learned.

REFERENCES

1. Medvei VC. The history of Cushing’s disease: a controversial tale. *J Royal Soc Med.* 1991;84(6):363–366.
2. Fisher DA. A short history of pediatric endocrinology in North America. *Pediatr Res.* 2004;55(4):716–726.
3. Randle PJ. Assay of plasma insulin activity by the rat-diaphragm assay. *Br Med J.* 1954;1(4873):1237–1240.

4. Salmon Jr WD, Daughaday WH. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J Lab Clin Med.* 1957;49(6):825–836.
5. Ranke MD, Wit JM. Growth hormone – past, present and future. *Nat Rev Endocrinol.* 2018;14(5):285–300.
6. Bach LA. What happened to the IGF binding proteins? *Endocrinology.* 2018;159(2):570–578.
7. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. *J Clin Invest.* 1960;39(5):1157–1175.
8. Guillemin R. Hypothalamic hormones a.k.a. hypothalamic releasing factors. *J Endocrinol.* 2005;184(1):11–28.
9. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat Rev Drug Discovery.* 2010;9(5):373–386.
10. Kleeman CR, Levine BS, Felsenfeld AJ. Fuller Albright: The consummate clinical investigator. *Clin J Am Soc Nephrol.* 2009;4(10):1541–1546.
11. Robbins DC, Tager HS, Rubenstein AH. Biological and clinical importance of proinsulin. *N Engl J Med.* 1984;310(18):1165–1175.
12. Kohane IS, Drazen JM, Campion EW. A glimpse of the next 100 years in medicine. *N Engl J Med.* 2012;367(26):2538–2539.
13. Holm IA, Agrawal PB, Ceyhan-Birsoy O, et al. The BabySeq project: implementing genomic sequencing in newborns. *BMC Pediatr.* 2018;18(1):225.
14. Ceyhan-Birsoy O, Machini K, Lebo MS, et al. A curated gene list for reporting results in newborn genomic sequencing. *Genet Med.* 2017;19(7):809–818.
15. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. *N Engl J Med.* 2018;379(5):464–473.
16. New MI, Tong YK, Yuen T, et al. Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma. *J Clin Endocrinol Metab.* 2014;99(6):E1022–E1030.
17. De Franco E, Flanagan SE, Houghton JA, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet.* 2015;386(9997):957–963.
18. Eichler F, Duncan C, Musolino PL, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. *N Engl J Med.* 2017;377(17):1630–1638.
19. Church G. Compelling reasons for repairing human germlines. *N Engl J Med.* 2017;377(20):1909–1911.
20. Roseboom TJ, Painter RC, van Abeelen AF, et al. Hungry in the womb: what are the consequences? Lessons from the Dutch famine. *Maturitas.* 2011;70(2):141–145.
21. Barker DJ. The Wellcome Foundation Lecture, 1994. The fetal origins of adult disease. *Proc Biol Sci.* 1995;262(1363):37–43.
22. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiological? *Physiol Rev.* 2014;94(4):1027–1076.
23. Nettle D, Bateson M. Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proc Biol Sci.* 2015;282(1812). 20151005.
24. Gilbert SF, Bosch TC, Ledon-Rettig C. Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. *Nat Rev Genet.* 2015;16(10):611–622.
25. Heindel JJ, Balbus J, Birnbaum L, et al. Developmental origins of health and disease: integrating environmental influences. *Endocrinology.* 2015;156(10):3416–3421.
26. Simeoni U, Armengaud JB, Siddeek B, et al. Perinatal origins of adult disease. *Neonatology.* 2018;113(4):393–399.
27. Frankenhuis WE, Nettle D, McNamara JM. Echoes of early life: recent insights from mathematical modeling. *Child Dev.* 2018;89(4):1504–1518.
28. Devaskar SU, Chu A. Intrauterine growth restriction: hungry for an answer. *Physiology (Bethesda).* 2016;31(2):131–146.
29. Morsi A, DeFranco D, Witchel SF. The hypothalamic-pituitary-adrenal axis and the fetus. *Horm Res Paediatr.* 2018;89(5): 380–387.
30. Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. *N Engl J Med.* 2018;378(14):1323–1334.
31. Kalish JM, Jiang C, Batolovei MS. Epigenetics and imprinting in human disease. *Int J Dev Biol.* 2014;58(2–4):291–298.
32. Bansal A, Pinney SE. DNA methylation and its role in the pathogenesis of diabetes. *Pediatr Diabetes.* 2017;18(3):167–177.
33. Lim AL, Ng S, Leow SC, et al. Epigenetic state and expression of imprinted genes in umbilical cord correlates with growth parameters in human pregnancy. *J Med Genet.* 2013;49(11):689–697.
34. Jett PL, Samuels MH, McDaniel PA, et al. Variability of plasma cortisol levels in extremely low birth weight infants. *J Clin Endocrinol Metab.* 1997;82(9):2921–2925.
35. Vermes I, Dohanics J, Toth G, et al. Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. *Hormone Res.* 1980;12(5):237–244.
36. Sippell WG, Becker H, Versmold HT, et al. Longitudinal studies of plasma aldosterone, corticosterone, deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol and cortisone determined simultaneously in mother and child at birth and during the early neonatal period. I. Spontaneous delivery. *J Clin Endocrinol Metab.* 1978;46(6):971–985.
37. Soliman AT, Taman KH, Rizk MM, et al. Circulating adrenocorticotrophic hormone (ACTH) and cortisol concentrations in normal, appropriate-for-gestational-age newborns versus those with sepsis and respiratory distress: Cortisol response to low-dose and standard-dose ACTH tests. *Metab Clin Exp.* 2004;53(2):209–214.
38. Kaplan SL, Grumbach MM, Shepard TH. The ontogenesis of human fetal hormones. I. Growth hormone and insulin. *J Clin Invest.* 1972;51(12):3080–3093.
39. Gurtunca N, Sperling MA. Growth hormone, prolactin, and placental lactogen in the fetus and newborn. In: Richad P, Abman S, Rowitch D, Benitz W, eds. *Fetal and Neonatal Physiology, Vol. 2.* 5th ed. Philadelphia: Elsevier; 2017:1470–1476.
40. Goodyer CG, Figueiredo RM, Krackovitch S, et al. Characterization of the growth hormone receptor in human dermal fibroblasts and liver during development. *Am J Physiol Endocrinol Metab.* 2001;281(6):E1213–E1220.
41. Romero GS, Stephan DA, Sperling MA, et al. Distinct sexual dimorphism in the effect of hypothyroidism on the expression of the growth hormone receptor and growth hormone-binding protein gene in rat liver. *Hormone Res.* 1996;45(6):273–278.
42. Menon RK, Sperling MA. Carbohydrate metabolism in neonatal adaptation: the transition to post-natal life. *Semin Perinatol.* 1988;12(2):157–162.
43. Stanley CA, Rozance PJ, Thornton PS, et al. Re-evaluation transitional neonatal hypoglycemia: mechanism and implications for management. *J Pediatr.* 2015;166(6):1520–1551.
44. Thornton PS, Stanley CA, De Leon DD, et al. Recommendations from the Pediatric Endocrine Society for evaluation and management of persistent hypoglycemia in neonates, infants and children. *J Pediatr.* 2015;167(2):238–245.
45. Ryabets-Lienhard A, Stewart C, Borchert M, Geffner ME. The optic nerve hypoplasia spectrum: review of the literature and clinical guidelines. *Adv Pediatr.* 2016;63(1):127–146.
46. Mehta A, Hindmarsh PC, Mehta H, et al. Congenital hypopituitarism: clinical, molecular and neuroradiological correlates. *Clin Endocrinol (Oxford).* 2009;71(3):376–382.
47. Szinnai G. Genetics of normal and abnormal thyroid development in humans. *Best Prac Res Clin Endocrinol Metab.* 2014;28(2):133–150.
48. Ferraz C, Paschke R. Inheritable and sporadic non-autoimmune hyperthyroidism. *Best Prac Res Clin Endocrinol Metab.* 2017;31(2):265–275.
49. Stamou MI, Cox KH, Crowley Jr WF. Discovery of genes essential to the regulation of human reproduction using a human disease model: adjusting to life in the “Omics” Era. *Endocr Rev.* 2016;2016(1):4–22.
50. Lord K, DeLeon DD. Hyperinsulinism in the neonate. *Clin Perinatol.* 2018;45(1):61–74.
51. Hussain K, Challis B, Rocha M, et al. An activating mutation of AKT2 and human hypoglycemia. *Science.* 2011;334(6055):474.
52. Leiter SM, Parker VER, Welters A, et al. Hypoinsulinemic, hypoketotic hypoglycemia due to mosaic genetic activation of PI3-Kinase. *Eur J Endocrinol.* 2017;177(2):175–186.
53. Bowman P, Sulen Å, Barbeti F, et al. Effectiveness and safety of long-term treatment with sulfonlyureas in patients with neonatal diabetes due to KCNJ11 mutations: an international cohort study. *Lancet Diabetes Endocrinol.* 2018;8:636–646.

54. Taylor SI, Accili D, Haft CR, et al. Mechanisms of hormone resistance: lessons from insulin resistant patients. *Acta Paediatr Suppl.* 1994;399:95–104.
55. Abuzzahab MJ, Schneider A, Goddard A, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med.* 2012;366(24):2211–2222.
56. Woods KA, Camacho-Hübner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor-1 gene. *N Engl J Med.* 1996;335(18):1363–1367.
57. Correa R, Salpea P, Stratakis CA. Carney complex: an update. *Eur J Endocrinol.* 2015;173(4): M85–87.
58. Hanna-Shmouni F, Trivelli NG, Stratakis CA. Genetics of acromegaly and gigantism. *Growth Hormone IGF-I Res.* 2016; 30–31:37–41.

2

Molecular Endocrinology, Endocrine Genetics, and Precision Medicine

Ram K. Menon, Massimo Trucco, Joseph A. Majzoub, Constantine A. Stratakis

CHAPTER OUTLINE

INTRODUCTION	9
BASIC MOLECULAR TOOLS	9
Isolation and Digestion of DNA and Southern Blotting	9
Restriction Fragment Length Polymorphism	10
Polymerase Chain Reaction	10
RNA Analysis	12
DETECTION OF MUTATIONS IN GENES	15
Direct Methods	15
Indirect Methods	19
CRISPR-CAS9 SYSTEM	19
POSITIONAL GENETICS IN ENDOCRINOLOGY	20
The Principles of Positional Genetics	20
Genomic Identification of “Endocrine” Genes	21
Impact of Modern Sequencing in Clinical Practice	21
CHROMOSOME ANALYSIS AND MOLECULAR CYTOGENETICS	22
Outline of Methods	22
Applications	22
Future Developments	22
PRINCIPLES OF INTERPRETATION OF GENETIC TESTS IN THE DIAGNOSIS AND MANAGEMENT OF PEDIATRIC ENDOCRINE DISEASES	23
GENOMIC MEDICINE, GENOME SEQUENCING, AND APPLICATIONS IN GENETIC TESTING	24
GENETICS IN CLINICAL PRACTICE AND NEXT-GENERATION SEQUENCING APPLICATIONS	24
Genome Versus Exome Sequencing, Variant Interpretation, Other Challenges	25
NGS and Cell-Free DNA in Cancer Medicine and Prenatal Testing	25
Next-generation sequencing, mosaicism, and its applications in detecting somatic genetic defects	26
PRECISION MEDICINE	26
RECOMBINANT DNA TECHNOLOGY AND THERAPY OF PEDIATRIC ENDOCRINE DISEASES	27
CONCLUDING REMARKS	28
References	28

INTRODUCTION

The study of the endocrine system has undergone a dramatic evolution since the 1990s, from the traditional physiologic studies that dominated the field for many years to the discoveries of molecular endocrinology and endocrine genetics.^{1,2} At the present time, the major impact of molecular medicine

on the practice of pediatric endocrinology relates to diagnosis and genetic counseling for a variety of inherited endocrine disorders. In contrast, the direct therapeutic application of this new knowledge is still in its infancy. Endocrine oncology has greatly benefited from the application of new drugs that were designed to target specific mutations in, for example, thyroid cancer. A notable recent therapeutic advancement that followed the identification of the molecular basis of an endocrine disorder is the development of monoclonal antibody biosimilars directed against the fibroblast growth factor-23 protein to treat X-linked hypophosphatemic rickets. This chapter is an introduction to the basic principles of molecular biology, common laboratory techniques, and some examples of the recent advances made in clinical pediatric endocrinologic disorders with an emphasis on endocrine genetics. Most new diagnostic testing, pharmacogenetics, and molecular therapies are discussed in the disease-specific chapters of this book, and only examples that highlight the principle/strategy under discussion are discussed in this chapter.

BASIC MOLECULAR TOOLS

Isolation and Digestion of DNA and Southern Blotting

The human chromosome comprises a long double-stranded helical molecule of deoxyribonucleic acid (DNA) associated with different nuclear proteins.^{3,4} As DNA forms the starting point of the synthesis of all the protein molecules in the body, molecular techniques using DNA have proven to be crucial in the development of diagnostic tools to analyze endocrine diseases. DNA can be isolated from any human tissue, including circulating white blood cells. About 200 µg of DNA can be obtained from 10 to 20 mL of whole blood, with the efficiency of DNA extraction being dependent on the technique used and the method of anticoagulation used. The extracted DNA can be stored almost indefinitely at an appropriate temperature. Furthermore, lymphocytes can be transformed with the Epstein-Barr virus (or other means) to propagate indefinitely in cell culture as “immortal” cell lines, thus providing a renewable source of DNA. For performing molecular genetic studies, transformed lymphoid lines are routinely the tissue of choice, because a renewable source of DNA obviates the need to obtain further blood from the family. Fibroblast-derived cultures can also serve as a permanent source of DNA or ribonucleic acid (RNA) (once transformed), but they have to be derived from surgical specimens or a biopsy. It should be noted that, because the expression of many genes is tissue specific, immortalized lymphoid or fibroblastoid cell lines cannot be used to analyze the abundance or composition of messenger RNA (mRNA) for a specific gene. Hence, studies involving mRNA necessitate the analysis of the tissue(s) expressing the gene, as outlined in the section on “RNA Analysis.” More recently, the problem of limited amounts of DNA obtainable from certain sources has been circumvented by the utilization of the polymerase chain reaction (PCR), a versatile way to faithfully multiply segments of the original DNA.

DNA is present in extremely large molecules; the smallest autosomal chromosome (chromosome 22) has about 50 million base pairs and the entire haploid human genome is estimated to comprise 3 to 4 billion base pairs. This extreme size precludes the analysis of DNA in its native form in routine molecular biology techniques. The techniques for identification and analysis of DNA became feasible and readily accessible with the discovery of enzymes termed *restriction endonucleases*. These enzymes, originally isolated from bacteria, cut DNA into smaller sizes on the basis of specific recognition sites that vary from two to eight base pairs in length.^{5,6} The term *restriction* refers to the function of these enzymes in bacteria. A restriction endonuclease destroys foreign DNA (such as bacteriophage DNA) by cleaving the DNA at specific sites, thereby “restricting” the entry of foreign DNA in the bacterium. Several hundred restriction enzymes with different recognition sites are now commercially available. Because the recognition site for a given enzyme is fixed, the number and sizes of fragments generated for a particular DNA molecule remain consistent with the number of recognition sites and provide predictable patterns after separation by electrophoresis.

The analysis of a few hundred base pairs of DNA in the region of interest is difficult when DNA from all human chromosomes is cut and separated on the same gel. These limitations are circumvented by the technique of Southern blotting (named after its originator, Edward Southern). Southern blotting involves digestion of DNA and separation by electrophoresis through agarose.⁷ After electrophoresis, the DNA is transferred to a solid support (such as nitrocellulose or nylon membranes), enabling the pattern of separated DNA fragments to be replicated onto the membrane (Fig. 2.1). The DNA is then

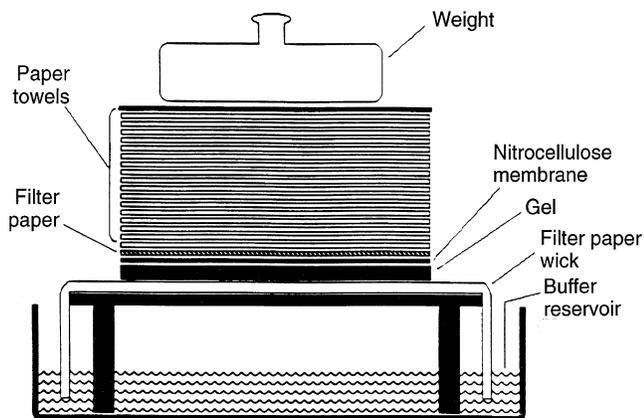


Fig. 2.1 Southern blot. Fragments of double-stranded deoxyribonucleic acid (DNA) are separated by size by agarose gel electrophoresis. To render the DNA single stranded (denatured), the agarose gel is soaked in an acidic solution. After neutralization of the acid, the gel is placed onto filter paper, the ends of which rest in a reservoir of concentrated salt buffer solution. A sheet of nitrocellulose membrane is placed on top of the gel and absorbent paper is stacked on top of the nitrocellulose membrane. The salt solution is drawn up through the gel by the capillary action of the filter paper wick and the absorbent paper towels. As the salt solution moves through the gel, it carries along with it the DNA fragments. Because nitrocellulose binds single-stranded DNA, the DNA fragments are deposited onto the nitrocellulose in the same pattern that they were placed in the agarose gel. The DNA fragments bound to the nitrocellulose are fixed to the membrane by heat or ultraviolet irradiation. The nitrocellulose membrane with the bound DNA can then be used for procedures, such as hybridization to a labeled DNA probe. Techniques to transfer DNA to other bonding matrices, such as nylon, are similar. (Modified from Turco, E., Fritsch, R., Trucco, M. (1990). Use of immunologic techniques in gene analysis. In: Herberman, R.B., Mercer, D.W. (eds.). Immunodiagnosis of cancer. New York: Marcel Dekker, p. 205.)

denatured (i.e., the two strands are physically separated), fixed to the membrane, and the dried membrane is mixed with a solution containing the DNA probe. A DNA probe is a fragment of DNA that contains a nucleotide sequence specific for the gene or chromosomal region of interest. For purposes of detection, the DNA probe is labeled with an identifiable tag, such as radioactive phosphorus (e.g., ³²P) or a chemiluminescent moiety; the latter has now almost exclusively replaced radioactivity. The process of mixing the DNA probe with the denatured DNA fixed to the membrane is called *hybridization*, the principle being that there are only four nucleic acid bases in DNA—adenine (A), thymidine (T), guanine (G), and cytosine (C)—that always remain complementary on the two strands of DNA, A pairing with T, and G pairing with C. Following hybridization, the membrane is washed to remove the unbound probe and exposed to an x-ray film either in a process called *radioautography* (also referred to as *autoradiography*) to detect radioactive phosphorus or in a process used to detect the chemiluminescent tag. Only those fragments that are complementary and have bound to the probe containing the DNA of interest will be evident on the x-ray film, enabling the analysis of the size and pattern of these fragments. As routinely performed, the technique of Southern analysis can detect a single copy gene in as little as 5 μg of DNA, the DNA content of about 10⁶ cells.

Restriction Fragment Length Polymorphism

Restriction fragment length polymorphism (RFLP) is a technique that is currently rarely used but is widely present in endocrine genetic literature, as a number of endocrine genetic discoveries over the last 2 to 3 decades were based on this technique. The number and size of DNA fragments resulting from the digestion of any particular region of DNA form a recognizable pattern. Small variations in a sequence among unrelated individuals may cause a restriction enzyme recognition site to be present or absent; this results in a variation in the number and size pattern of the DNA fragments produced by digestion with that particular enzyme. Thus this region is said to be polymorphic for the particular enzyme tested—that is, an RFLP (Fig. 2.2). The value of RFLP is that it can be used as a molecular tag for tracing the inheritance of the maternal and paternal alleles. Furthermore, the polymorphic region analyzed does not need to encode the genetic variation that is the cause of the disease being studied, but only to be located near the gene of interest. When a particular RFLP pattern can be shown to be associated with a disease, comparing the offspring’s RFLP pattern with the RFLP pattern of the affected or carrier parents can determine the likelihood of an offspring inheriting the disease. The major limitation of the RFLP technique is that its applicability for the analysis of any particular gene is dependent on the prior knowledge of the presence of convenient (“informative”) polymorphic restriction sites that flank the gene of interest by at most a few kilobases. Because these criteria may not be fulfilled in any given case, the applicability of RFLP cannot be guaranteed for the analysis of a given gene.

Polymerase Chain Reaction

PCR is a technique that was developed in the late 1980s and revolutionized molecular biology (Fig. 2.3). PCR allows the selective logarithmic amplification of a desired fragment of DNA from a complex mixture of DNA that theoretically contains at least a single copy of the target fragment. In the typical application of this technique, some knowledge of the DNA sequences in the region to be amplified is necessary, so that a pair of short (approximately 18–25 bases in length) specific oligonucleotides (“primers”) can be synthesized. The primers are synthesized in such a manner that they define the limits of the

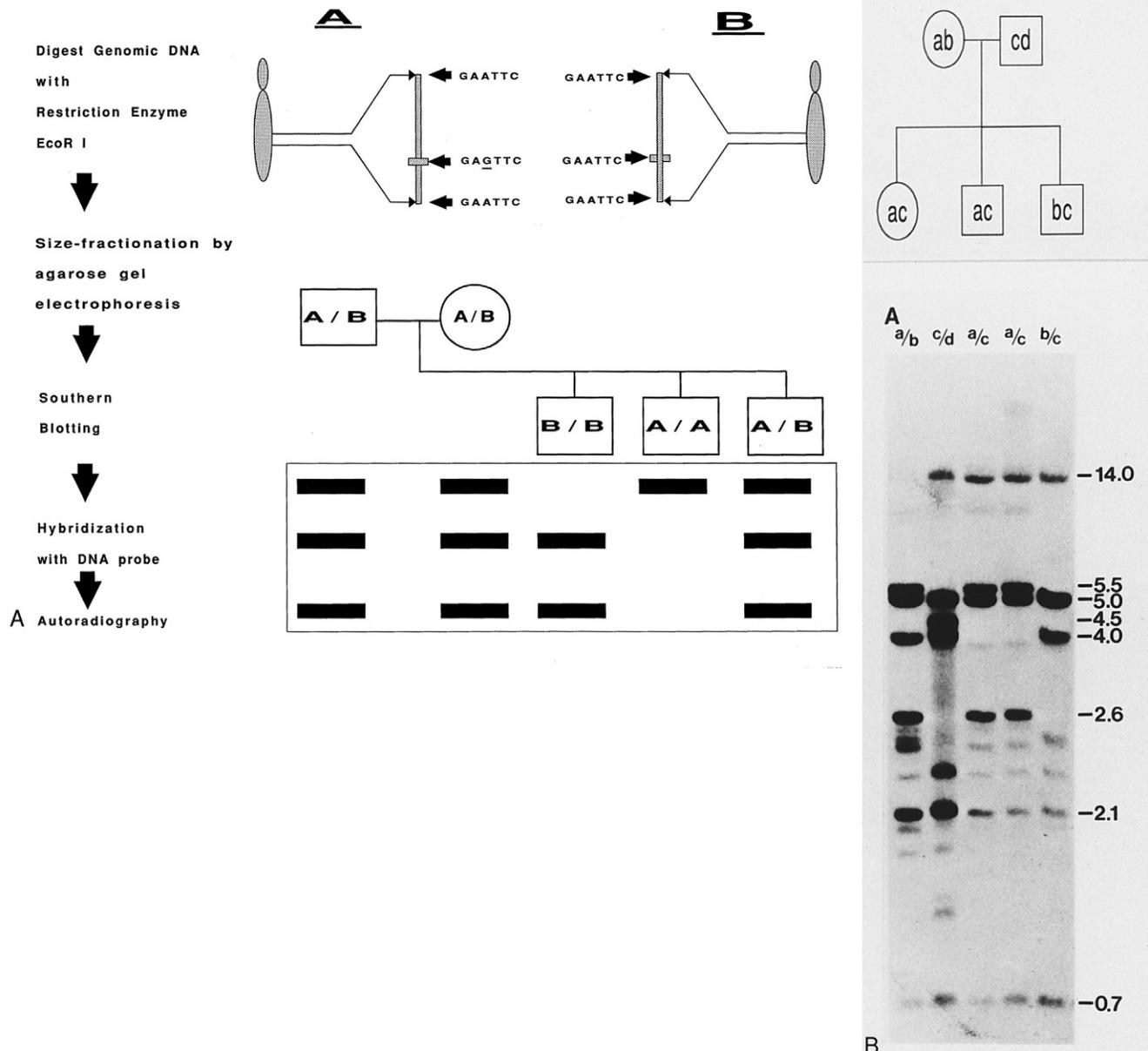


Fig. 2.2 Restriction fragment length polymorphism (RFLP). A, Schematic illustration. A and B represent two alleles that display a polymorphic site for the restriction enzyme EcoR I. EcoR I will cut deoxyribonucleic acid (DNA) with the sequence “GAATTC”; hence, allele B will be cut by EcoR I at three sites to generate two fragments of DNA, whereas allele A will be cut by EcoR I only twice and not at the site (indicated by horizontal bar) where nucleotide G (underlined) replaces the nucleotide A present in allele B. Following digestion, the DNA is size-fractionated by agarose gel electrophoresis and transferred to a membrane by Southern blot technique (see Fig. 2.1 for details). The membrane is then hybridized with a labeled DNA probe, which contains the entire sequence spanned by the three EcoR I sites. Radioautography DNA probe membrane will detect the size of the DNA fragments generated by the restriction enzyme digestion. In this particular illustration, both parents are heterozygous and possess both A and B alleles. Matching the pattern of the DNA bands of the offspring with that of the parents will establish the inheritance pattern of the alleles. For example, if allele A represents the abnormal allele for an autosomal recessive disease, then examination of the Southern blot will establish that (from left to right) the first offspring (B/B) is homozygous for the normal allele, the second offspring (A/A) is homozygous for the abnormal allele, and the third offspring (A/B) is a carrier. B, RFLP analysis of the *DQ-beta* gene of the human leukocyte antigen (HLA) locus. Genomic DNA from the members of the indicated pedigree was digested with restriction enzyme Pst I, size-fractionated by agarose gel electrophoresis, and transferred to nitrocellulose membrane by Southern blot technique. The membrane was then hybridized with a complementary DNA probe specific for the *DQ-beta* gene; the excess probe was removed by washing at appropriate stringency and was analyzed by radioautography. The sizes of the DNA fragments (in kilobases, kb) are indicated on the right. The pedigree chart indicates the polymorphic alleles (a, b, c, d) and the bands on the Southern blot corresponding to these alleles (a [5.5 kb], b [5.0 kb], c [14.0 kb], d [4.5 kb]) indicate the inheritance pattern of these alleles. (Modified from Turco, E., Fritsch, R., Trucco, M. (1998). First domain encoding sequence mediates human class II beta-chain gene cross-hybridization. *Immunogenetics* 28:193.)

region to be amplified. The DNA template containing the segment that is to be amplified is heat denatured, such that the strands are separated and then cooled to allow the primers to anneal to the respective complementary regions. The enzyme

Taq polymerase, a heat stable enzyme originally isolated from the bacterium *Thermophilus aquaticus*, is then used to initiate synthesis (extension) of DNA. The DNA is repeatedly *denatured*, *annealed*, and *extended* in successive cycles in a machine called

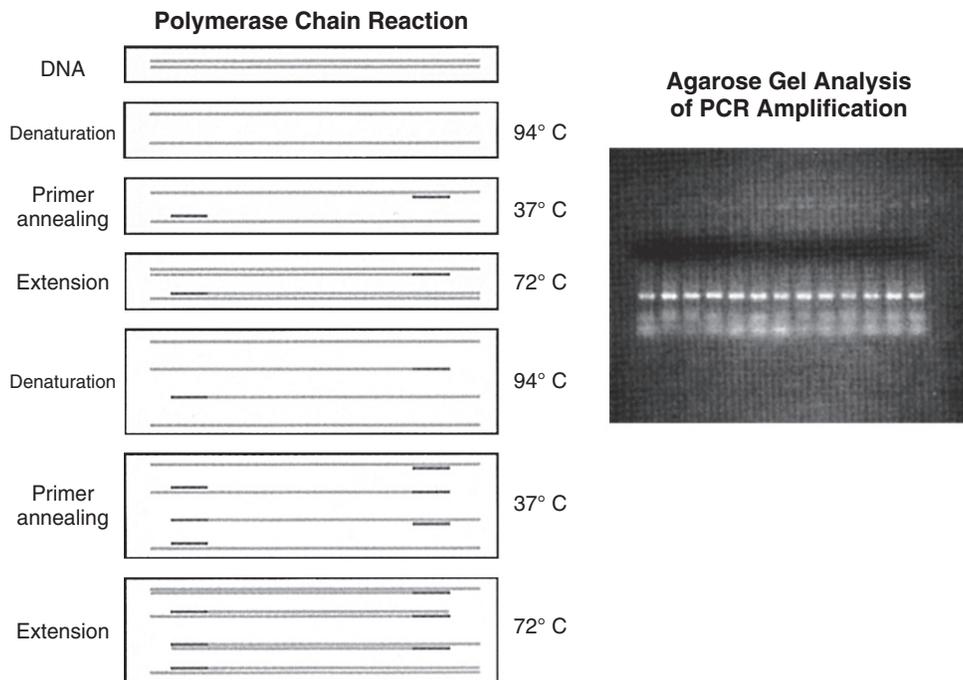


Fig. 2.3 Polymerase chain reaction (PCR). A pair of oligonucleotide primers (solid bars), complementary to sequences flanking a particular region of interest (shaded, stippled bars), are used to guide deoxyribonucleic acid (DNA) synthesis in opposite and overlapping directions. Repeated cycles of DNA denaturation, primer annealing, and DNA synthesis (primer extension) by DNA polymerase enzyme result in an exponential increase in the target DNA (i.e., the DNA sequence located between the two primers) such that this DNA segment can be amplified 1×10^6 – 7 times after 30 such cycles. The use of a thermostable DNA polymerase (i.e., Taq polymerase) allows for this procedure to be automated. Inset: The amplified DNA can be used for subsequent analysis (i.e., size-fractionation by agarose gel electrophoresis). (Modified from Trucco, M. (1992). To be or not to be ASP 57, that is the question. *Diabetes Care* 15:705.)

the *thermocycler* that permits this process to be automated. In the usual assay, these repeated cycles of denaturing, annealing, and extension result in the synthesis of approximately 1 million copies of the target region in about 2 hours. To establish the veracity of the amplification process, the identity of the amplified DNA can be analyzed by electrophoresis, hybridization to RNA or DNA probes, digestion with informative restriction enzyme(s), or subjected to direct DNA sequencing. The relative simplicity combined with the power of this technique has resulted in widespread use of this procedure and has spawned a wide variety of variations and modifications that have been developed for specific applications.^{8,9} From a practical point of view, the major drawback of PCR is the propensity to get cross-contamination of the target DNA. This drawback is the direct result of the extreme sensitivity of the method that permits amplification from one molecule of the starting DNA template. Thus unintended transfer of amplified sequences to items used in the procedure will amplify DNA in samples that do not contain the target DNA sequence (i.e., a false positive result). Cross-contamination should be suspected when amplification occurs in negative controls that did not contain the target template. One of the most common modes of cross-contamination is via aerosolization of the amplified DNA during routine laboratory procedures, such as vortexing, pipetting, and manipulation of microcentrifuge tubes. Meticulous care to experimental technique, proper organization of the PCR workplace, and inclusion of appropriate controls are essential for the successful prevention of cross-contamination during PCR experiments.

In general, PCR applications are either directed toward the identification of a specific DNA sequence in a tissue or body fluid sample or used for the production of relatively large amounts of DNA of a specific sequence, which then are used in further studies. Examples of the first type of application

are common in many fields of medicine, such as in microbiology, wherein the PCR technique is used to detect the presence of DNA sequences specific for viruses or bacteria in a biological sample. Examples of such an application in pediatric endocrinology include the use of PCR of the *SRY* gene for detecting Y chromosome material in patients with karyotypically defined Turner syndrome and the rapid identification of chromosomal gender in cases of fetal or neonatal sexual ambiguity¹⁰ (Fig. 2.4).

Most PCR applications, both as research tools and for clinical use, are directed toward the production of a target DNA or the complementary DNA of a target RNA sequence. The DNA that is made ("amplified") is then analyzed by other techniques, such as DNA sequencing.

RNA Analysis

The majority (>95%) of the chromosomal DNA represents noncoding sequences. These sequences harbor regulatory elements, serve as sites for alternate splicing, and are subject to methylation and other epigenetic changes that affect gene function. However, at present most disease-associated mutations in the human gene have been identified in coding sequences. An alternate strategy to analyze mutations in a given gene is to study its mRNA, which is the product (via transcription) of the remaining 5% of chromosomal DNA that encodes for proteins. In addition, because the mRNA repertoire is cell and tissue specific, the analyses of the mRNA sequences provide unique information about tissue-specific proteins produced in a particular organ/tissue.

There are many techniques for analyzing mRNA. The oldest and most widely used in the past, although now rarely used, is Northern blotting (so named because it is based on the same principle as the Southern blot), which is one of the original

to all the PCR reaction tubes serves as an internal control for the efficiency of the PCR process, and the yield of the mutant DNA in the various tubes can be used for the equalization of the yield of the DNA by PCR. It is important to ensure for accurate quantitation of the DNA of interest that the concentrations of the mutant and target template should be nearly equivalent. Because the use of mutated DNA for normalization does not account for the variability in the efficiency of the RT enzyme, a variation of the original method has been developed. In this modification, competitive mutated RNA transcribed from a suitably engineered RNA expression vector is substituted for the mutant DNA in the reaction before initiating the synthesis of the cDNA. Competitive RT-PCR can be used to detect changes of the order of two- to threefold of even very rare mRNA species. The major drawback of this method is the propensity to get inaccurate results because of the contamination of samples with the mRNA of interest. In theory, as the technique is based on PCR, contamination by even one molecule of mRNA of interest can invalidate the results. Hence, scrupulous attention to laboratory technique and set up is essential for the successful application of this technique.

In general, two types of methods are used for the detection and quantitation of PCR products: the “end-point”

measurements of products and the newer “real-time” techniques. End-point determinations (e.g., the competitive RT-PCR technique described earlier) analyze the reaction after it is completed, whereas real-time determinations are made during the progression of the amplification process. In general, the real-time approach is more accurate and is currently the preferred method. Advances in fluorescence detection technologies have made the use of real-time measurement possible for routine use in the laboratory. One of the popular techniques that takes advantage of real-time measurements is the TaqMan (fluorescent 5' nuclease) assay (Fig. 2.5).^{16,17} The unique design of TaqMan probes, combined with the 5' nuclease activity of the PCR enzyme (Taq polymerase), allows direct detection of PCR product by the release of a fluorescent reporter during the PCR amplification by using specially designed machines (ABI Prism 5700/7700). The TaqMan probe consists of an oligonucleotide synthesized with a 5'-reporter dye (e.g., FAM; 6-carboxy-fluorescein) and a downstream, 3'-quencher dye (e.g., TAMRA; 6-carboxy-tetramethyl-rhodamine). When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Forster-type energy transfer. During PCR, forward and reverse primers hybridize to a specific sequence

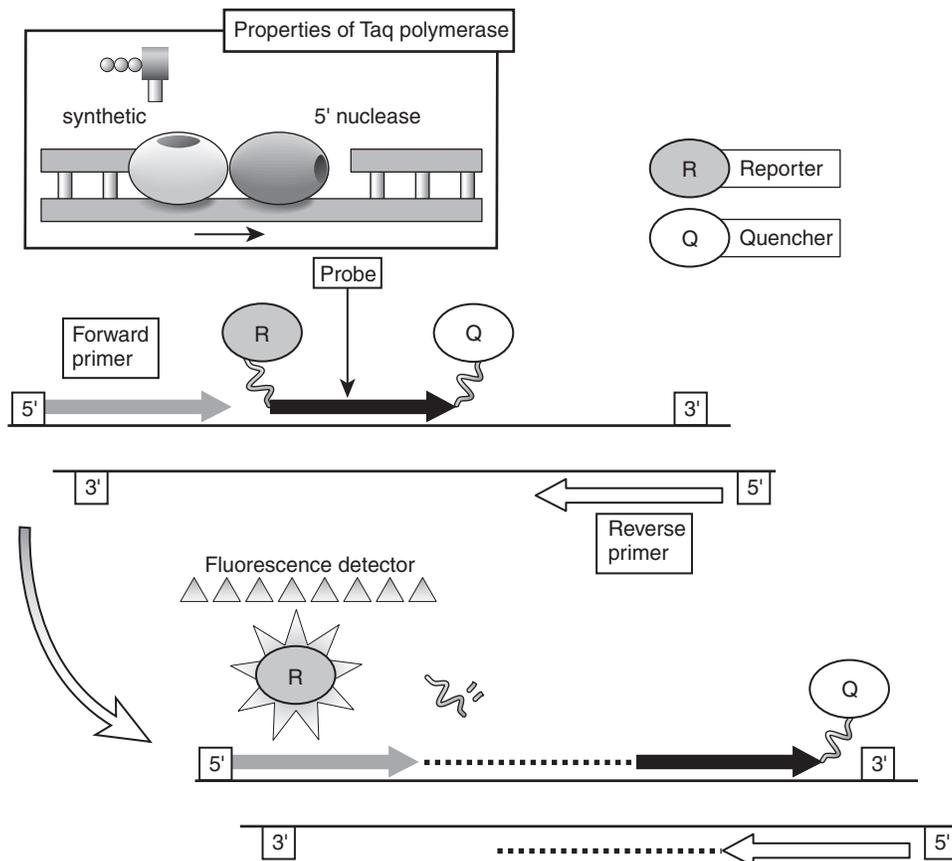


Fig. 2.5 Fluorescent 5' nuclease (TaqMan) assay. Three synthetic oligonucleotides are used in a fluorescent 5' nuclease assay. Two oligonucleotides function as “forward” and “reverse” primers in a conventional polymerase chain reaction (PCR) amplification protocol. The third oligonucleotide, termed the TaqMan probe, consists of an oligonucleotide synthesized with a 5'-reporter dye (e.g., FAM; 6-carboxy-fluorescein) and a downstream, 3'-quencher dye (e.g., TAMRA; 6-carboxy-tetramethyl-rhodamine). When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Forster-type energy transfer. During PCR, forward and reverse primers hybridize to a specific sequence of the target deoxyribonucleic acid (DNA). The TaqMan probe hybridizes to a target sequence within the PCR product. The Taq polymerase enzyme, because of its 5'-3' exonuclease activity, subsequently cleaves the TaqMan probe. The reporter dye and the quencher dye are separated by cleavage, resulting in increased fluorescence of the reporter dye as a direct consequence of target amplification during PCR. Both primers and probe must hybridize to the target for amplification and cleavage to occur. Hence the fluorescence signal is generated only if the target sequence for the probe is amplified during PCR. Fluorescent detection takes place through fiberoptic lines positioned above the caps of the reaction wells. Inset: The two distinct functions of the enzyme Taq polymerase: the 5'-3' synthetic polymerase activity and the 5'-3' polymerase-dependent exonuclease activity.

of the target DNA. The TaqMan probe hybridizes to a target sequence within the PCR product. The Taq polymerase enzyme, because of its 5'-3' nuclease activity, subsequently cleaves the TaqMan probe. The reporter dye and the quencher dye are separated by cleavage, resulting in increased laser-stimulated fluorescence of the reporter dye as a direct consequence of target amplification during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. Both primer and probe must hybridize to the target for amplification and cleavage to occur. The fluorescence signal is generated only if the target sequence for the probe is amplified during PCR. Because of these stringent requirements, non-specific amplification is not detected. Fluorescent detection takes place through fiber optic lines positioned above optically nondistorting tube caps. Quantitative data are derived from a determination of the cycle at which the amplification product signal crosses a preset detection threshold. This cycle number is proportional to the amount of starting material, thus allowing for a measurement of the level of specific mRNA in the sample. An alternate machine (Light Cycler) also uses fluorogenic hydrolysis or fluorogenic hybridization probes for quantification in a manner similar to the ABI system.

MicroRNA

One of significant advances in the early 2000s in the field of RNA biology is the discovery of small (20–30 nucleotide) noncoding RNAs.¹⁸ In general, there are two categories of small noncoding RNAs: microRNA (miRNA) and small interfering RNA (siRNA). miRNAs are expressed products of an organism's own genome, whereas siRNAs are synthesized in the cells from foreign double-stranded RNA (e.g., from viruses or transposons or from synthetic DNA introduced into the cell to study the function of a particular gene/process). In addition, there are differences in the biogenesis of these two classes of small nucleotide RNAs. These differences notwithstanding, the overall biological effect of these small nucleotide RNAs is translational repression or target degradation and gene silencing by binding to complementary sequences on the 3' untranslated region of target mRNA; positive regulation of gene expression via such a mechanism is distinctly uncommon. The complexity of the phenomenon is increased by the fact that in a cell- or tissue-specific context, a single miRNA can target multiple RNAs and more than one miRNA can recognize the same mRNA target to amplify and strengthen the translational repression of the target gene. It is estimated that this phenomenon is present in several cell types and the human genome codes for more than 1000 miRNAs that could target 60% to 70% of mammalian genes. miRNA-mediated events have been implicated in regulation of cell growth and differentiation, cell growth, apoptosis, and other cellular processes. To date, the major impact of the discovery of miRNA has been in the fields of developmental biology, organogenesis, and cancer.¹⁹ miRNA and miRNA-related events (e.g., proteins involved in miRNA processing) have been directly implicated in only a small number of non-neoplastic endocrine disorders (e.g., diGeorge syndrome and X-linked mental retardation). It is predicted that as we learn more about the basic biology of this process, small nucleotide noncoding RNAs will be implicated in the pathogenesis of a wider spectrum of endocrine diseases.

DETECTION OF MUTATIONS IN GENES

Changes in the structural organization of a gene that impact its function involve deletions, insertions, or transpositions of relatively large stretches of DNA, or more frequently single-base substitutions in functionally critical regions. High throughput or next-generation sequencing (NGS) has revolutionized the identification of mutations in genes.²⁰

Direct Methods

DNA sequencing is the current gold standard for obtaining unequivocal proof of a point mutation. However, DNA sequencing has its limitations and drawbacks. A clinically relevant problem is that current DNA sequencing methods do not reliably and consistently detect all mutations. For example, in many cases where the mutation affects only one allele (heterozygous), the heights of the peaks of the bases on the fluorescent readout corresponding to the wild-type and mutant allele are not always present in the predicted (1:1) ratio. This limits the discerning power of "base calling" computer protocols and results in inconsistent or erroneous assignment of DNA sequence to individual alleles. Because of this limitation, clinical laboratories routinely determine the DNA sequence of both the alleles to provide independent confirmation of the absence/presence of a putative mutation. DNA sequencing can be labor intensive and expensive, although advances in pyrosequencing (discussed later), for example, have made it technically easier and cheaper.

Although the first DNA sequences were determined with a method that chemically cleaved the DNA at each of the four nucleotides,²¹ the enzymatic or dideoxy method developed by Sanger and colleagues in 1977 became the most commonly used for routine purposes (Fig. 2.6).²² This method uses the enzyme DNA polymerase to synthesize a complementary copy of the single-stranded DNA ("template") whose sequence is being determined. Single-stranded DNA can be obtained directly from viral or plasmid vectors that support the generation of single-stranded DNA or by partial denaturing of double-stranded DNA by treatment with alkali or heat. The enzyme DNA polymerase cannot initiate synthesis of a DNA chain de novo but can only extend a fragment of DNA. Hence the second requirement for the dideoxy method of sequencing is the presence of a "primer." A primer is a synthetic oligonucleotide, 15 to 30 bases long, whose sequence is complementary to the sequence of the short corresponding segment of the single-stranded DNA template. The dideoxy method exploits the observation that DNA polymerase can use both dNTP and 2',3'-dideoxynucleoside triphosphates (ddNTPs) as substrates during elongation of the primer. Whereas DNA polymerase can use dNTP for continued synthesis of the complementary strand of DNA, the chain cannot elongate any further after addition of the first ddNTP, because ddNTPs lack the crucial 3'-hydroxyl group. To identify the nucleotide at the end of the chain, four reactions are carried out for each sequence analysis, with only one of the four possible ddNTPs included in any one reaction. The ratio of the ddNTP and dNTP in each reaction is adjusted so that these chain terminations occur at each of the positions in the template where the nucleotide occurs. To enable detection by radioautography, the newly synthesized DNA is labeled, usually by including in the reaction mixture radioactively labeled dATP (for the older manual methods) or, most commonly, currently fluorescent dye terminators in the reaction mixture (now in use in automated techniques). The separation of the newly synthesized DNA strands manually is done via high-resolution denaturing polyacrylamide electrophoresis or with capillary electrophoresis in automatic sequencers. Fluorescent detection methods have enabled automation and enhanced throughput. In capillary electrophoresis, DNA molecules are driven to migrate through a viscous polymer by a high electric field to be separated on the basis of charge and size. Although this technique is based on the same principle as that used in slab gel electrophoresis, the separation is done in individual glass capillaries rather than gel slabs, facilitating loading of samples and other aspects of automation. Whereas manual methods allow the detection of about 300 nucleotides of sequence information with one set of sequencing reactions, automated methods using

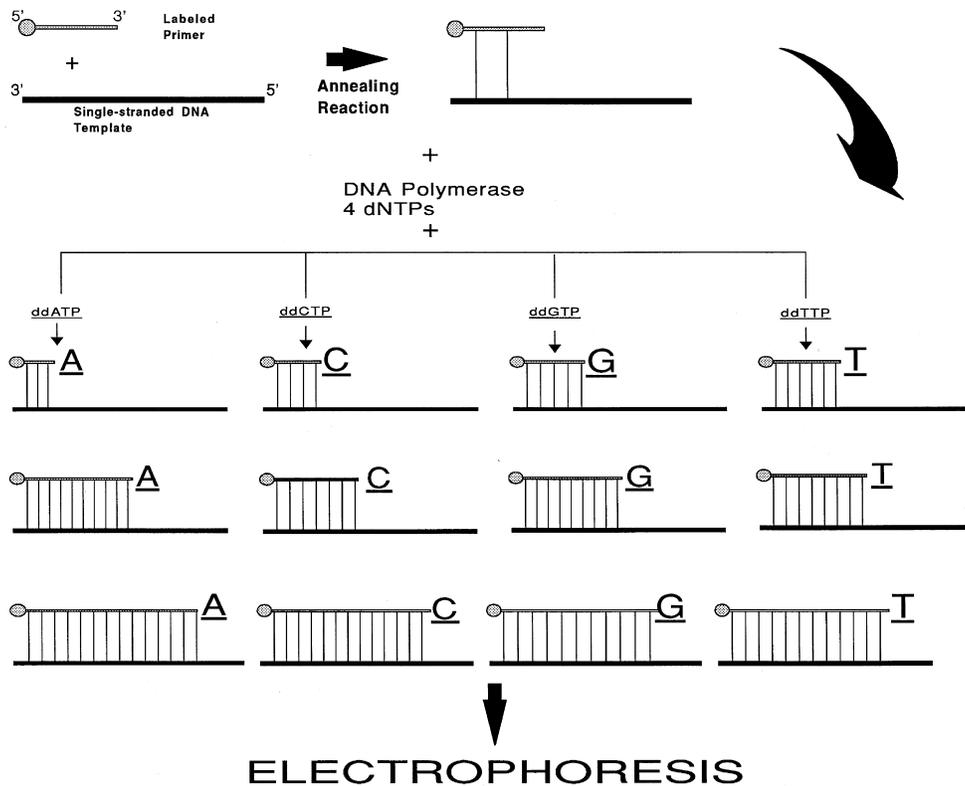


Fig. 2.6 Deoxyribonucleic acid (DNA) sequencing by the dideoxy (Sanger) method. A 5'-end-labeled oligonucleotide primer with sequence complementarity to the DNA that is to be sequenced (DNA template) is annealed to a single-strand of the template DNA. This primer is elongated by DNA synthesis initiated by the addition of the enzyme DNA polymerase in the presence of the four 2'-deoxynucleoside triphosphates (dNTPs) and one of the 2',3'-dideoxynucleoside triphosphates (ddNTPs); four such reaction tubes are assembled to use all the four ddNTPs. The DNA polymerase enzyme will elongate the primer using the dNTPs and the individual ddNTP present in that particular tube. Because ddNTPs are devoid of the 3' hydroxyl group, no elongation of the chain is possible when such a residue is added to the chain. Thus each reaction tube will contain prematurely terminated chains ending at the occurrence of the particular ddNTP present in the reaction tube. The concentrations of the dNTPs and the individual ddNTP present in the reaction tubes are adjusted so that the chain termination takes place at every occurrence of the ddNTP. Following the chain elongation-termination reaction, the DNA strands synthesized are size-separated by acrylamide gel electrophoresis and the bands visualized by radioautography.

fluorescent dyes and laser technology can analyze 7500 or more bases per reaction. To sequence larger stretches of DNA, it is necessary to divide the large piece of DNA into smaller fragments that can be individually sequenced. Alternatively, additional sequencing primers can be chosen near the end of the previous sequencing results, allowing the initiation point of new sequence data to be moved progressively along the larger DNA fragment.

One of the seminal technological advances has been the introduction of microarray-based methods for detection, and analysis of nucleic acids.²³ Microarrays contain thousands of oligonucleotides deposited or synthesized in situ on a solid support, typically a coated glass slide or a membrane. In this technique, a robotic device is used to print DNA sequences onto the solid support. The DNA probes immobilized on the microarray slide as spots can either be cloned cDNA or gene fragments (expressed sequence-tags [ESTs]), or oligonucleotides corresponding to known genes or putative open reading frames. The arrays are hybridized with fluorescent targets prepared from RNA extracted from tissue/cells of interest; the RNA is labeled with fluorescent tags, such as Cy3 and Cy5. The prototypic microarray experimental paradigm consists of comparing mRNA abundance in two different samples. One fluorescent target is prepared from control mRNA and the second target with a different fluorescent label is prepared from mRNA isolated from the treated cells or tissue under investigation. Both targets are mixed and hybridized to the microarray

slide, resulting in target gene sequences hybridizing to their complementary sequences on the microarray slide. The microarray is then excited by laser, and the fluorescent intensity of each spot is determined with the relative intensities of the two colored signals on individual spots being proportional to the amounts of specific mRNA transcripts in each sample (Fig. 2.7). Analysis of the fluorescent intensity data yields an estimation of the relative expression levels of the genes in the sample and control sample. Microarrays enable individual investigators to perform large-scale analyses of model organisms and to customize arrays for special genome applications.

The method of choice for global expression profiling depends on several factors, including technical aspects, labor, price, time, and effort involved, and, most important, the type of information that is sought. Technical advances in the development of expression arrays, their abundance and commercial availability, and the relative speed with which analysis can be done are all factors that make arrays more useful in routine applications. In addition, array content can now be readily customized to cover from gene clusters and pathways of interest to the entire genome: some studies examine series of tissue-specific transcripts or genes known to be involved in particular pathology; others directly use arrays covering the whole genome. Another factor that needs to be considered before embarking on any high-throughput approach is whether individual or pooled samples will be investigated. Series of pooled samples reduce the price, the time spent, and the number of the

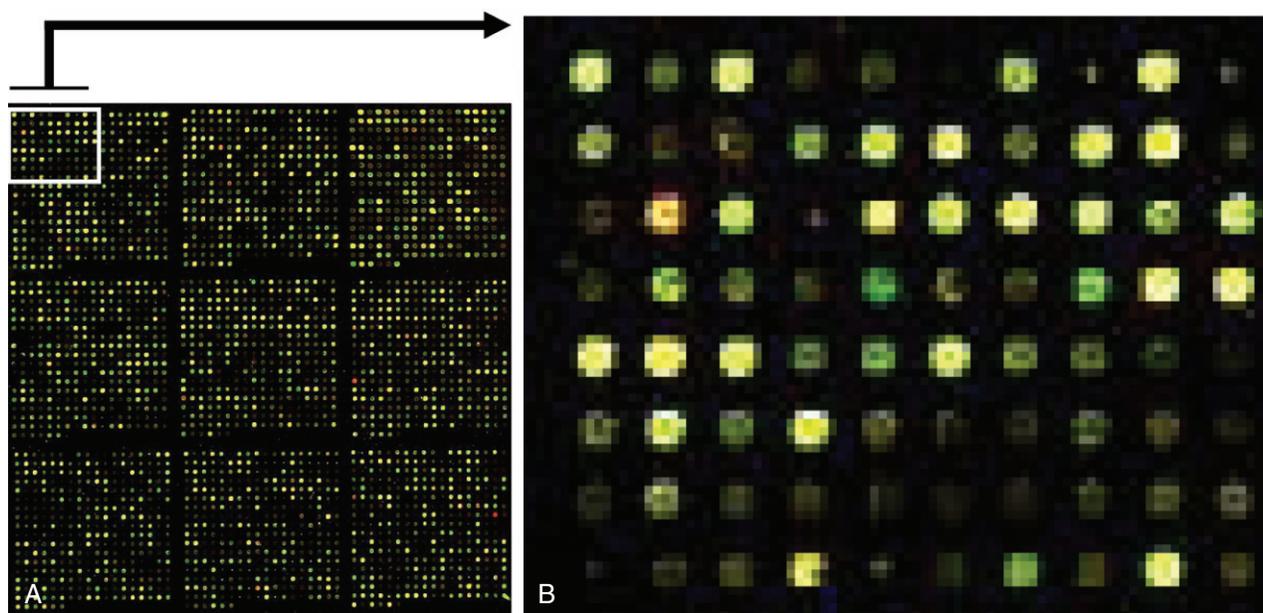


Fig. 2.7 A, Complementary deoxyribonucleic acid (cDNA) microarray, fluorescent labeled cDNA targets, adrenocorticotropic hormone (ACTH)-independent bilateral macronodular adrenal hyperplasia (Cy3), and ACTH-dependent hyperplasia (Cy5) were hybridized to glass slides containing genes involved in oncogenesis. Following laser activation of the fluorescent tags, fluorescent signals from each of the DNA “spots” are captured and subjected to analysis. B, Magnified view of the microarray platform displaying the fluorescent signals; green (Cy3) and red (Cy5) with yellow represent overlap of these two colors.

experiments down to the most affordable. Investigating individual samples, however, is important for identifying unique expression ratios in a given type of tissue or cell. There are limitations of microarray-based techniques; for example, similar to direct DNA sequencing methods, microarray-based methods also suffer from the disadvantage of not being able to reliably and consistently detect heterozygous mutations. Furthermore, microarrays cannot be used to detect insertions of multiple nucleotides without exponentially increasing the number of oligonucleotides that must be immobilized on the glass slides.

A more reliable technique in mutation identification is pyrosequencing, which is based on an enzymatic real-time monitoring of DNA synthesis by bioluminescence²⁴ (Fig. 2.8). Pyrosequencing is performed by the addition of dNTPs individually, in a predefined dispensation order, so that the nascent nucleotide chain is extended one nucleotide residue per dispensation event. Detection of nucleotide sequence is performed by way of a chain of enzymatic reactions involving the activities of DNA polymerase, apyrase, ATP sulfurylase, and luciferase, respectively, allowing for the incorporation of complementary nucleotide, degradation of unused dNTP, generation of luciferase-substrate from pyrophosphate and adenosine 5'-phosphosulfate, and emission of light from the ATP-driven conversion of luciferin to oxyluciferin. Incorporation of a particular nucleotide is displayed graphically in the form of a chart recording of nucleotide dispensation event versus the intensity of emitted light. This cascade of enzyme reactions is quantitative, in that increased light intensity is produced upon incorporation of multiple nucleotides.

Pyrosequencing, introduced in the early 2000s, provided the background for the explosion of new techniques collectively known as *high-throughput NGS* or *massively parallel sequencing*. NGS provides longer read length and cheaper price per base of sequencing compared with Sanger sequencing. NGS is based on the uncoupling of the traditional nucleotide-identifying enzymatic reaction and the image capture and doing so in an ever-speedier way allows for essentially unlimited capacity.

The first discoveries of gene mutations for endocrine diseases exploiting NGS were published in 2011.²⁵ Currently, many similar systems are being used for NGS. Illumina[®] workflows, for example, include four basic steps: the first consists in the random fragmentation of the DNA (or cDNA) sample, followed by 5' and 3' adapter ligation. These adapter short sequences encompass binding sites, indices (necessary for performing multiplex reactions), and segments complementary to the oligos fixed on a flow cell. Adapter-ligated fragments are then PCR amplified and gel purified to generate a “library.” Then the generated library is loaded into the flow cell, where its fragments are captured on oligos complementary to the library adapters, immobilized onto a solid support at a dilute concentration. Each fragment is then further amplified into distinct, clonal clusters through bridge amplification. “Bridges” are formed by the annealing of the terminal, still free adapter sequence present at the other, not yet captured extremity of each single fragment, bending to the adjacent complementary immobilized oligo. A polymerase then generates a complementary strand. After denaturation, both strands bind again, pairing with other complementary primers anchored to the floor of the plate. This step is repeated a number of times, generating millions of each fragment’s copies. When this “cluster generation” is complete, the templates are ready for “sequencing.” Here also, as per conventional pyrosequencing, the reversible terminator-based method detects single bases as they are incorporated into DNA template strands, generating light emissions under excitation with a laser. Each colony of distinct fragments is sequenced through the capture of high-resolution images that reflect the base-by-base addition of nucleotides. In contrast to conventional pyrosequencing, all four reversible terminator-bound dNTPs are present during each sequencing cycle, each labeled with a different fluorescent dye. Natural competition minimizes incorporation bias, which reduces raw error rates. Finally, a sophisticated computer program aligns the newly identified sequence reads to a reference genome sequence, grouped, in the case of multiplex reactions, by the index

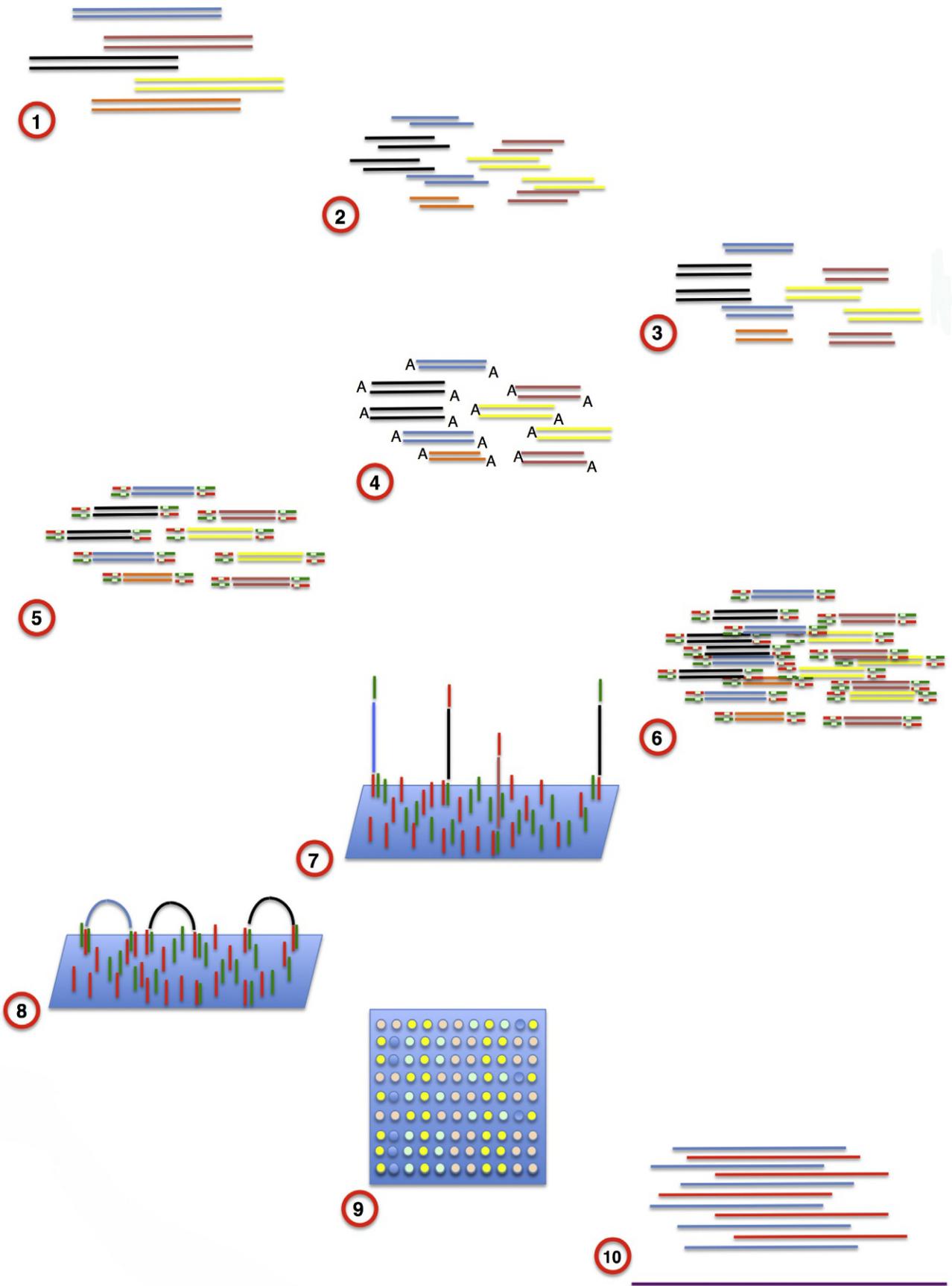


Fig. 2.8 Steps in pyrosequencing. (1) Deoxyribonucleic acid (DNA) extraction. (2) Genomic DNA shearing. (3) Fragment end repair. (4) Adenine ligation. (5) Adaptor ligation. (6) PCR amplification. (7) Fragment binding, via the adaptors, to complementary DNA segments anchored to a solid surface. (8) Clonal cluster formation through bridge amplification. (9) High-resolution images capture. (10) Alignment of multiple reads to a reference genome.

segments to each different DNA source. The extremely high number of equal sequences guarantees the correctness of the obtained results and the exclusion of the ones only sporadically represented. This dramatic rise of data output, together with the progressive reduction of sequencing cost, paved the way for “personalized medicine” in which the genome of each individual can be easily obtained and compared with normal genome sequences to detect possibly present, known, or new, disease-linked mutations.

Semiconductor and nanotechnology-based systems are currently under use in massive sequencing efforts and promise an even cheaper and faster way of determining mutations and other abnormalities of the human genome.

A requirement of all high-throughput screening approaches is confirmation of findings (expression level of a given gene/sequence) by other independent methods. A select group of genes are tested usually; these genes are picked from the series of sequences that were analyzed either because they were found to have significant changes or because of their particular interest with regard to their expression in the studied tissue or their previously identified relationship to pathology or developmental stage. The confirmation process attempts to support the findings on three different levels: (1) reliability of the high-throughput experiment (for this purpose the same samples examined by the microarrays are used); (2) trustfulness of the observations in general (to achieve that, larger number of samples are examined, assessment of which by high-throughput approaches is often unaffordable price- or labor-wise); and (3) verification of the expression changes at the protein level. A commonly used confirmatory technique is qRT-PCR. For verification at the protein level, immunohistochemistry (IHC) and Western blot are the two most commonly chosen techniques. IHC is not quantitative but has the advantage of allowing for the observation of the exact localization of a signal within a cell (cytoplasmic versus nuclear) and the tissue (identifying histologically the tissue that is stained). Modern Western blot methods require a smaller amount of protein lysate than older techniques and have the advantage of offering high-resolution quantitation of expression without the use of radioactivity.

Indirect Methods

In the mid-1980s, the need for rapid, high-throughput, accurate, and economical mutation analysis systems led to the development of several technologies, as an alternative to analysis by direct sequencing, that allowed detection of single mutations in long stretches of DNA (200–600 bp). However, screening for mutations by indirect methods has fallen out of favor because NGS and Sanger sequencing have become faster and cheaper methods for identifying gene mutations. We only refer to them here briefly and for historical purposes, because some of these techniques are still in use sporadically, and understanding the literature published since the 1980s requires the knowledge of the principles underlying these techniques.

Indirect methods of mutation identification included restriction endonuclease digestion of PCR products (PCR-RFLP), denaturing-gradient gel electrophoresis, single-strand conformation polymorphism, dideoxy fingerprinting, and heteroduplex mobility assay. Most of these methods used PCR to amplify a region of the DNA, a physical or chemical treatment of amplified DNA (e.g., by denaturation or restriction enzyme digestion), separation of the amplicons by denaturing or non-denaturing gel electrophoresis, and visualization of the separated sequence strands (by radioautography or fluorescence-based detection). Modifications in some of these techniques allowed simultaneous separation and detection of DNA fragments with the use of sophisticated equipment, such as HPLC and capillary electrophoresis.

CRISPR-CAS9 SYSTEM

The CRISPR-Cas9 system is a newly discovered relatively simple, yet very efficient approach to physically correct mutations present on selected genes or to mutate at will a target gene sequence²⁶ (Fig. 2.9). It is faster, cheaper, and more accurate than previous techniques of editing DNA and has a wide range of potential applications both for basic science and clinical medicine. This is a unique technology that enables geneticists and medical researchers to edit parts of the genome by removing, adding, or altering sections of the DNA sequence. It is based on a novel restriction system present in some bacteria that evolved to respond to invading pathogens. The system consists of two key molecules: an enzyme called *Cas9* and a segment of RNA called *guide RNA* (gRNA). *Cas9* acts as a pair of “molecular scissors” that can cut the two strands of DNA at a specific location in the genome, so that bits of DNA can then be added or removed in the same DNA site. gRNA is a short RNA sequence (about 20 bases long) able to bind to DNA at a predefined sequence. The gRNA binding to a specific sequence in the DNA, “guides” *Cas9* to the selected part of the genome where it should cut the DNA. The DNA repair machinery of the cell will then complete the repair process resulting in the refashioning of the targeted DNA segment that

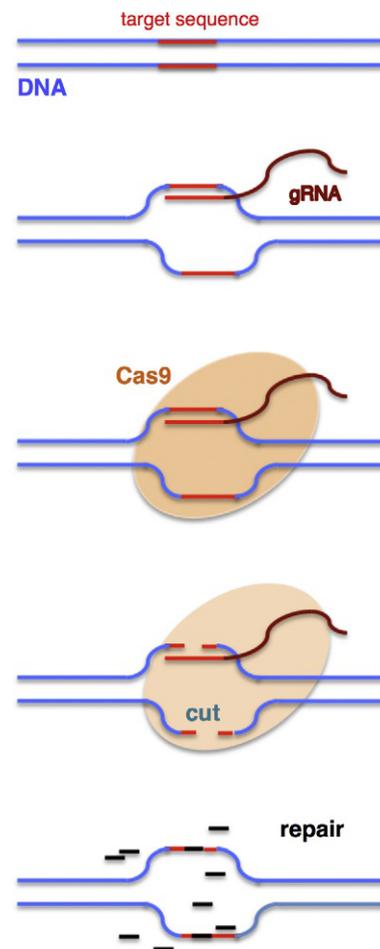


Fig. 2.9 CRISPR-Cas9 system. Cas9 enzyme acts as a pair of ‘molecular scissors’ that can cut the two strands of deoxyribonucleic acid (DNA) at a preselected location in the genome, guided in situ by a short ribonucleic acid sequence (gRNA). The machinery of the cell will then complete the DNA repair process introducing a segment of choice or disrupting the target sequence.

will not include the sequence to be eliminated or include a segment of choice.

Although the CRISPR-Cas9 system has a lot of potential as a tool for editing the genomes of somatic (nonreproductive) cells, there is considerable debate concerning its potential to edit germline (reproductive) cells. The conceptual possibility is tempered by ethical implications, as the gene changes will be transmitted to future generations of the treated individual. Furthermore, there are concerns about the specificity of the gRNA in guiding a targeted change in the DNA sequence of interest, as some data indicate that different and/or larger-than-intended DNA segments could be removed, introducing potentially unwanted DNA (off target) alterations that could be pathologic to the targeted cells(s). In several countries, for example the United Kingdom, the performance of these germline manipulations are for the time being prohibited in humans. The World Health Organization has convened a panel of experts to discuss the issue and give appropriate guidelines for possible future clinical considerations.

POSITIONAL GENETICS IN ENDOCRINOLOGY

The Principles of Positional Genetics

For the purpose of disease gene identification, the candidate gene approach relies on partial knowledge of the genetic basis of the disease under investigation. This process was successful in identifying disease genes whose function was obvious. For example, the genetic defects of most of the hereditary enzymatic disorders, including congenital adrenal hyperplasia (CAH) syndromes, became known in the late 1980s, when the introduction of PCR made the tools of molecular biology widely available to the medical and genetic research community. However, at about the same time, research on diseases without any obvious candidate genes (e.g., the multiple endocrine neoplasia [MEN] syndromes) or diseases in which the screening of obvious candidate genes failed to reveal mutations was ongoing. It was in these diseases that the application of “reverse genetics,” or more appropriately termed *positional cloning*, yielded information regarding the genetic basis of these disease states.^{27,28} Positional cloning is complemented by the Human Genome Project (HGP) and the worldwide web in

making available, in a fast and controlled manner, information that would otherwise be inaccessible.²⁹

The process of positional genetics is outlined in Fig. 2.10. The first step is the collection of clinical information from families with affected members, the determination of the mode of inheritance of the defect (autosomal dominant or recessive, X-linked, complex inheritance), and the phenotyping of subjects (or tissues), following well-established criteria for the diagnosis of the disorder. If inheritance is not known, formal segregation analysis needs to be performed to determine the autosomal, or X-linked, and the recessive or dominant nature of the inheritance.³⁰ Once this determination is made and the penetrance of the disorder is known, appropriate linkage software may be used.³¹ Linkage is examined with polymorphic markers that span the entire human genome²⁸; any marker that shows polymorphism and is known to lie close to or within a putative disease gene may be used. Genetic linkage can be defined as the tendency for alleles close together on the same chromosome to be transmitted together as an intact unit through meiosis. The strength of linkage can then be used as a unit of measurement to find out how close genetically different loci are to each other. This unit of map distance is an approximation of physical distance but is also highly dependent on other factors (e.g., the frequency of recombination is sexually dimorphic, and different among various chromosomes and along the length of a specific chromosome). The likelihood (logarithm of odds [or LOD]) score method is widely used for linkage analysis.

Once a locus on a chromosome has been identified, the region (which is usually several thousands of base pairs in length) is narrowed by analyzing informative recombinations in the cohort of patients and families available for study. The disease region may harbor already mapped genes. Online databases such as Gen Bank, ENSEMBL (www.ensembl.org), and others, and especially for clinicians, the Online Mendelian Inheritance in Man (OMIM),³² may provide all the necessary information. If a transcript is a reasonable candidate, mutation screening may identify the disease gene. If, however, these steps fail to identify the disease gene, screening new sequences from the area may be needed; today, this is done typically by NGS, followed by Sanger sequencing for confirmation. Chromosomal maps are linked by sequence-tagged sites (STSs) that are present in more than one genomic clone, thus providing

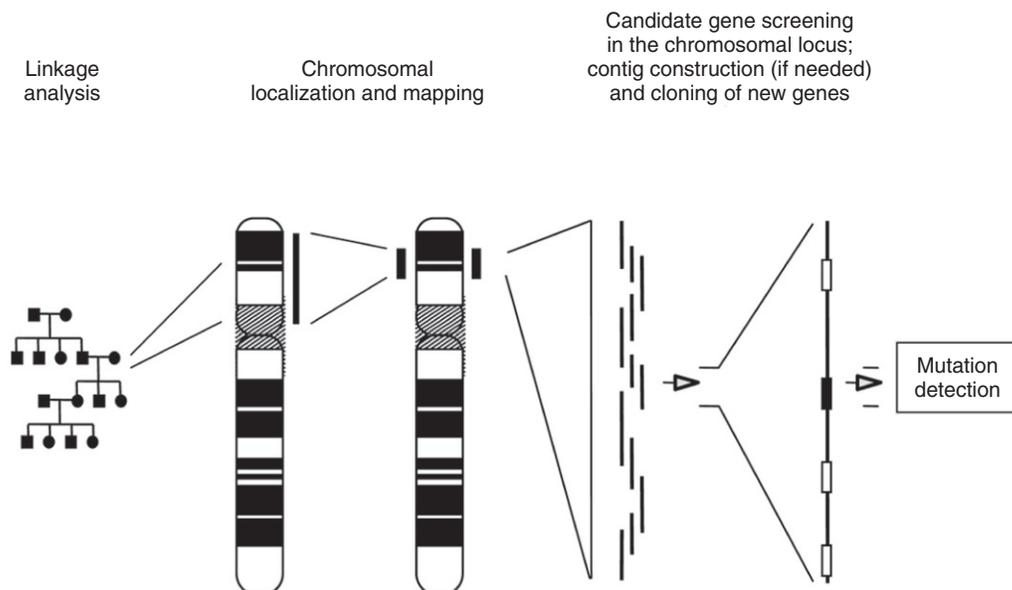


Fig. 2.10 The steps of positional cloning (see text).

critical information that allows for the proper aligning of DNA in a given locus. Polymorphic markers (including those that were used for the linkage part of the process) are the most useful STSs because they provide a direct link between the genetic and the physical mapping data. Individual clones can be sequenced; genes are identified in this process through their unique sequence features or through *in vitro* translation. In the past, ESTs provided information for what gene sequences are expressed from this area. Today, almost all the genes are fully sequenced; however, ESTs are still useful to look at, especially when one is trying to identify a new gene for a given disease-linked locus. Each one of the newly identified genes may be screened for mutations, as long as the expression profile of the identified transcript matches the spectrum of the tissues affected by the disease under investigation. Although this is helpful for most diseases, for others the expression profile may even be misleading; thus the presence of a transcript in an affected tissue is not always necessary. Complete segregation of the disease with an identified mutation, functional proof, or mutations in two or more families with the same disease are usually required as supportive evidence that the cloned sequence is the disease gene.

Genomic Identification of “Endocrine” Genes

In the 1990s positional cloning was used to identify a number of genes relevant to endocrinology; today, this is being achieved by NGS and other genome-wide methods. Endocrine tumor syndromes, despite their rarity and modest overall impact on everyday clinical endocrine practice, are seminal examples of diseases whose molecular etiology was elucidated by positional cloning. Identification of these genes was greatly assisted by the use of neoplastic tissue for studies, such as loss-of-heterozygosity (LOH), comparative genomic hybridization, and fluorescent *in situ* hybridization (FISH) applications. These techniques narrowed the genetically defined chromosomal regions and thus facilitated the identification of the responsible genes; LOH studies were critical in the identification of von Hippel-Lindau disease (*VHL-elongin*),³³ MEN1

(*menin*),³⁴ Cowden disease (*PTEN*),³⁵ Peutz-Jeghers syndrome (*STK11/LKB1*),^{36,37} and Carney complex (*PRKARIA*)³⁸ genes.

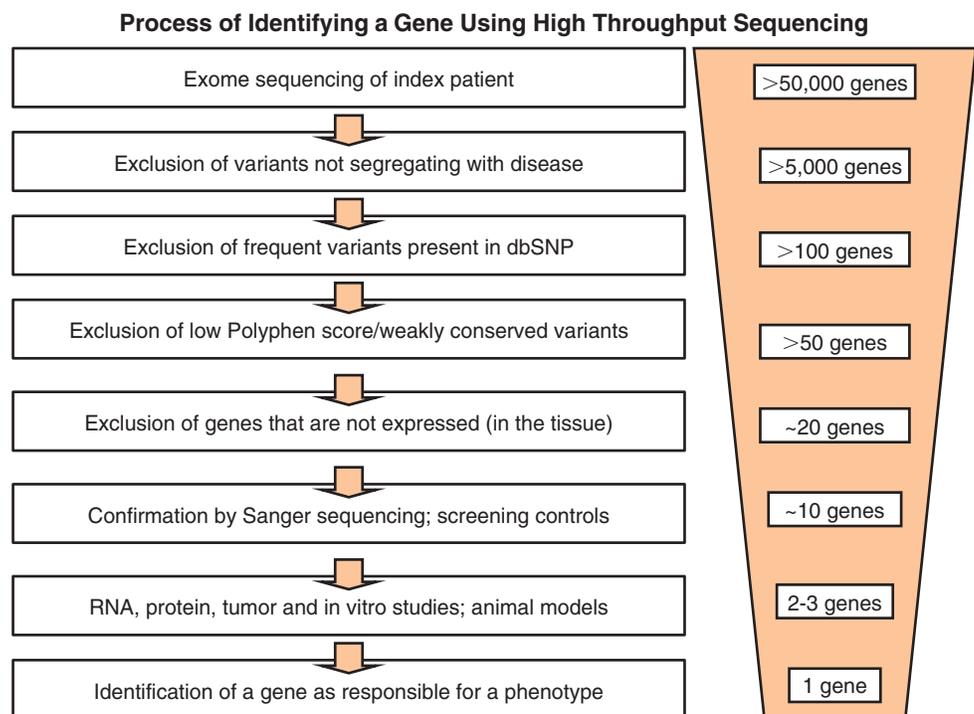
NGS is used today to sequence the entire genome (whole-genome sequencing [WGS]) or only the expressed genes (whole exome sequencing [WES]) of a proband (or his family members) with the intention to identify the mutations responsible for a disease after the methods described here have positionally identified a locus of interest. In the absence of linkage or other positioning information (such as, for example, in the absence of family history, tumor material, or additional DNA samples) an individual’s genomic DNA (or tissue derived from the individual) may be sequenced by WGS or WES. This, however, leads to the identification of many mutations and other variants that need to be excluded to identify the disease-specific gene(s). A typical flowchart for analyzing such studies is shown in Fig. 2.11.

Impact of Modern Sequencing in Clinical Practice

Genome-sequencing studies indicate that each human subject carries as many as 100 loss-of-function mutations, with more than 20 genes being completely inactivated.³⁹ Many genetic variants are found to have previously unsuspected functional redundancies; thus a patient may have many genetic variants within a singular signaling pathway leading to graded phenotypes.^{39,40}

An example of the application of the techniques of modern DNA sequencing in pediatric endocrinology that resulted in the elucidation of the disease mechanism is in the so-called *white Addison disease* (WAD) phenotype.⁴¹ It has been recognized that patients presenting with primary adrenal insufficiency (pAI) are not always pigmented; this variant of pAI was called WAD. Increased pigmentation in pAI is attributed to the binding of high levels of adrenocorticotropin (ACTH) to the melanocortin (MC) 1 receptor (MC1R). MC1R, a molecule with a high degree of sequence similarity to the MC2R, the ACTH receptor, binds to its regular ligand, α -melanocyte-stimulating hormone (α -MSH) and ACTH with an almost equal affinity.⁴² α -MSH stimulates melanogenesis in cultured human melanocytes

Fig. 2.11 Whole genome or exome sequencing is the currently preferred deoxyribonucleic acid (DNA) sequencing technology for gene identification. However, even in the genome of phenotypically normal human subjects, these sequencing techniques yield a multitude of sequencing variants, including bona fide protein-truncating mutations, hence the need to use a systematic and careful filtering process to identify the causative gene(s). The steps in the analysis of gene mutations identified by high-throughput sequencing techniques that culminate in the assignment of a gene to a specific phenotype are illustrated.



and acts specifically to increase the synthesis of eumelanin. Both α -MSH and ACTH are splice products of the same precursor pituitary protein, proopiomelanocortin (POMC), and play a role in determining pigmentation in humans, as homozygous or compound heterozygote mutations in the POMC gene are associated with hypopigmentation and red hair. In pAI that is not appropriately treated with glucocorticoids, POMC expression increases, and both α -MSH and ACTH increase. The important role of MC1R in the determination of human skin and hair pigmentation is beyond doubt: reduced function MC1R alleles lead to red hair, freckling, sun sensitivity, and an increased risk of cutaneous cancers, including melanoma.^{42,43}

Familial glucocorticoid deficiency (FGD) caused by ACTH resistance consists of at least three distinct genetic syndromes that are all inherited as autosomal recessive (AR) traits: inactivating mutations of the ACTH receptor (the *MC2R* gene) and its accessory protein (MRAP); isolated ACTH resistance (iACTHR) without *MC2R*, *MRAP*, or any other known mutation; and Allgrove syndrome.^{44,45} Turan and colleagues⁴¹ described a patient with FGD without pigmentation: she was born with red hair, which gradually darkened during early childhood; despite repeated episodes of hypoglycemia, she was not diagnosed with pAI and was thus exposed to high ACTH levels until she was 6 years old. *MC2R* sequencing showed a homozygous T152K mutation that is known to affect trafficking of the receptor, like most ACTH receptor defects causing FGD. *MC1R* sequencing showed a homozygous R160W mutation that is among the most common genetic variants of the receptor in red-haired individuals. In a fascinating confirmation of the roles of ACTH (and possibly other POMC-derived peptides) and MC1R in determining not only skin but also hair pigmentation, the patient's hair lightened and "reverted to a reddish color" following proper replacement with hydrocortisone and a decrease of serum ACTH levels.

With genetic variants responsible for even subtler effects (than, for example, red hair), DNA sequencing data challenge the practicing clinician to incorporate systems biology information in clinical practice. Clearly not all sequence defects identified by WGS or WES cause diseases; quite the opposite. The evidence is that there is redundancy and an exquisitely delicate and complex molecular balance in human biology. But clinicians have to incorporate genetics in their daily practice, and educators have to introduce molecular pathways and their genetic variability in classic physiology and pathophysiology lectures.⁴⁶

CHROMOSOME ANALYSIS AND MOLECULAR CYTOGENETICS

Chromosomes represent the most condensed state in metamorphosis of the genome during a cell cycle. Condensation of the genetic material at metaphase stage is a crucial event that provides precise and equal segregation of chromosomes between the two daughter's newborn cells during the next step, anaphase. This is followed by relaxation of the genetic content after cell division. This ability of the genome to transform from a molecular level (DNA) to a materialistic submicroscopic stage (chromosome) provides a unique opportunity to visualize the genome of an individual cell of an organism. Different chromosomal abnormalities related to particular diseases or syndromes can be detected at this stage by karyotyping chromosomes.

Chromosomes can be individually recognized and classified by size, by shape (ratio of the short/long arm), and by using differential staining techniques. In the past, identification of chromosomes was restricted to chromosome groups only. The introduction of the chromosome banding technique

revolutionized cytogenetic analysis.⁴⁷ The banding patterns are named by the following abbreviations: G for Giemsa, R for reverse, Q for quinacrine, and DAPI for 4'6'-diamino-2-phenylindole; the last two give a pattern similar to G-banding. Further development of high-resolution banding techniques enabled the study of chromosomes at earlier stages of mitosis, prophase, and prometaphase.^{48,49} Chromosomes are longer and have an enriched banding pattern at those stages, providing great details for the identification of chromosomal aberrations.

Outline of Methods

Preparation of good-quality chromosomes is an art. Many different methods for chromosome isolation have been developed in cytogenetics since the 1960s. The main principle behind all methods is to arrest cells at metaphase by disruption of the cell spindle. Metaphase spindle is a structure composed of tubular fibers formed in the cell to which the chromosomes are attached by kinetochores (centrosomes). The spindle separates the chromosomes into the two daughter cells. The agent commonly used for spindle disruption is Colcemid. The exposure time to Colcemid varies depending on the proliferative activity of cells. Cells with a high proliferative index need a shorter time of exposure to a high concentration of Colcemid, 0.1 to 0.07 $\mu\text{g}/\text{mL}$ for 10 to 20 minutes. Slow-growing cells require longer exposure, 1 to 4 hours or overnight with a lower concentration, 0.01 to 0.05 $\mu\text{g}/\text{mL}$. Prolonged exposure to Colcemid or the use of high concentrations increases the proportion of chromosomes at late metaphase, resulting in shortening of the chromosomes. Conversely, a short exposure with a high concentration of Colcemid reduces the total yield of metaphases. The optimum strikes a balance of these parameters. There are some additional modifications that allow for the enrichment of long (prometaphase) chromosomes by using agents that prevent DNA condensation, such as actinomycin D, ethidium bromide, or BrDU. Cell synchronization techniques can also significantly increase the total yield of metaphase chromosomes.

Applications

Chromosomes are invaluable material for the evaluation of the genome integrity and its preservation at the microscopic chromosomal level. The areas of application include prenatal diagnostics, genetic testing of multiple familial syndromes, including cancer, positional cloning of the genes, and physical mapping (assignment of the genes on chromosomes and subchromosomal regions). The number and morphology of all 23 chromosome pairs in humans can be examined using G-banding differential staining of chromosomes obtained from a peripheral blood sample. Aberrations in the number of chromosomes or visible chromosomal alterations, such as translocations, deletions, and inversions involving extended regions, can be detected by this method. Advances, such as spectral karyotyping, allow for better visualization of aneuploidy and translocations between different chromosomes. Subtle rearrangements, such as submicroscopic deletions or cryptic translocations (an exchange of the small distal telomeric regions between the two nonhomologous chromosomes), can be visualized using specific probes in the FISH technique (Fig. 2.12).⁵⁰

Future Developments

Chromosome analysis will remain a powerful analytic tool in clinical and research fields for the foreseeable future. Possible strategies to improve existing methods include automatization and linearization of the genetic content by increasing resolution to visualize at the level of the chromosome, chromatin,

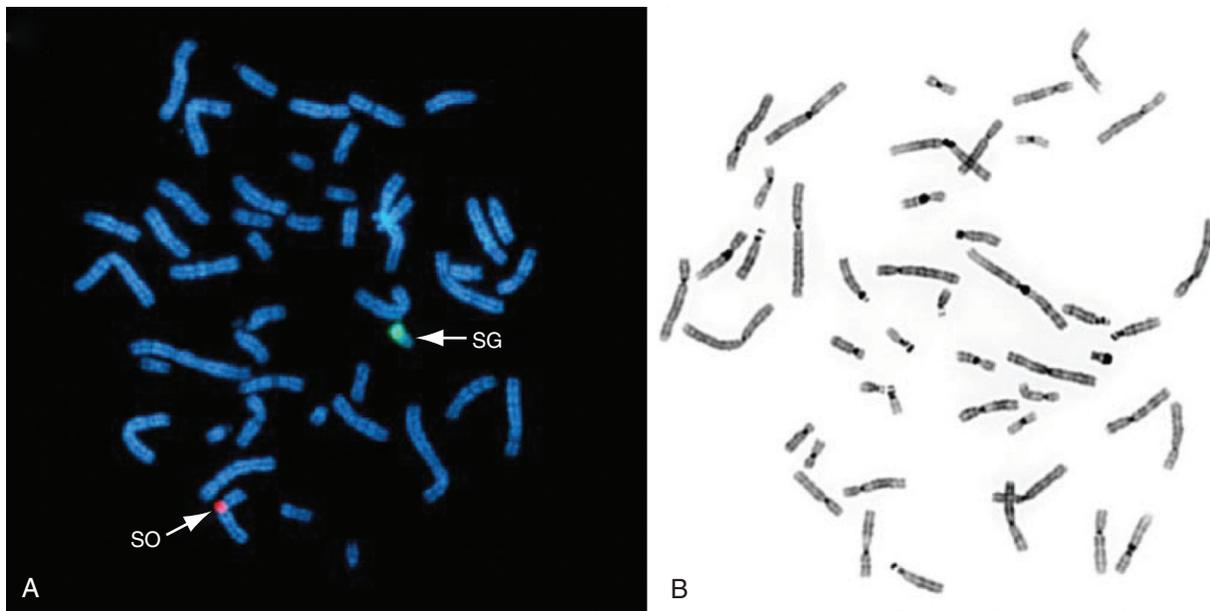


Fig. 2.12 Human metaphase chromosomes (A) after fluorescent in situ hybridization (FISH) using the chromosome X-specific centromeric probe labeled with Spectrum Orange (SO) and chromosome Y-specific heterochromatin labeled with Spectrum Green (SG) and (B) with the inverted DAPI banding (similar to G-banding) allowing chromosomal identification.

DNA, and gene. Another possible direction of development is functional analysis of the genome using constitutional chromosomes and labeled expressed sequences from particular tissues mapped directly to their original positions on chromosomes.

PRINCIPLES OF INTERPRETATION OF GENETIC TESTS IN THE DIAGNOSIS AND MANAGEMENT OF PEDIATRIC ENDOCRINE DISEASES

In the past, genetic tests were usually carried out by research laboratories that had a specific interest in the disease/syndrome under investigation. Under this scenario, the medical provider could generally rely on the expertise of the research laboratory to help with the identification of the appropriate test/test panel and interpretation of the results of the tests. However, with the increasing use of commercial laboratories for these tests, these responsibilities are being transferred to the medical providers. Hence it is now essential for medical providers to become familiar with issues, such as choosing of the appropriate test, the potential usefulness of the information provided by the test including false-positive and false-negative results, available preventive or treatment options, and social and behavioral issues related to genetic testing. The following are some of the points that should be considered when ordering or interpreting a genetic test for a pediatric endocrine disorder:

Limitations of commonly used PCR-based assays. In general routine PCR-based assays (whether they involve electrophoresis or DNA sequencing of the product) cannot reliably differentiate between the two alleles of an individual gene. Hence in a given instance, the detection of a mutation in an autosomal recessive disease could be either caused by the mutation being present in only one of the alleles (and thus not likely to have clinical manifestation) or the result of the mutation being present in a single allele with the other allele being absent (e.g., because of gene deletion) in which case the mutation in the one allele present would clinically manifest. In a similar manner, the inability to amplify a loss-of-function mutant allele by PCR could either be

caused by the presence of two normal alleles (hence excluding the genetic defect) or because of the absence of both the alleles (attributed to gene deletion) in which case the genetic defect would be symptomatic. In many cases, the latter scenario can be excluded by testing using alternative techniques, such as Southern blotting.

Germline versus somatic mutation. Germline mutations are present in every cell descended from the zygote to which that mutant gamete (ova/sperm) contributed. In contrast, somatic mutations occur in a somatic cell (e.g., liver or bone marrow or skin) and hence are not present in other cell types in the body. Most common examples of inherited mutations are germline mutations. Examples of somatic mutations causing endocrine disorders are rarer—for example, in McCune-Albright syndrome, the mutation in the *GNAS* gene may only be detectable in the skin (café au lait) or the bone (fibrous dysplasia) lesion.

Disorders of imprinting. In many instances, the genetic basis of the endocrine disorder is not caused by a mutation but rather by an abnormality in genomic imprinting. Genomic imprinting is the modification of gene expression dependent on whether the genetic material is inherited from the mother or the father. Classic examples are Prader-Willi syndrome (chromosome 15q12 deletion inherited from father) and Angelman syndrome (chromosome 15q12 deletion inherited from mother). Other examples of endocrine disorders in which imprinting is implicated are pseudohypoparathyroidism and Albright hereditary osteodystrophy, Russell-Silver syndrome, Beckwith-Wiedemann syndrome, the focal form of persistent hyperinsulinemic hypoglycemia of infancy, and transient neonatal diabetes.

Penetrance and expressivity. Penetrance is defined as the percentage of people who have the gene and who develop the cognate phenotype. Expressivity is the extent to which a gene is expressed in one person. For example, when a gene has 50% expressivity, only half the features are present or the disease severity is only half of what can occur with full expression. The variable penetrance of many of the neoplastic components of the MEN syndrome is an example of this phenomenon.

One gene, multiple diseases. Examples of mutations in the same gene causing different disease (e.g., *lamin A/C* gene [LMNA] mutation causing Emery-Dreifuss muscular dystrophy, Hutchinson-Gilford progeria, Charcot-Marie-Tooth type 2, familial partial lipodystrophy syndrome, and dilated cardiomyopathy). In many such instances, the variability can be attributed to specific mutation(s) for each of the different clinical manifestations or sets of mutations within different domains/regions of the gene. A similar example is mutations in the *RET* oncogene implicated in MEN2 syndromes, nonsynchondroma paraganglioma, and in Hirschsprung disease.

One disease, multiple genes (phenocopy). Phenocopy refers to the development of disease manifestations that are usually associated with mutations of a particular gene but instead are caused by another gene/etiology. Such a scenario could confound clinical diagnosis and management of a suspected hereditary endocrine disorder. For example, it has been reported that MEN1 syndrome, which is usually caused by mutation in the *MEN1* gene, can be mimicked by familial hypocalciuric hypercalcemia caused by an inactivating mutation in the calcium-sensing receptor, the hyperparathyroidism-jaw tumor syndrome resulting from a mutation in the gene responsible for hyperparathyroidism type 2 (HRPT2), and mutations in the p27 (*CDKN1B*) gene that cause a MEN1-like syndrome that has been called MEN4.

GENOMIC MEDICINE, GENOME SEQUENCING, AND APPLICATIONS IN GENETIC TESTING

As mentioned earlier, in the last 3 decades, there have been two major advances in modern molecular genetics. The first was theoretical; it was the introduction of the concept of “positional cloning,” which was explained previously. The second was technical; the technique of PCR was also extensively discussed in the preceding pages. Cancer medicine and traditional human genetics were the fields that benefited most from the first applications of the new genomic concepts and technologies. The HGP was completed in 2003 using mostly PCR-based Sanger sequencing. The latter was expensive, laborious, and impractical for studying whole genomes; thus HGP technologies that were grown out of necessity led to the development of NGS methods that are now widely available.⁵¹ The HGP also determined that the human genome is composed of 3.3 billion pairs of nucleotide bases and that all human beings share 99.9% similarity at the DNA level with only 0.1% genetic variation, the latter mostly caused by single-nucleotide polymorphisms (SNPs). Thus there are about 10 million SNPs in the human genome. On average, these SNPs occur once in every 300 nucleotides, mostly in noncoding DNA that is located between the approximately 20,000 to 25,000 coding genes. Decades after the first successful disease-causing gene identifications in endocrinology, the gene mutations in CAH syndromes, the insulin and steroid hormone receptors, and *RET*, *GNAS*, *menin*, *PTEN*, and *PRKARIA* in the various forms of multiple endocrine tumor syndromes, a number of other genetic causes have been identified in diseases affecting the pituitary, thyroid, parathyroid, pancreas, adrenal, the gonads, and so on.⁵² In fact, progress is so fast that we are already talking about postgenomic medicine or endocrinology.⁵³ However important these discoveries were in helping us understand cellular processes, glandular development, disease pathophysiology, even leading in some cases to new, molecularly designed treatments, it is only now that genomic medicine is in fact altering clinical practice. The changes are fast and far-reaching, from defining the genotypes of each one of us, to linking electronic medical records (EMRs) to genomic data, to direct-to-consumer genetic testing and its implications for patient-doctor interactions, disease

surveillance, ethics and beyond. Hippocrates, again, noted that “Medicine cannot be learned quickly because it is impossible to create any established principle in it, the way that a person who learns writing according to one system that people teach understands everything; for all who understand writing in the same way, do so because the same symbol does not sometimes become opposite, but is always steadfastly the same and not subject to chance. Medicine, on the other hand, does not do the same thing at this moment and the next, and it does opposite things to the same person, and at that things that are self-contradictory.”⁵⁴ The continuous shifting of ideas and practices is indeed very real in modern medicine and endocrinology and is caused by the advances of genetics.

GENETICS IN CLINICAL PRACTICE AND NEXT-GENERATION SEQUENCING APPLICATIONS

The vast majority of patients encountered in daily endocrine practice were not, up to recently, candidates for genetic testing. For most practicing endocrinologists, encountering a patient with familial glucocorticoid resistance (FGR), for example, would be almost unheard of. In these rare cases, a referral to a research academic center would be the solution; there the patient of our example, with FGR, would undergo genetic testing that could lead to the identification of the causative mutation in the glucocorticoid receptor gene.⁵⁵ The discovery would lead to a publication but would remain relatively obscure for the average clinician. This was the case up to recently for most of medicine.

However, today, there are more than 10,000 rare diseases and even though each is extraordinarily rare (like FGR), collectively, these diseases affect large numbers of patients and represent an exceedingly disproportionate share of healthcare costs.⁵⁶ The International Rare Disease Research Consortium estimates that a significant number of patients with rare diseases are seen and remain undiagnosed at clinical practices.⁵⁶ Several of our patients with complex symptoms and signs are now offered genetic testing in routine clinical settings. In addition, the chances are that most clinicians in developed countries will encounter patients that have already been the recipients of direct-to-consumer genetic testing (DCGT).⁵⁷ In February 2019 it was estimated that over 26 million people have taken home DNA tests. In 2015 a company known as “23andme” was granted approval by the US Food and Drug Administration (FDA) to include health risk assessments in genetic reports. There are several such companies today from those providing DCGT for ancestry and various traits, for a relatively inexpensive fee, to others that provide full genome sequence analysis data and discuss health risks for a higher price.

In another front, it is not unusual for the average clinician practitioner to encounter pharmacogenomics (PGx).⁵⁸ For example, in testing for abnormal cortisol levels in response to the 1 mg overnight dexamethasone (DEX) test, it is essential to know whether the patient is a rapid or slow DEX metabolizer. There are several testing sites that now test for the cytochrome P450 liver enzymes that are responsible for DEX metabolism. In areas of medicine other than endocrinology, PGx is already in daily practice from infectious diseases and the right choice of an antibiotic for a rapid or slow metabolizer, respectively, to how certain ethnic groups respond to a drug, based on gene-specific and ethnicity-related genetic variants. There are now more than 200 drugs that have PGx information included in their FDA-approved labels and new clinical practice guidelines, based on PGx, are now published frequently.⁵⁹

Finally, there is increasing use of genetic screening for preventive health and for prenatal diagnostic testing. There are several efforts across the United States and other countries and in

several health systems, private or government-run, to link cancer, cardiovascular, and other risk factors to medical records (EMR and other types of records) with the intent to lead to behavior, diet, and other modifications, diagnostic and even invasive procedures that prevent disease.⁵⁸ Noninvasive prenatal testing (NIPT) relies on the detection of cell-free DNA (cfDNA) on parental samples for the detection of trisomies and increasingly other genetic defects.⁶⁰ NIPT represents one of the fastest adopted tests by every day clinical practice in the history of medicine and has revolutionized the detection of fetal aneuploidies and other disorders, obviating the need for amniocentesis and a number of other procedures.^{51,60}

The aforementioned screening and testing strategies were possible because of advances emanating from the HGP, in particular massively parallel DNA sequencing technologies, which were first introduced in 2005 and keep improving and becoming cheaper every day.⁵¹ Collectively, these new DNA sequencing technologies can generate billions of short sequencing reads within hours; the current cost for a near-comprehensive determination of one's genome by NGS for both frequent and rare variants is approximately \$1000, whereas the cost is significantly less for exome-only versions and even less if specific regions or a collection of genes are targeted.^{61,62}

In addition to NGS, a product of HGP was high-density DNA microarrays based on SNPs. They were first introduced in the late 1990s; however, at the beginning, these microarrays were mostly used in population-based or other large cohort-based research studies of genotype human genomes for genetic variation and other association studies. Now, high-density DNA microarrays are also used in clinical practice for the identification of structural variants of the human genome and for the rapid identification of any sequence variation: in fact, the most popular genetic tests today are not sequencing-based tests, but genotyping tests using technology derived from the DNA microarrays that were first developed by HGP researchers.^{61,62} It is hard to predict where NGS is heading.⁵¹ It is safe to say, however, that it will expand in ways and applications that will intrude in every aspect of medicine and indeed life. It is therefore essential for endocrinologists to understand the basics of NGS, how it is applied today, and its impact on how we practice medicine.

Genome Versus Exome Sequencing, Variant Interpretation, Other Challenges

The goal of NGS is to provide a high-quality map of genome variation for each sample. WGS provides exactly that with short-read NGS technology, which is widely available today.⁶² Yet, it remains prohibitively expensive for clinical use and cumbersome in data management. Because nearly 99% of the roughly 3.3 billion nucleotides constituting the human genome do not code for proteins, and most human diseases are caused by variations of or defects of coding genes (the exome), the alternative approach, as mentioned earlier, is WES, which currently costs less than \$1000 and is widely available in developed countries.

Single nucleotide variants (SNVs) and small insertion or deletion variants (indels) that are less than 50 bp long represent the vast majority of variants in the human genome: there are 3 to 4 million SNVs and 0.4 to 0.5 million indels in every human sample compared with the reference genome.⁶² However, only a few more than 100 lead to a premature stop codon for a coding gene and only about 20 or so are potentially deleterious. Nevertheless, practically every variant can cause a phenotype not only through changing the protein by an amino acid substitution, a deletion, or an insertion, but also by changing binding of transcription factors, interactions with other molecules,

RNA stability, and so on. Sequence variants that are identified by WGS or WES are reported therefore as variants of unknown significance (VUS) because unless there is previously existing data documenting their function, they may not be called mutations and cannot be definitely linked to disease or even a risk factor.^{61,62}

Structural variation (SV) is another form of genome variation, by definition it is larger than 50 bp, which includes copy number variants (CNV) that represent amplifications or deletions of variable size DNA segments, chromosomal rearrangements, and mobile element insertions.⁶² SVs account for only about 0.2% of total variants, but they more frequently effect phenotype, representing, for example, as many as 4% to 12% of high-impact coding alleles. Their phenotypic effect is typically proportionate to their size, although exceptions to this rule exist. SVs are not easily detectable by short read WGS and even less so by WES. Their architectural diversity also poses additional challenges in their detection. For one to see the impact of methodology, a typical human genome contains as many as 10,000 SVs detectable by the commonly used short-read WGS but more than 20,000 SVs identified by long-read WGS. The complexity of variant interpretation increases when one considers repetitive elements, such as short or variable number tandem repeats and mobile element insertions.

Functional annotation for each one of the variants, whether it is a VUS, SV (CNV or otherwise), or a repeat variant, is constantly updated based on information in publicly available databases to which genotypic and phenotypic data are added daily.⁵⁸ It is estimated that up to 25% of patients with a rare disease may be found to have a causative defect upon first application and reading of a WES test in specific cohorts studied by experienced clinical centers.⁵⁶ Because of continuously available new information, reanalysis of previously "negative" WES data may increase yield by more than 10% and even higher when family and other data are added to the analysis.^{56,62}

Variants may be classified as pathogenic or likely pathogenic variants and benign or likely benign based on structural effect on DNA and/or the coding gene or protein, functional studies, in silico data, variant frequency in control populations, family studies (segregation analysis), and other factors.⁶³ There are stringent and explicit guidelines followed now by all genetic testing laboratories and endorsed by professional societies. Variant assessment also includes searching carefully the available literature but one has to be careful with older nomenclature, different gene symbols, sequence numbering, and phenotypic interpretations.

During NGS, one may also identify what have been called *secondary findings*, which is understandable given the number of variations in the human genome, as stated earlier. Currently, the American College of Medical Genetics and Genomics (ACMG) and other organizations have identified 59 medically actionable genes in which, if variants are found, they need to be reported as secondary findings and may lead to additional testing and even invasive procedures.^{61,63} Examples include the *BRCA1* and *BRCA2* genes, associated with early breast, ovarian, and other cancers; *BMPRIA* and *SMAD4* associated with increased risk for colorectal and other cancers; and other genes.⁶¹

NGS and Cell-Free DNA in Cancer Medicine and Prenatal Testing

Plasma-borne DNA was first described in 1948, but for years its origin and significance were unknown.⁶¹ In the 1980s, it was realized that tissues such as tumors, the placenta, and the human fetus, shed DNA and RNA. In cancer medicine, cfDNA

and cfRNA can lead to early detection, identification of the mutational profile of a given tumor, and provide means for follow-up as a tumor marker and in response to therapy. The notion of “liquid biopsy” is now well accepted in many oncological settings despite the challenges that remain: the relatively low proportion of tumor-derived cfDNA and cfRNA in the circulation, the difficulty in assessing such abnormalities early in the course of malignancy, and the high rates of false positives.⁶¹

On the other hand, sequence analysis of cfDNA and cfRNA fragments that circulate in the blood of pregnant women, along with the translation of this method into screening for fetal chromosome abnormalities, is clearly a great success story of modern genetics.^{51,60} As of late 2017, a total of 4 to 6 million pregnant women had had DNA from their plasma analyzed to screen for fetal aneuploidy.⁶⁰ During pregnancy, DNA fragments are released from the placenta into the maternal circulation; plasma contains both maternal and placental DNA and one calculates the ratio of placental to total cfDNA, which is known as the “fetal fraction” and increases as pregnancy advances. Testing is performed from the 10th week of gestation onward. The testing has revolutionized aneuploidy detection and obviated the need for more invasive procedures (i.e., amniocentesis). However, there are issues with a relatively high rate of false positives and the identification of secondary findings governed by the same ACMGG-sponsored guidelines referred to in the previous section.⁶³

Next-generation sequencing, mosaicism, and its applications in detecting somatic genetic defects

A “negative” WGS or WES in a tissue sample may be caused by some of the issues discussed earlier and caused by the increasingly frequent issue of mosaicism: most commercially available NGS technologies may not detect mosaicism for a pathogenic variant because the latter may not be present in the tissue tested (e.g., in peripheral blood in most cases). Picking a variant at low levels from the “noise” of a sequence is often impossible (unless one increases the “depth” of the sequence), and mosaicism levels for a variant change with aging at various tissues (sequence variants detectable in blood in infancy may not be there in adolescence).⁶¹ There are new diagnostic tools under development to detect mosaicism, but for most cases it remains a difficult issue to solve.

On the other hand, NGS is used frequently for the detection of the mutational spectrum in cancers.⁶¹ Tumors have a hugely variable genome that offers diagnostic and treatment opportunities if identified by NGS. Just like what was mentioned earlier in cfDNA analysis, challenges include the relatively high heterogeneity of tumor samples, low frequency of single clonal DNA changes, and the high rate of false positivity.

In endocrine diseases, McCune-Albright syndrome is a classic disorder caused by postzygotic mosaicism for an activating mutation of the *GNAS* gene.⁶⁴ NGS may be used for the detection of mosaicism in peripheral tissues, because frequently the mutation is not detectable in peripheral blood. It is expected that the wider application of NGS will lead to the identification of many more instances of mosaicism in endocrine diseases, just like it did in other areas of medicine.

PRECISION MEDICINE

As noted in the preceding section of this chapter, genetic testing for monogenic, mendelian disorders is becoming an increasingly important tool for the pediatric endocrinologist. It can by itself reveal a diagnosis and inform prognosis and treatment. For example, a heterozygous loss-of-function mutation in *ABCC8* inherited from the father may be evidence for congenital hyperinsulinism caused by a focal pancreatic lesion

treatable by resection of the lesion, whereas compound heterozygous loss-of-function mutations in the same gene inherited from both parents most likely would require near-total pancreatectomy. The diagnosis of monogenic diabetes may allow patients previously diagnosed with type 1 diabetes to transition from insulin to an oral sulfonylurea. In boys with primary adrenal insufficiency, the presence of a pathogenic mutation in *ABCD1* is diagnostic of adrenoleukodystrophy and predicts a very different prognostic and therapeutic trajectory than would be the case for an autoimmune etiology. Moreover, different mutations in the same gene may have different implications, as in CAH caused by mutations in *CYP21A2*, in which salt-wasting, virilizing, or nonclassical phenotypes can often be predicted by specific mutations. In addition, as specific gene- and cell-based therapies for endocrine diseases emerge, such as gene-editing, base-editing, and anti-sense oligonucleotide (ASO) therapies, it will become important to document a patient’s precise genetic lesion to correctly devise and deliver the appropriate treatment. Endocrinologists should understand the genetic basis of endocrine diseases and their treatments so they can advise their patients, participate in therapeutic decisions, and monitor the therapeutic consequences.

However, despite the growing importance of genetic testing in pediatric endocrinology, several barriers exist. First and foremost, many physicians have a knowledge deficit in genetic medicine. Most physicians, especially those who finished medical school in the past 25 years, have had robust education in basic and clinical genetics, but because this knowledge was not carried through residency, fellowship training, or faculty development, they are not comfortable applying clinical genetic principles to clinical practice. Physicians should (1) be proficient in recognizing when a patient might have a genetic basis for their disease when it appears at a very young age, when it is very severe, when it is part of a syndrome, and when its inheritance segregates within a family; (2) recognize different inheritance patterns of autosomal dominance, autosomal recessiveness, X-linked inheritance, and imprinted parental transmission from mother or father; (3) recognize consequences of loss of function, gain of function, and dominant negative mutations, and that different mutations in the same gene may have different consequences; (4) recognize the specific endocrine diseases that may have a monogenic basis, as well as the responsible genes; (5) understand what types of genetic tests are available (single gene, gene panel, exome, genome, microarray, cytogenetic/karyotype, PCR, FISH, etc.) and how to use these; (6) know how to interpret different DNA sequence results (benign versus pathogenic versus uncertain significance); and (7) understand that the interpretation of a patient’s test result may change over time as new knowledge emerges, and know how to recontact the original testing laboratory and search public databases (ClinVar, Human Mutation Gene Database, OMIM, etc.) for new information.

The proper interpretation of genetic test results by a testing laboratory requires it to have accurate phenotype information, which must be supplied by the physician who orders the test. For single genes and gene panel tests, different classes of mutations in the same gene have different consequences. For example, different variants in *CASR* cause either familial hypocalcemic hypercalcemia (loss of function) or hypocalcemia (gain of function). The testing laboratory would be reluctant to interpret the significance of a variant without knowing which phenotype to associate it with. When evaluating exome or genome DNA sequence results, the testing laboratory is guided by the phenotype (often documented in human phenotype ontology or HPO terms) to select which variants within the roughly 20,000 human genes to evaluate for pathogenicity. Incorrect or incomplete phenotype information may misdirect this analysis.

Patients (and if a child, their guardians) should provide informed consent for all genetic tests. Even for single gene and gene panel tests, they should be informed how the test may aid diagnosis, prognosis, or treatment. They should understand that a result may not be informative, may provide or confirm a diagnosis, may be of uncertain significance, or may require reanalysis at a future time. They should understand that if parental DNA is also analyzed (for example, a trio study using DNA from the patient and both assumed biological parents), there is a chance that nonpaternity may be revealed. They should understand that genetic test results may result in discrimination in obtaining life insurance, or provide evidence of preexisting disease, which under some future scenarios may result in denial of health insurance. Patients should also realize that exome and genome studies may reveal pathogenic findings outside of the scope of the purpose for the test, such as a predisposition to cancer, which they must decide either to be informed of or not. Finally, patients should understand their financial liability for genetic testing, as it is variably covered by health insurers. Before a genetic test is ordered, it is desirable to understand if the patient's health insurance provider will approve the testing, and if so, whether the patient will be responsible for copay or deductible costs.

When the physician discusses with the patient both the initial recommendation to perform genetic testing, as well as the test results, it is often helpful to do this in conjunction with a genetics counselor, who is trained in all of the aforementioned areas, including how to effectively discuss these issues with patients and their families.

Precision medicine therapeutics hold great promise for the future of pediatric endocrinology. Gene replacement of mutant genes coupled with autologous stem cell transplantation is being investigated in patients with adrenoleukodystrophy.⁶⁵ In vivo treatment of spinal muscular atrophy using an ASO approach⁶⁶ has recently been approved by the FDA.⁶⁷ In the future, direct correction of human gene defects in somatic cells in vivo may be possible by CRISPR-assisted gene- and base-editing strategies.⁶⁸ Knowledge of the specific genetic basis of a patient's disease will become the necessary first step in considering these future potential therapies.

RECOMBINANT DNA TECHNOLOGY AND THERAPY OF PEDIATRIC ENDOCRINE DISEASES

From a therapeutic point of view, recombinant DNA technology can be exploited to either tailor pharmacotherapy according to the genotype of a patient (i.e., targeted pharmacotherapy), manipulate genes within the human body (gene therapy), or engineer prokaryotic or eukaryotic cells to produce proteins, such as hormones, which can then be administered for therapy or diagnosis. Whereas targeted pharmacotherapy and gene therapy are mostly restricted to the research arena, the use of hormones produced by recombinant DNA technology is well established in clinical endocrinology. Historically, insulin was the first hormone synthesized by recombinant DNA technology to be approved for clinical use.^{69,70} At present, a variety of recombinant hormones including growth hormone (GH), luteinizing hormone, follicular-stimulating hormone, thyroid-stimulating hormone (TSH), parathyroid hormone, and erythropoietin are being used clinically or are in advanced stages of clinical trials.

On a theoretic basis, it should be possible to synthesize any protein hormone whose gene has been cloned and DNA sequence determined. Thus recombinant DNA technology makes it possible to insert the gene coding for a particular protein hormone into a host cell such that the protein is produced by the host cell's protein-synthesizing machinery. The

synthesized protein is then separated from the rest of the host cell proteins to obtain the pure form of the hormone of interest. Both prokaryotic and eukaryotic cells can serve as the host cell for the production of proteins by this technology. Because post-translational modifications, such as glycosylation, may be essential for the optimal action of a protein hormone, the choice of the specific cell system used for the production of a particular protein hormone is critical. Prokaryotic cell systems, such as *Escherichia coli*, are suitable for the production of protein hormones that do not need posttranslational modifications, such as GH.⁷¹ Eukaryotic cell systems, such as Chinese hamster ovary cells that are capable of posttranslational modification of the protein, are useful for the production of hormones, such as TSH, that require glycosylation for optimal bioefficacy.⁷² In addition, eukaryotic cells are capable of synthesizing proteins that undergo the appropriate folding, a step that is not carried out by prokaryotic cells. The advantages of the use of recombinant DNA for the production of these proteins include the possibility of a limitless supply of a highly pure form of a protein and the absence of the risk of contamination with biological pathogens associated with the extraction of proteins from human or animal tissue. In addition, this technology permits the development of hormone analog and antagonists with much greater ease than conventional protein synthesis protocols.

The influence of genetic factors on the metabolism of various drugs is a well-established phenomenon with the effect of various isoenzymes of cytochrome p450 on the circulating half-life of drugs, such as anticonvulsants, which are a classic example of this interaction. Another example of the role of genotype on the choice of pharmacotherapy is the phenomenon of drug-induced hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency. The genomic revolution has allowed for the exploitation of computational approaches to identify polymorphisms of known genes encoding proteins with different functional characteristics, as discussed previously. Investigators can now predict the response of a particular individual to a class of drugs/chemicals. This pharmacogenomic approach to clinical therapeutics has been successful in demonstrating an association between specific polymorphisms in the β -adrenergic receptor and response to β -agonists in patients with bronchial asthma, and polymorphisms in hydroxytryptamine receptors and response to neuroleptic drugs. The widespread application of the tools of molecular biology to unravel the molecular basis of action of hormones has also yielded benefits by allowing for customization of the pharmacotherapy of endocrine diseases and syndromes based on the specific individual genetic defect. One such example is the report of directed pharmacologic therapy of an infant with ambiguous genitalia, resulting from a mutation in the androgen receptor.⁷³ In this infant with an M807T mutation in the androgen receptor, in vitro functional studies had indicated that the mutant receptor exhibited loss of binding capacity for testosterone with retention of binding for dihydrotestosterone. Furthermore, this differential binding was also reflected in the better preservation of the transactivation potential of dihydrotestosterone (DHT) compared with testosterone. These in vitro findings were exploited to treat the infant with DHT, resulting in restoration of male genital development. This case illustrates that in selected cases, in vitro functional assays can help identify subsets of patients with ambiguous genitalia and androgen insensitivity who would respond to targeted androgen therapy. It can be anticipated that in the coming years, more examples of such innovative therapeutic strategies and "customized" hormonal treatment protocols will become routine and implemented in the practice of clinical pediatric endocrinology.

CONCLUDING REMARKS

The application of recombinant DNA technology has resulted in a tremendous increase in our understanding of physiologic processes and pathological conditions. Achievements, such as the full sequence of the human genome in normal subjects,⁷⁴ have resulted in a paradigm shift in the way that we think about genetic causes and predisposition to disease⁴⁶ and in the analysis of the function of hormones and related proteins.⁷⁵ In the traditional paradigm, investigators seeking to discover new genes or to analyze the function of known proteins needed to devote a significant part of their time to conducting “bench research” in “wet laboratories.” The new approach in this “postgenomic” era takes advantage of the unprecedented power of computational biology to “mine” nucleotide, protein sequence, and other related databases. In the future, most researchers will deal with abstract models and data sets stored in computer databases. Hence initial discoveries of novel genes or novel interactions between known proteins or intracellular signaling pathways could be made using the analytic power of computational software tools (functional genomics); these initial insights can then be verified and expanded upon by traditional laboratory bench methods. The advantages of this new paradigm are obvious with computational approaches taking a significantly shorter time, with less demand on manpower, and can easily expand the scope of the search to include multiple molecules and organisms (phylogenetic profiling). Several public-domain web-accessible databases are currently serving as the major repositories for this information. GenBank is the major repository for sequence information and is currently supported by the National Institutes of Health. One of the main sources of the physical location, clinical features, inheritance patterns, and other related information of specific gene defects is the OMIM operated by Johns Hopkins University in Baltimore, Maryland. Johns Hopkins University also operates the online Genome Data Base, which allows scientists to identify polymorphisms and identify contacts for gene probes and other related research tools. The ever-expanding number of endocrine (and other) disorders that can be attributed to changes in the nucleotide sequence of specific genes has also increased the necessity for the availability of accurate, reliable, and timely genetic tests, such as mutation detection. One source for such information is a collaborative website (www.genetests.org) that maintains an up-to-date catalog of commercially available and research-based tests for inherited disorders.

With the ubiquitous use of these powerful tools in laboratories around the world, genes are being cloned and genetic diseases are being mapped at a rapid pace. In all of these exciting developments, one still needs to keep in mind that whereas this “new” science has allowed for hitherto inaccessible areas of human biology to be probed and studied, a lot remains to be understood with respect to individual disease processes. Hence at the present time, we have only a rudimentary understanding of the correlations between phenotype and genotype in many of the common genetic diseases, such as CAH. These lacunae in our knowledge dictate that clinicians should be cautious about basing therapeutic decisions solely on the basis of molecular and genetic studies. This is especially true in the area of prenatal diagnosis and recommendation for termination of pregnancy based on genetic analysis. As we improve our understanding of the molecular and genetic basis of disease and translate this knowledge into gains at the bedside, it behooves us, both as individuals and as a society, to be cognizant of critical issues relating to the privacy of health data and to remain vigilant against misuse by inappropriate disclosure of this powerful knowledge.

REFERENCES

- Bolander F. *Molecular Endocrinology*. 3rd ed. Academic Press; 2004.
- Shupnik MA. *Gene Engineering in Endocrinology*. New York: Garland Science; 2000.
- Alberts B, et al. *Molecular Biology of the Cell*, New York. Garland Science; 2000.
- Lodish H, et al. *Molecular Cell Biology*. 5th ed. New York: W. H. Freeman; 2003.
- Roberts RJ. Restriction enzymes and their isoschizomers. *Nucleic Acids Res*. 1989;17(suppl):347–388.
- Nathans D, Smith HO. Restriction endonucleases in the analysis and restructuring of DNA molecules. *Ann Rev Biochem*. 1975;44:273–293.
- Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*. 1975;98:503–517.
- Waters DLE, Shapter FM. The polymerase chain reaction (PCR): general methods. *Methods Mol Biol (Clifton, NJ)*. 2014;1099:65–75.
- Ishmael FT, Cristiana Stellato C. Principles and applications of polymerase chain reaction: basic science for the practicing physician. *Ann Allergy Asthma Immunol*. 2008;101(4):437–443.
- Kocova M, et al. Detection of Y chromosome sequence in Turner's syndrome by Southern blot analysis of amplified DNA. *Lancet*. 1993;342:140–143.
- Lehrach HD, et al. RNA molecular weight determinations by gel electrophoresis under denaturing conditions. *Biochemistry*. 1977;16:4743–4751.
- Thomas PS. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc Natl Acad Sci USA*. 1980;77:5201–5205.
- Wagner EM. Monitoring gene expression: quantitative real-time RT-PCR. *Methods Mol Biol (Clifton, NJ)*. 2013;1027:219–245.
- Freeman WM, et al. Quantitative RT-PCR: pitfalls and potential. *BioTechniques*. 1999;26(1):112–122, 124–125.
- Innis MA, et al. eds. *PCR Protocols: A Guide to Methods and Applications*. San Diego, CA: Academic Press; 1990.
- Navarro E, et al. Real-time PCR detection chemistry. *Clin Chim Acta; Int J Clin Chem*. 2015;439:231–250.
- Giulietti A, et al. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. *Methods (San Diego, Calif)*. 2001;25(4):386–401.
- Mohr AM, Mott JL. Overview of MicroRNA biology. *Semin Liver Dis*. 2015;35(1):3–11.
- Wójcicka A, et al. Mechanisms in endocrinology: microRNA in diagnostics and therapy of thyroid cancer. *Eur J Endocrinol*. 2016;174(3):R89–R98.
- Reuter JA, et al. High-throughput sequencing technologies. *Mol Cell*. 2015;58(4):586–597.
- Maxam AM, Gilbert W. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol*. 1980;65:499–560.
- Sanger F, et al. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA*. 1977;74:5463–5467.
- Marzancola MG, et al. DNA microarray-based diagnostics. *Methods Mol Biol (Clifton, NJ)*. 2016;1368:161–178.
- Harrington CT, et al. Fundamentals of pyrosequencing. *Arch Pathol Lab Med*. 2013;137(9):1296–1303.
- Choi M, et al. K⁺ Channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science (New York, NY)*. 2011;331(6018):768–772.
- Zhang F, et al. CRISPR/Cas9 for genome editing: progress, implications and challenges. *Hum Mol Genet*. 2014;23(R1):R40–R46.
- Collins FS. Positional cloning: let's not call it reverse anymore. *Nat Genet*. 1992;1:3–6.
- Collins FS. Positional cloning moves from perdictional to traditional. *Nat Genet*. 1995;9:347–350.
- Borsani G, et al. A practical guide to orient yourself in the labyrinth of genome databases. *Hum Mol Genet*. 1998;7:1641–1648.
- Khoury MJ, et al. *Fundamentals of Genetic Epidemiology*, 1st ed. Oxford, UK: Oxford University Press; 1993.
- Speer MC. Use of LINKAGE programs for linkage analysis. *Curr Protocol Hum Genet*. 2006. 1(1.7).
- “Online Mendelian Inheritance in Man, OMIM.” National Library of Medicine, McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine

- (Bethesda, MD). Center for Medical Genetics. In: *Johns Hopkins University and National Center for Human Genome Research*; 2007. <http://www.ncbi.nlm.nih.gov/omim/>.
33. Linehan WM, et al. Identification of the von Hippel-Lindau (VHL) gene. *JAMA*. 1995;273:564–570.
 34. Chandrasekharappa SC, et al. Positional cloning of the gene for multiple endocrine neoplasia-Type 1. *Science*. 1997;276:404–407.
 35. Liaw D, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*. 1997;16:64–67.
 36. Hemminki A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*. 1998;391:184–187.
 37. Jenne DE, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. *Nat Genet*. 1998;18:38–43.
 38. Kirschner LS, et al. Mutations of the gene encoding the protein kinase A type i-alpha regulatory subunit in patients with the Carney complex. *Nat Genet*. 2000;26:89–92.
 39. 1000 Genomes Project Consortium, Adam Auton, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68–74.
 40. 1000 Genomes Project Consortium, Gonçalo, R. A., et al. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467(7319):1061–1107.
 41. Turan S, et al. An atypical case of familial glucocorticoid deficiency without pigmentation caused by coexistent homozygous mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metabol*. 2012;97(5):E771–E774.
 42. Millington GWM. Proopiomelanocortin (POMC): the cutaneous roles of its melanocortin products and receptors. *Clin Exp Dermatol*. 2006;31(3):407–412.
 43. Rees JL, Harding RM. Understanding the evolution of human pigmentation: recent contributions from population genetics. *J Invest Dermatol*. 2012;132(3 Pt 2):846–853.
 44. Clark AJL, et al. The genetics of familial glucocorticoid deficiency. *Best Pract Res Clin Endocrinol Metabol*. 2009;23(2):159–165.
 45. Brooks BP, et al. Genotypic heterogeneity and clinical phenotype in triple a syndrome: a review of the NIH Experience 2000-2005. *Clin Genet*. 2005;68(3):215–221.
 46. Stratakis CA. 'Patients can have as many gene variants as they damn well please': why contemporary genetics presents us daily with a version of Hickam's Dictum. *J Clin Endocrinol Metabol*. 2012;97(5):E802–E804.
 47. Caspersson T, et al. Analysis of human metaphase chromosome set by aid of DNA-binding fluorescent agents. *Exp Cell Res*. 1999;253(2):302–304.
 48. Yunis JJ, Lewandowski RC. High-resolution cytogenetics. *Birth Defects Orig Art Ser*. 1983;19(5):11–37.
 49. Yunis JJ. High resolution of human chromosomes. *Science*. 1976;191:1268–1270.
 50. Mateuca RA, et al. Cytogenetic methods in human biomonitoring: principles and uses. *Methods Mol Biol (Clifton NJ)*. 2012;817:305–334.
 51. Green ED, et al. The future of DNA sequencing. *Nature*. 2017;550(7675):179–181.
 52. Stratakis CA. Clinical genetics of multiple endocrine neoplasias, Carney Complex and related syndromes. *J Endocrinol Invest*. 2001;24(5):370–383.
 53. Stratakis CA. Genetics and the New (Precision) Medicine and Endocrinology: in Medias Res or Ab Initio? *Endocrinol Metabol Clin North Am*. 2017;46(2):xv–xvi.
 54. Potter P. *Hippocrates "Places in Man."* Cambridge, MA: Harvard University Press; 1995.
 55. Stratakis, C.A., et al. (1994). Glucocorticosteroid resistance in humans. elucidation of the molecular mechanisms and implications for pathophysiology. *Ann N. Y. Acad Sci*, 746, 362–374; discussion 374–376.
 56. Boycott KM, et al. A diagnosis for all rare genetic diseases: the horizon and the next frontiers. *Cell*. 2019;177(1):32–37.
 57. Crow D. A new wave of genomics for all. *Cell*. 2019;177(1):5–7.
 58. Abul-Husn NS, Kenny EE. Personalized medicine and the power of electronic health records. *Cell*. 2019;177(1):58–69.
 59. Sirugo G, et al. The missing diversity in human genetic studies. *Cell*. 2019;177(4):1080.
 60. Bianchi DW, Chiu RWK. Sequencing of circulating cell-free DNA during pregnancy. *N Engl J Med*. 2018;379(5):464–473.
 61. Shendure J, et al. Genomic medicine-progress, pitfalls, and promise. *Cell*. 2019;177(1):45–57.
 62. Lappalainen T, et al. Genomic analysis in the age of human genome sequencing. *Cell*. 2019;177(1):70–84.
 63. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424.
 64. Salpea P, Stratakis CA. Carney complex and McCune Albright syndrome: an overview of clinical manifestations and human molecular genetics. *Mol Cell Endocrinol*. 2014;386(1–2):85–91.
 65. Eichler F, et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. *N Engl J Med*. 2017;377(17):1630–1638.
 66. Chiriboga CA, et al. Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in children with spinal muscular atrophy. *Neurology*. 2016;86(10):890–897.
 67. Sheridan C. Roche's splashes \$2.4. billion on foundation medicine's cancer platform. *Nat Biotechnol*. 2018;36(8):669–670.
 68. Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat Rev Genet*. 2018;19(12):770–788.
 69. Goeddel DV, Kleid DG, et al. Expression in Escherichia Coli of chemically synthesized genes for human insulin. *Proc Natl Acad Sci USA*. 1979;76(1):106–110.
 70. Riggs AD. Bacterial production of human insulin. *Diabetes Care*. 1981;4(1):64–68.
 71. Goeddel DV, Heyneker HL, et al. Direct expression in Escherichia Coli of a DNA sequence coding for human growth hormone. *Nature*. 1979;281(5732):544–548.
 72. Gesundheit N, Weintraub BD. Mechanisms and regulation of TSH glycosylation. *Adv Exp Med Biol*. 1986;205:87–105.
 73. Ong YC, et al. Directed pharmacological therapy of ambiguous genitalia due to an androgen receptor gene mutation. *Lancet*. 1999;354:1444–1445.
 74. MacArthur DG, et al. A systematic survey of loss-of-function variants in human protein-coding genes. *Science (New York, NY)*. 2012;335(6070):823–828.
 75. Hsu SY, Hsueh AJW. Discovering new hormones, receptors, and signaling mediators in the genomic era. *Mol Endocrinol*. 2000;14(5):594–604.

3

Receptor Transduction Pathways Mediating Hormone Action

Bassil Kublaoui, Michael A. Levine

CHAPTER OUTLINE

INTRODUCTION	30
BASIC PRINCIPLES OF RECEPTOR ACTION	31
G PROTEIN–COUPLED RECEPTORS	31
CLASS A RECEPTORS THAT TRANSDUCE HORMONE ACTION	36
The Peptide Receptor Group	36
The Glycoprotein Hormone Receptor Group	38
The Gonadotropin-Releasing Hormone Receptor Group	41
The Thyrotropin-Releasing Hormone and Secretagogue Receptor Group	42
Other Class A Receptors That Transduce Hormone Action	42
CLASS B RECEPTORS THAT TRANSDUCE HORMONE ACTION	44
Growth Hormone–Releasing Hormone Receptor	44
Gastric Inhibitory Polypeptide Receptors	44
Parathyroid Hormone and Parathyroid Hormone–Related Peptide Receptors	45
Other Class B Receptors That Transduce Hormone Action	45
CLASS C RECEPTORS THAT TRANSDUCE HORMONE ACTION	45
Calcium-Sensing Receptors	45
G PROTEIN GENE DISORDERS	47
Inactivating Mutations of the <i>GNAS</i> Gene	47
Activating Mutations of the <i>GNAS</i> Gene	47
CYTOKINE RECEPTORS	48
Structure and Function of Type 1 Cytokine Receptors	48
Cytokine Receptors That Transduce Hormone Action	48
LEPTIN RECEPTORS	49
RECEPTOR TYROSINE KINASES	50
INSULIN RECEPTOR TYROSINE KINASE FAMILY	50
THE INSULIN RECEPTOR	51
THE INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR	52
THE FIBROBLAST GROWTH FACTOR RECEPTOR FAMILY	52
Fibroblast Growth Factor Receptor 1	53
Fibroblast Growth Factor Receptors 2–4	53
NUCLEAR RECEPTORS	53
General Structure of the Nuclear Receptors	54
SUBFAMILY 1 NUCLEAR RECEPTORS: THYROID HORMONE, VITAMIN D₃, AND PEROXISOME PROLIFERATOR–ACTIVATED RECEPTORS	54
Thyroid Hormone Receptors	54
Vitamin D Receptor	57
Peroxisome Proliferator-Activating Receptor γ	58
SUBFAMILY 2 NUCLEAR RECEPTORS: HEPATOCYTE NUCLEAR FACTOR AND RETINOID X RECEPTORS	58
Hepatocyte Nuclear Factor Receptors	58
SUBFAMILY 3 NUCLEAR RECEPTORS: THE STEROID RECEPTORS AND GLUCOCORTICOID, ANDROGEN, ESTROGEN, AND MINERALOCORTICOID RECEPTORS	58
Glucocorticoid Receptors	58
Androgen Receptors	59
Estrogen Receptors	59
Mineralocorticoid Receptors	60
SUBFAMILY 0 NUCLEAR RECEPTORS: DAX1	60
DAX1	60
SUMMARY	61
References	61

INTRODUCTION

Hormones exert their actions by binding to specific receptor proteins, a process that induces conformational changes or compartmental redistribution of these proteins. The activated receptor is now capable of inducing positive (or negative) intracellular effects that ultimately are recognized as a physiologic response. The specificity of hormone action is determined by the affinity of hormones for different receptors, the cell-specific expression of the receptor, and the unique responses induced by ligand occupancy.

Since the early 2000s, our understanding of hormone action has advanced rapidly with the success of genomics and advanced molecular biologic techniques. This combined approach has led to the discovery and classification of an

unexpectedly large number of receptors, some quite novel and others even unanticipated, that are members of large families of genetically conserved proteins. Moreover, our understanding of receptor action has been clarified by the identification and detailed characterization of postreceptor signaling proteins and signaling mechanisms. Four major receptor superfamilies have been identified that are distinguished by protein structure, cellular localization, and effector systems. These families include the G protein–coupled receptors (GPCRs), cytokine receptors, tyrosine kinase receptors (RTKs), and nuclear receptors (Table 3.1). This chapter reviews major features of these important receptor families. Mutations influencing receptor function leading to endocrine disorders are also highlighted.

TABLE 3.1 Major Types of Hormone Receptors

Receptor Class	Hormone Receptors
G protein-coupled receptors	ACTH and other melanocortins, V2 vasopressin, LH, FSH, TSH, GnRH, TRH, GHRH, corticotropin-releasing factor, somatostatin, glucagon, oxytocin, gastric inhibitory peptide, type 1 PTH, free fatty acid, GPR54, orexin, ghrelin, melanin-concentrating, calcitonin, glucagon-like peptide-1, and calcium-sensing receptors
Type 1 cytokine receptors	Growth hormone, prolactin, and leptin receptors
Receptor tyrosine kinases	Insulin, IGF-1, and fibroblast growth factor receptors
Nuclear receptors	Thyroid hormone, vitamin D ₃ , PPAR γ , HNF4A, glucocorticoid, androgen, estrogen, mineralocorticoid, and DAX1 receptors

ACTH, adrenocorticotropic hormone; DAX1, dosage-sensitive sex-reversal adrenal hypoplasia congenital critical region on the X chromosome gene 1; FSH, follicle-stimulating hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin-releasing hormone; GPR54, KISS-1 derived peptide receptor 54; HNF4A, hepatocyte nuclear factor-4 α ; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; PPAR γ , peroxisome proliferator-activating receptor γ ; PTH, parathyroid hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

BASIC PRINCIPLES OF RECEPTOR ACTION

A molecule that binds to a receptor is called a *ligand*. When ligand binding leads to activation of signaling processes inside cells that ligand is called an *agonist*. The response of a receptor to its ligand is generally assessed by two characteristics of the ligand: potency and efficacy. Potency describes the concentration of ligand needed to cause the biological effect by binding to the receptor. A potent agonist activates the receptors at a low concentration, typically in the nanomolar range. Efficacy describes the maximal effect induced by a ligand. Potency is generally described by using the EC₅₀, which is the concentration of ligand that induces a half-maximal effect. When a synthetic agonist exceeds the efficacy of the natural ligand it is called a *super agonist*. When receptor ligands (natural or synthetic) do not induce full activation, they are called *partial agonists*. An antagonist is a molecule that blocks the natural ligand from binding to its receptor. If it binds to the same site as the natural agonist it is called an *orthosteric antagonist*. If it binds to a different site than the natural agonist, it is called an *allosteric antagonist*. The potency of the antagonist is described by the IC₅₀, which is the concentration that causes half-maximal inhibition. A receptor that has constitutive basal activity can be bound by a ligand that inhibits the receptor's activity in the absence of the natural ligand. In this case the ligand is called an *inverse agonist*. A scale has been formulated to express the continuity in receptor ligand function—from -1 (representing a full inverse agonist), to 0 (representing a neutral antagonist), to +1 (representing a full agonist).^{1,2}

Receptors have multiple possible conformations that are constantly changing. Receptors exist in some conformations more than others, based on the free energy of each conformation. Agonists act as modulators that stabilize a given conformation of a receptor by reducing the energy needed to enter that conformation. This conformation is associated with activation of downstream signaling. Partial agonists stabilize the receptor in conformations less efficient at activation of downstream signaling. Super agonists stabilize the receptor in conformations more efficient at activation of downstream signaling. Conformational changes in a receptor can also affect the number and type of signals that a receptor generates. In the classic paradigm, a GPCR was believed to function as a binary switch that could be activated by agonist binding or inhibited by antagonist blockade of agonist binding. We now know that GPCR signaling is more complex than a simple binary switch model (i.e., "on" and "off"), and that different ligands can bind the same GPCR and selectively activate one downstream pathway versus another. This ability of different ligands to induce different conformations of a receptor that activate (or inhibit) selective downstream signals is called *biased signaling*. Ligands that bind to the native site of a receptor but produce different signaling events are termed *orthostatic ligands*. An example of this is provided by the follicle-stimulating hormone (FSH)

receptor, a classic GPCR.³ Fully glycosylated FSH is more acidic and acts as a full agonist at FSH receptors, activating Gs-coupled signaling and generation of cyclic adenosine monophosphate (AMP). Partially glycosylated FSH is more basic and acts as a partial agonist or a biased ligand as it activates both Gs and Gi pathways, which compete. Deglycosylated FSH has no effect on signaling but binds to the receptor and so acts as a competitive antagonist. These differently glycosylated variants exist in the circulation and are a means for fine-tuning the signal from the pituitary to the gonads. In certain cases, a neutral ligand can bind to the receptor at a different site than the natural ligand's binding site and by doing so, influence the conformation of the receptor such that the ligand's efficacy is increased, decreased, or biased. These are called *allosteric modulators*. They do not activate or inhibit the receptors on their own. Similarly, there are allosteric synthetic ligands for the FSH receptor that demonstrate signaling bias ranging from full agonists to reverse agonists.

Receptors are generally found associated with other proteins whether at the cell surface or in the cytoplasm. These associated proteins influence receptor conformation. An example is receptor activity modifying proteins or RAMPs. Calcitonin receptor and calcitonin-like receptor are GPCRs that can bind to several ligands (calcitonin, adrenomedullin, amylin, and calcitonin gene-related protein). RAMP1, RAMP2, and RAMP3 associate with both the calcitonin receptor and calcitonin-like receptor, and depending on which RAMP is associated determines selectivity for one of the aforementioned ligands.

Conformational fluidity of receptors and interactions with multiple ligands and modulators leads to greater complexity, specificity, and fine-tuning, as well as overall efficiency.

G PROTEIN-COUPLED RECEPTORS

More than 1% of the genome of vertebrates encodes a large protein family of receptors that sense molecules outside the cell and activate signal transduction pathways and, ultimately, cellular responses. These receptor proteins are embedded in the plasma membrane and are coupled to intracellular signal generating systems by heterotrimeric G proteins (i.e., GPCRs).⁴ GPCRs are also known as *seven-transmembrane domain receptors*, *7TM receptors*, *heptahelical receptors*, and *serpentine receptors*. They are called transmembrane receptors because they pass through the cell membrane, and they are called seven-transmembrane receptors because they have alpha helical regions that pass through the cell membrane 7 times. The human genome encodes roughly 950 GPCRs. GPCRs are involved in many diseases and are also the target of approximately 40% of all modern medicinal drugs.⁵ Approximately 150 of the GPCRs found in the human genome have unknown functions. Most GPCRs are odorant and pheromone receptors.⁴ Also important to note is that most hormones bind to GPCRs, and hence G protein-dependent signal transduction represents the most common mechanism for hormone action (Table 3.2).

TABLE 3.2 G Protein–Coupled Receptors and Clinical Conditions Associated With Receptor Mutations

Receptor	Germline Mutation	Endocrine Disorder
ACTH/melanocortin-2 receptor	Inactivating mutations (homozygous, compound heterozygous)	Familial glucocorticoid deficiency type 1
Melanocortin-4 receptor	Inactivating mutations (most heterozygous, some homozygous)	Obesity
V2 vasopressin receptor	Inactivating mutations (most X-linked recessive, rarely X-linked dominant)	X-linked nephrogenic diabetes insipidus
LH receptor	Inactivating mutations (homozygous, compound heterozygous) Activating mutations (heterozygous)	Males: types I and II Leydig cell hypoplasia Females: asymptomatic or hypergonadotropic hypogonadism with primary amenorrhea Males: male limited precocious puberty
FSH receptor	Inactivating mutations (homozygous, compound heterozygous)	Females: autosomal recessive hypergonadotropic ovarian dysgenesis or milder hypergonadotropic hypogonadism Males: variable impairment of spermatogenesis
TSH receptor	Inactivating mutations (most homozygous or compound heterozygous, rarely heterozygous) Activating mutations (heterozygous)	Resistance to TSH Autosomal-dominant inherited nonautoimmune hyperthyroidism/toxic adenomas
GnRH receptor	Inactivating mutations (homozygous or compound heterozygous)	Isolated hypogonadotropic hypogonadism
TRH receptor	Inactivating mutations (compound heterozygous)	Central hypothyroidism
GPR54	Inactivating mutations (homozygous, compound heterozygous)	Normosmic isolated hypogonadotropic hypogonadism
Ghrelin	Inactivating mutations (homozygous, possible heterozygous)	Short stature because of decreased growth hormone secretion
GHRH receptor	Inactivating mutations (homozygous/compound heterozygous)	Isolated growth hormone deficiency
Type 1 PTH receptor	Inactivating mutations (homozygous, heterozygous) Activating mutations (heterozygous)	Blomstrand chondrodysplasia if homozygous and rarely if heterozygous; enchondromatosis if heterozygous Jansen metaphyseal chondrodysplasia
Calcium-sensing receptor	Inactivating mutations (heterozygous, homozygous) Activating mutations (heterozygous)	Familial benign hypocalciuric hypercalcemia typical if heterozygous, neonatal severe hyperparathyroidism rarely if heterozygous, typical if homozygous Autosomal-dominant hypocalcemic hypocalciuria, Bartter syndrome type V

ACTH, Adrenocorticotropic hormone; *FSH*, follicle-stimulating hormone; *GHRH*, growth hormone–releasing hormone; *GnRH*, gonadotropin-releasing hormone; *GPR54*, KISS-1 derived peptide receptor 54; *LH*, luteinizing hormone; *PTH*, parathyroid hormone; *TRH*, thyrotropin-releasing hormone; *TSH*, thyroid-stimulating hormone.

The GPCR superfamily is divided into eight major classes.^{4,6} These receptors contain an amino-terminal extracellular domain that is frequently called the *ectodomain* or *exodomain*.⁷ These receptors also contain seven putative transmembrane spanning alpha helices (TM-I to TM-VII). The alpha helices are connected by three intracellular (i1–i3) and three extracellular (e1–e3) loops that are often collectively called the *serpentine region* (Fig. 3.1).^{7,8} The carboxy-terminal intracellular region is usually referred to as the *endodomain*.⁷

GPCRs are activated by a wide variety of signals, including proteins, nucleotides, amino acid residues, Ca²⁺, light photons, and odorants (see Fig. 3.1).⁴ It is postulated that ligand binding alters the conformation of transmembrane domains and intracellular loops, increasing the affinity of the receptor for specific heterotrimeric guanosine nucleotide binding proteins (G proteins) (see Fig. 3.1).^{9,10} G proteins share a common heterotrimeric structure consisting of an α subunit and a tightly coupled $\beta\gamma$ dimer.^{11,12} The α subunit interacts with detector and effector molecules, binds guanosine 5'-triphosphate (GTP), and possesses intrinsic GTPase activity. There are 16 genes in mammals that encode some 20 different α chains. The $G\alpha$ subunits are categorized in four classes and include $G\alpha_s$ (G stimulatory), $G\alpha_i$ (G inhibitory) and $G\alpha_o$ (G other), $Gq/11\alpha$, and $G12/13\alpha$. They behave differently in the recognition of the effector but share similar structures and mechanism of activation. The $G\alpha$ subunits consist of two domains: a GTP-binding domain and a helical insertion domain. The GTP-binding domain is homologous to Ras-like small GTPases and includes switch regions I and II, which change conformation during activation. The switch regions are loops of alpha helices with conformations

sensitive to guanine nucleotides. The helical insertion domain is inserted into the GTP-binding domain before switch region I and is unique to heterotrimeric G proteins. This helical insertion domain sequesters the guanine nucleotide at the interface with the GTP-binding domain and must be displaced to enable nucleotide dissociation.

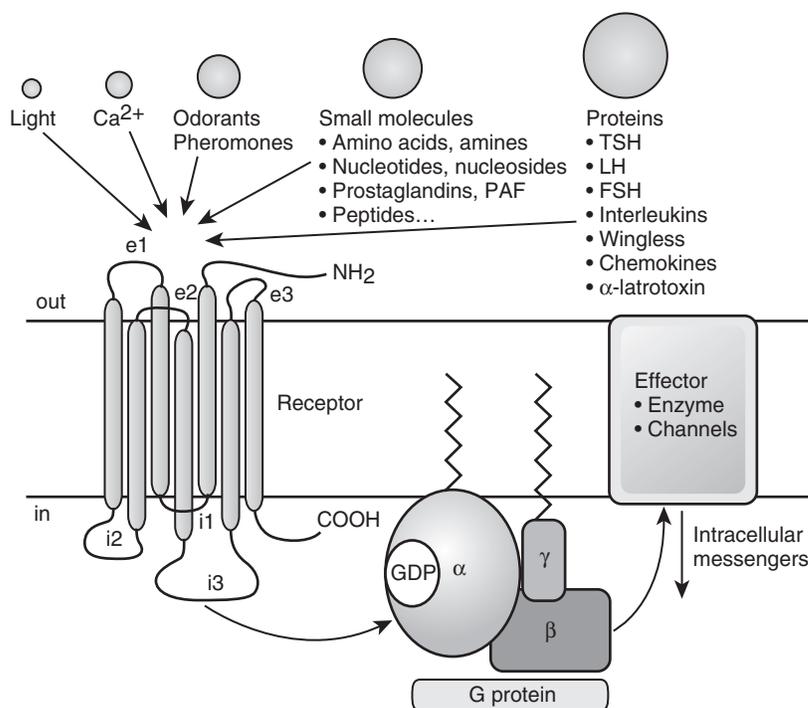
The α subunits associate with a smaller group of $\beta(5)$ and $\gamma(12)$ subunits.^{11,13} Combinatorial specificity in the associations between various G protein subunits provides the potential for enormous diversity and may allow distinct heterotrimers to interact selectively with only a limited number of GPCRs and effector proteins.^{9,10,14}

There are two principal signal transduction pathways involving the GPCRs: the cyclic AMP signal pathway and the phosphatidylinositol signal pathway. G protein–induced signal generation is regulated by a “molecular timer” that is determined by the rate of GTP exchange and hydrolysis. In the inactive state, G proteins exist in the heterotrimeric form with guanosine 5'-diphosphate (GDP) bound to the α chain. Interaction of a ligand-bound receptor with a G protein leads to release of GDP, with subsequent binding of GTP to the α chain. The binding of GTP to the α chain leads to dissociation of the α chain from the $\beta\gamma$ dimer, allowing the now free α -GTP chain to interact with target enzymes and ion channels. The $\beta\gamma$ dimers also participate in downstream signaling events through interaction with an everwidening array of targets, including certain forms of adenylyl cyclase and phospholipase C, potassium channels, and GPCR kinases.

G protein signaling is terminated by the hydrolysis of α -GTP to α -GDP by an intrinsic GTPase. A group of proteins, called

Fig. 3.1 G protein–coupled receptor (GPCR) structure and function.

GPCRs have an amino-terminal extracellular domain, seven putative transmembrane domains separated by three extracellular loops (e1–e3) and three intracellular loops (i1–i3), and a carboxy-terminal intracellular domain. Ligand binding results in the exchange of guanosine 5'-triphosphate (GTP) for guanosine 5'-diphosphate (GDP), which induces dissociation of the G protein into a GTP α subunit and a $\beta\gamma$ subunit. Then these subunits alter the activity of intracellular effector enzymes and transmembrane channels, resulting in the alteration of intracellular levels of second messengers that can include cyclic adenosine monophosphate and calcium. (Modified from Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G-protein–coupled receptors: an evolutionary success. *Embo J*, 18, 1724. With permission.)



regulators of G protein signaling (RGs), acts as GTPase-activating proteins (GAPs), specific for $G\alpha$ subunits. These proteins accelerate hydrolysis of GTP to GDP and terminate the transduced signal. In some cases, the effector itself may possess intrinsic GAP activity, which helps deactivate the pathway. This is true in the case of phospholipase C β , which possesses GAP activity within its carboxy-terminal region. This is an alternate form of regulation for the $G\alpha$ subunit. However, it should be noted that the GAPs do not have catalytic residues to activate the $G\alpha$ protein. Rather, GAPs reduce the required activation energy for the reaction to take place. After hydrolysis of GTP, the $G\alpha$ -GDP chain reassociates with the $\beta\gamma$ dimer; the reassociated heterotrimeric G protein is now capable of participating in another cycle of receptor-activated signaling.^{4,9,10,14}

Specificity in ligand binding is conferred by variations in the primary structures of the extracellular and intracellular domains.⁴ Specificity of effector responses is conferred by the variations in the primary structure of intracellular domains and isoforms of the $G\alpha$ subunits of G proteins.^{15,16} Some GPCRs couple predominantly with $G\alpha_i/G\alpha_o$ subunits that act primarily to decrease adenylyl cyclase activity.^{16–19} Other GPCRs couple predominantly with $G\alpha_s$ subunits that increase adenylyl cyclase activity or $G\alpha_q/G\alpha_{12}$ subunits that increase phospholipase C activity.^{16,20,23}

Interestingly, data show that cytoskeletal proteins may modulate receptor–G protein coupling. For example, the erythrocyte membrane cytoskeletal protein 4.1G can interfere with A1 adenosine receptor signal transduction.²¹ 4.1G also influences metabotropic glutamate receptor 1 α -mediated cyclic AMP accumulation, increases the ligand-binding ability of metabotropic glutamate receptor 1 α , and alters its cellular distribution.²² 4.1G may also play a role in receptor–receptor dimerization.

Receptor agonist-independent and agonist-induced homo- and heterodimerization have increasingly been recognized as important determinants of GPCR function.²⁴ For example, the GPCR somatostatin receptor 5 (SSTR5) primarily exist as monomers in the absence of an agonist. However, they form homodimers in the presence of an agonist.²⁵ Furthermore, it has been

shown that SSTR5 can form heterodimers with type 2 dopamine receptors (DRD2)—another GPCR—in the presence of hst2 agonist or dopamine.²⁶ Agonist-induced activation of SSTR5-DRD2 heterodimers in Chinese hamster ovary (CHO) cells expressing SSTR5 and DRD2 is increased, when compared with agonist-induced activation of monomers and homodimers in CHO cells expressing only SSTR5 or DRD2.²⁶ Heterodimerization of receptors may also lead to inactivation of one of the receptors in the complex. For example, heterodimerization of somatostatin receptor 2A (sst2A) with somatostatin receptor 3 (SSTR3) appears to lead to inactivation of the heterodimerized SSTR3, without inactivating the heterodimerized SSTR2.²⁷

GPCRs can form heterodimers with nonreceptor transmembrane proteins. Both the calcitonin receptor (CALCR) and the calcitonin receptor-like protein (CALCRL) can form heterodimers with three different accessory proteins that are termed “RAMPs”: RAMP1, RAMP2, and RAMP3.^{28–30} Whereas CALCRs can be activated by ligand in the absence of heterodimerization with a RAMP, CALCRLs are only activated by ligand if heterodimerized with a RAMP.^{28,29} RAMPs alter the ligand specificity of the heterodimerized receptor.

CALCRs that are not in heterodimers with RAMPs are activated by calcitonin and thus constitute the classic CALCR.^{28,29} However, CALCRs heterodimerized with RAMP1, RAMP2, and RAMP3 bind amylin and constitute amylin1, amylin2, and amylin3 receptors, respectively.^{28,29} CALCRLs dimerized with RAMP1 bind calcitonin gene-related peptide and constitute the calcitonin gene-related peptide receptor.^{28,29} CALCRLs dimerized with RAMP2 and RAMP3 bind adrenomedullin and constitute adrenomedullin1 and adrenomedullin2 receptors, respectively.^{28,29} RAMPs alter function of other GPCRs that transduce hormone action. The distribution and function of parathyroid hormone 1 and 2 receptors are altered by binding to RAMP2 and RAMP3, respectively.³¹ The distribution and function of the glucagon receptor is altered by binding to RAMP2.³¹ Dimerization/heterodimerization may occur in the endoplasmic reticulum (ER), shortly after protein synthesis occurs.³² The ER plays a role in determining whether or not a

protein will be expressed elsewhere in the cell, thus protecting the cell from misfolded and (likely) mutant proteins.³² The nonheterodimerized CALCRL is an orphan receptor because the CALCRLs cannot leave the ER for the cell membrane, unless heterodimerized with RAMPs.³³

The melanocortin receptors also use accessory proteins. Circulating adrenocorticotropin hormone (ACTH) binds to five different forms of the melanocortin receptor (types 1–5), but only the melanocortin 2 receptor (MC2R) in the adrenal cortex leads to release of adrenal steroids. MC2R interacts with Gs, which leads to activation of adenylyl cyclase and formation of cyclic AMP. The MC2R is the smallest GPCR known to date and belongs to a family of melanocortin receptors (types 1–5) that bind to various derivatives of proopiomelanocortin, especially α -melanocyte-stimulating hormone (α -MSH). The accessory protein melanocortin 2 receptor-associated protein (MRAP) is required for MC2R function, as it is critical for the translocation of the receptor from the ER to the cell surface.³⁴ Moreover, MRAP facilitates signaling of the MC2R.³⁵ Loss of function of MRAP thus prevents membrane expression of MC2R and completely prevents ACTH signaling. MRAP-deficient mice die at birth, unless rescued with glucocorticoids, but have normal mineralocorticoid and catecholamine production.³⁶ The adrenal glands of MRAP-deficient adult mice are small with abnormal adrenal morphology, abnormal cortex zonation, and abnormal adrenal progenitor cell differentiation. Interestingly, MRAP forms a unique antiparallel homodimer in close proximity to the MC2R.³⁷ The MRAP accessory protein can also interact with other melanocortin receptors, particularly MC5R, but exerts negative effects on their signaling. Expression of MRAP was shown to be predominantly present in the zona fasciculata in the rat adrenal gland, consistent with its facilitating role in glucocorticoid production. Hence mutations in MC2R³⁸ or MRAP³⁴ can lead to familial glucocorticoid deficiency secondary to ACTH resistance. In contrast, MRAP2, a protein with 39% amino acid homology to MRAP, shares the MC2R-trafficking function of MRAP but does not appear to play a major supportive role in adrenocortical ACTH signaling. On the contrary, *in vitro* studies have shown that overexpression of MRAP2 can suppress MC2R activation. MRAP2 appears to play a role in energy homeostasis as MRAP2 KO mice develop obesity.³⁹ MRAP2 interacts with MC4R in the paraventricular nucleus of the hypothalamus (PVN) where PVN-specific MRAP2 KO duplicates the global MRAP2 KO phenotype.

Failure of the ER to export mutant GPCR homodimers and mutant GPCR wild-type GPCR heterodimers to the cell membrane has been found to be the cause of dominant negative endocrine conditions. A dominant negative mutation is a heterozygous mutation that results in a phenotype that would be expected by a loss of function in both alleles. Some heterozygous MC4R mutations cause dominantly inherited obesity because of interaction of wild-type MC4R with the mutant receptor, and this specific effect of protein-protein interaction results in a dominant-negative effect.^{40,41} In addition, some heterozygous mutations in the gene encoding the V2 vasopressin receptor cause nephrogenic diabetes insipidus via production of mutant proteins that interfere with transit of normal receptors to the cell's membrane.⁴² These mutant receptors interfere with cell-surface expression of wild-type receptors by forming heterodimers with the wild-type receptors that cannot be exported from the ER to the cell membrane.⁴³ This finding explains why females heterozygous for these V2 vasopressin receptor gene mutations do not concentrate their urine with even high doses of desmopressin, a synthetic V2 vasopressin receptor agonist, in spite of being able to produce wild-type V2 vasopressin receptors.⁴⁴ A similar phenomenon explains dominant transmission of partial thyroid-stimulating hormone (TSH) receptor resistance in patients heterozygous for

some inactivating TSH receptor mutations.⁴⁵ In these patients, mutant TSH receptors form oligomers with wild-type receptors and prevent export of wild-type receptors from the ER to the cell membrane.⁴⁵

Similarly, misfolding and misrouting of some mutant gonadotropin-releasing hormone (GnRH) receptors in the ER (as well as oligomerization of these mutant GnRH receptors with wild-type GnRH receptors) decrease cell membrane expression of wild-type GnRH receptors.^{46–48} This phenomenon, however, has not been found to have clinical implications in patients who are heterozygous for mutations that cause autosomal recessive isolated hypogonadotropic hypogonadism (IHH), as the heterozygous individuals demonstrate an intact GnRH-gonadotropin axis and do not have clinical signs of IHH. Thus in these individuals, enough wild-type GnRH receptors do not oligomerize with mutant GnRH receptors and are transported to the cell membrane to maintain sufficiently normal GnRH-GnRH receptor interactions to avoid development of IHH.⁴⁷

Most GPCRs activate G proteins at very low levels in the absence of ligand binding. Some GPCRs have much higher constitutive (i.e., ligand-independent) activity, such as luteinizing hormone, TSH, thyrotropin-releasing hormone (TRH), glucagon-like peptide-1, melanocortin, and cannabinoid receptors. These receptors can activate G proteins in the absence of ligand binding,^{1,49} demonstrating constitutive activity that increases linearly with increased cell-surface expression of the receptors.⁵⁰ As described earlier, inverse agonists decrease the activity of these receptors.² In receptors without constitutive activity, genetic mutations that lead to substitution of a single amino acid can also greatly increase the interaction rate of the unliganded receptor for its G protein. It is possible that inverse agonists may play a role in treating medical conditions caused by GPCR mutations that lead to increased constitutional activation of the receptor.¹

Receptor desensitization and resensitization play a role in GPCR activity. Three processes for receptor desensitization have been described.^{51,52} The first receptor desensitization process is rapid uncoupling of the G protein from GPCRs.⁵² This process occurs within seconds to minutes after initiation of the process and occurs as a result of phosphorylation of GPCRs.⁵² G protein receptor kinases (GRKs) have been increasingly recognized as playing a major role when this process involves homologous desensitization.⁵¹ GRK-mediated phosphorylation of serine and threonine residues in the third intracellular loop, or the carboxy-terminal intracellular domain leads to activation of β -arrestins, which in turn inactivate adenylyl cyclase (Fig. 3.2).^{51–54} Second-messenger-dependent protein kinases also contribute to receptor desensitization when this process involves homologous desensitization, but they also participate in receptor desensitization when desensitization involves heterologous desensitization. Heterologous or agonist-independent desensitization occurs as a result of activation of a different receptor from the one that is desensitized.⁵²

The second receptor desensitization process is internalization/sequestration of GPCRs. This process is slower than receptor phosphorylation-induced uncoupling of the G protein from GPCRs and occurs within minutes to hours after initiation of the process. In addition to phosphorylation, both the GPCR and β -arrestins are modified posttranslationally in several ways, including by ubiquitination. This process is reversible because the receptors can be recycled to the cell surface (see Fig. 3.2).⁵² GRKs and β -arrestins play a role in initiating internalization/sequestration of β 2-adrenergic, luteinizing hormone (LH), FSH, TSH, TRH, vasopressin V2, angiotensin II type 1A, and other GPCRs in clathrin-coated vesicles (see Fig. 3.2).^{51,55–62} Dephosphorylation of the sequestered receptor, followed by disassociation of the receptor from β -arrestin, is necessary for

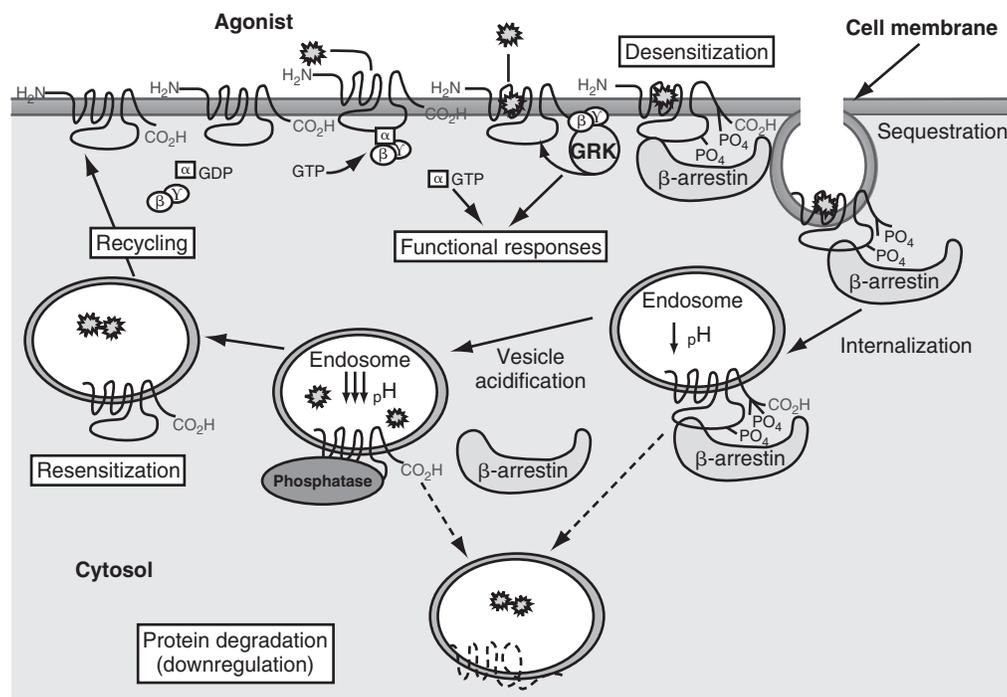


Fig. 3.2 Desensitization and recycling of G protein-coupled receptors (GPCRs). Shortly after an agonist binds a GPCR, G protein receptor kinases phosphorylation of serine and threonine residues in the third intracellular loop or the carboxy-terminal intracellular domain leads to activation of β -arrestin. Activation of β -arrestin inactivates adenylyl cyclase and initiates sequestration of the GPCR in clathrin-coated vesicles. Dephosphorylation of the sequestered receptor and subsequent disassociation of the receptor from β -arrestin is followed by recycling of the GPCR to the cell membrane. Alternatively, once sequestered, the GPCR can be destroyed in lysosomes. (Modified from Saunders, C., & Limbird, L. E. (1999). Localization and trafficking of α_2 -adrenergic subtypes in cells and tissues. *Pharmacol Ther*, 84, 200. With permission.)

the receptor to be recycled to the cell membrane and resensitized (see Fig. 3.2).²⁰

The third receptor desensitization process is downregulation. With downregulation, the number of intracellular GPCRs decreases because of increased lysosomal degradation and decreased synthesis of the receptors caused by alteration of transcriptional and posttranscriptional regulatory mechanisms (see Fig. 3.2).^{63,64} Downregulation is a slow process that occurs within several hours to days after initiation of the processes that lead to its development.⁶⁵ For many GPCRs, receptor ubiquitination promotes degradation of agonist-activated receptors in the lysosomes. Other proteins also play important roles in desensitization, including phosphodiesterases, RGS family proteins, and A-kinase-anchoring proteins. Together, this intricate network of kinases, ubiquitin ligases, and adaptor proteins orchestrate the acute and prolonged desensitization of GPCRs.

One of the ways the Arg137His V2 vasopressin receptor mutation interferes with mutant receptor function and causes X-linked nephrogenic diabetes insipidus is by altering desensitization and recycling of the mutant receptor.⁶⁶ In vitro studies have revealed that the mutant receptor is constitutively phosphorylated. Thus even in the absence of ligand binding, the mutant receptor is bound by β -arrestin—which in turn leads to sequestration of the mutant receptor within clathrin-coated vesicles. Recycling of the mutant receptor back to the cell membrane requires the mutant receptor to be dephosphorylated and disassociated from β -arrestin. However, the mutant receptor does not undergo dephosphorylation, while sequestered, and thus cannot be disassociated from β -arrestin and recycled to the cell membrane—thereby reducing cell membrane expression of the mutant receptor.

Of the eight classes of GPCRs, only classes A, B, and C contain receptors for mammalian hormones and

neurotransmitters (Fig. 3.3).⁶ Class A receptors contain the rhodopsin-like receptors and are divided into at least 15 groups.^{6,67} Four of these groups contain receptors activated by hormones. These are the peptide receptor, hormone protein receptor, GnRH receptor, and the TRH and secretagogue receptor groups.⁶

The peptide receptor group includes the angiotensin, ACTH/melanocortin, oxytocin, somatostatin, and vasopressin receptors.⁶ The hormone protein receptor group includes the receptors for glycoprotein hormones, including FSH, LH, and thyrotropin (TSH) receptors.⁶ These receptors have large extracellular amino-terminal domains and ligand-binding sites that include the first and third extracellular loops (see Fig. 3.3).^{4,6} There is also much similarity in amino acid sequence among these receptors (see Fig. 3.3).⁴ The GnRH receptor group only contains the GnRH receptor.⁵ The TRH and secretagogue receptor group includes the TRH receptor and the growth hormone (GH) secretagogue receptor.⁶

Class B GPCRs are structurally similar to members of the hormone protein receptor group (see Fig. 3.3).⁴ However, unlike the glycoprotein hormone receptors, class B GPCRs do not share similar amino acid sequences.⁴ This family contains receptors for higher molecular weight hormones, including calcitonin, glucagon, gastric inhibitory peptide, parathyroid hormone (PTH), and corticotrophin-releasing factor (CRF).^{4,6,68}

Class C receptors have a large extracellular domain, with two lobes separated by a hinge region that closes on the ligand (see Fig. 3.3).⁶⁹ This region has also been called the *Venus flytrap* domain or module because of the trapping mechanism of the hinge region.⁷⁰ This family includes the calcium-sensing receptor (CASR).⁷¹

Most GPCR-inactivating mutations can be classified into one of five classes.⁷² Class I inactivating mutations interfere with receptor biosynthesis. Class II inactivating mutations interfere

CLASS A

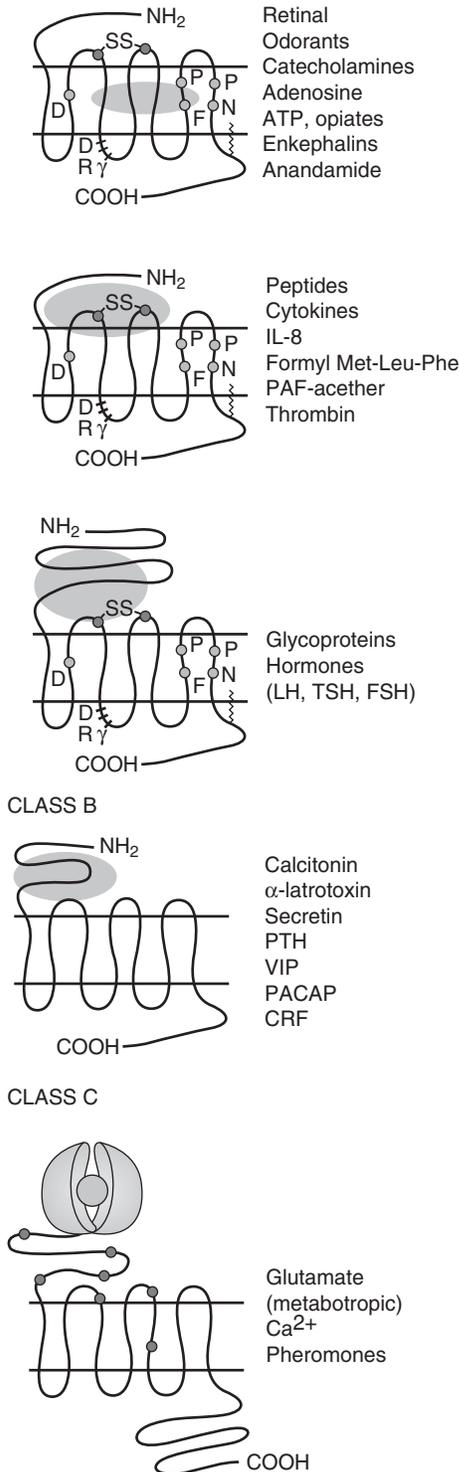


Fig. 3.3 Examples of class A, B, and C G protein-coupled receptors. The oval represents the ligand. These receptors can differ in amino acid sequence, in length of the amino-terminal extracellular and carboxy-terminal cytoplasmic domains, and in the receptor regions involved with ligand-receptor interactions. (Modified from Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *Embo J*, 18, 1275. With permission.)

with receptor trafficking to the cell surface. Class III inactivating mutations interfere with ligand binding. Class IV inactivating mutations impede receptor activation. Class V inactivating mutations do not cause discernible defects in receptor biosynthesis,

trafficking, ligand binding, or activation, but may cause medical disorders. There are also inactivating mutations that interfere with receptor function via multiple mechanisms and thus cannot be placed into one class.

CLASS A RECEPTORS THAT TRANSDUCE HORMONE ACTION

The Peptide Receptor Group

Adrenocorticotropin and Melanocortin-2 Receptors

An alternative name for the ACTH receptor is *melanocortin-2 receptor* (MC2R) because the ACTH receptor is one of five members of the melanocortin receptor family of GPCRs, all of which couple to G_s to activate generation of intracellular cyclic AMP.⁷² For the purpose of clarity, when discussing interactions between ACTH and its receptor, the older name will be used for the remainder of this chapter. The ACTH receptor gene is located on small arm of chromosome 18 (18p11.2).⁷³ The ACTH receptor has a small extracellular and intracytoplasmic domain. Adrenocorticotropin-induced activation of the ACTH receptor in the zona fasciculata and zona reticularis of the adrenal cortex stimulates G_s , resulting in increased intracellular cyclic AMP levels that stimulate steroidogenesis by activating cyclic AMP-dependent kinases.^{74–76}

Hereditary isolated glucocorticoid deficiency, resistance to ACTH, and familial glucocorticoid deficiency (FGD) are the same names for an autosomal recessive syndrome that consists of glucocorticoid deficiency accompanied by normal mineralocorticoid secretion. FGD has been classified further as FGD types 1 and 2 and the triple A syndrome.³⁸ Patients with FGD type 1 have biallelic MC2R mutations, resulting in ACTH receptors with abnormal function, and account for 25% of FGD cases.^{38,77–82} In contrast, patients with FGD type 2 have ACTH resistance caused by mutations in MRAP.⁸³ Triple A (Allgrove syndrome) is an autosomal recessive syndrome characterized by ACTH-resistant adrenal insufficiency, achalasia, and alacrima—which is caused by mutations in the achalasia-adrenocortical insufficiency-alacrima syndrome (AAAS) gene encoding the protein ALADIN.⁸⁴ ALADIN is thought to regulate nuclear pore complexes, and nucleocytoplasmic transport⁸⁵ plays a role in modulating the oxidative stress response in adrenal cells.⁸⁶

The spectrum of disease in patients with FGD type 1 varies from presentation as a neonate with hypoglycemia, seizures, or circulatory collapse to presentation in childhood with fatigue and increased susceptibility to infection.^{79,87–91} Less commonly, patients may present with childhood asthma that resolves with treatment with physiologic doses of glucocorticoids.^{38,79,87} Hyperpigmentation thought to be caused by increased ACTH levels acting on the MC1R may be seen as early as the first month of life, but usually becomes apparent after the fourth month of life.^{77–79,87–89,92} There is one reported case of FGD type 1 without hyperpigmentation, despite elevated ACTH levels, in a patient with homozygous mutations in both the MC2R and the MC1R.⁹³ The assumption that hyperpigmentation is caused by increased ACTH levels acting on the MC1R (both in FGD type 1 and Addison disease) was substantiated in this patient whose MC1R mutation had previously been implicated in red hair and pale skin phenotypes. Neonates with FGD type 1 may also suffer from jaundice.^{79,89,91,94} Tall stature accompanied by an advanced or dissociated bone age, in spite of normal age of onset of puberty, appears to be common in children with FGD type 1.^{38,79,82,87,88,92} The pathophysiology of tall stature has not been definitively elucidated. One theory is that the anabolic effects of GH are unopposed by cortisol.

Patients with FGD1 exhibit absent adrenarche, confirming the importance of ACTH in the induction and maintenance

of adrenarache.⁸³ At presentation, plasma cortisol, androstenedione, and dihydroepiandrosterone levels are low or low normal—and plasma ACTH levels are elevated.^{38,79,87–91} When supine, patients with FGD type 1 have renin and aldosterone levels that are near normal.^{38,88,90,91} Histologically, the zona fasciculata and zona reticularis are atrophied with FGD.⁸⁷ However, demonstrating the lack of an essential role for ACTH in the embryologic development and maintenance of the zona glomerulosa, adrenal cortices in patients with all types of FGD contain zona glomerulosa cells.^{38,87,88,92,95,96}

FGD type 2 is caused by mutations in MRAP1 as described earlier and accounts for 20% of all FGD cases. Patients with FGD type 2 present with severe symptoms at an earlier age than patients with FGD type 1 (median age 0.08 years vs. 2 years).⁸³ Patients can present with severe neurologic disability, seizures, and microcephaly thought to be caused by unrecognized hypoglycemia.⁹⁷ Patients with FGD type 2 have normal heights.^{38,98} This is thought to be caused by early treatment with glucocorticoids. Mutations are splice site or nonsense mutations predicted to produce proteins without the transmembrane domain, which is necessary for interaction with MC2R.

Abnormalities in ACTH receptor expression may be seen in other conditions. Evidence suggests that the ACTH receptor- α_s -adenylyl cyclase-cyclic AMP cascade maintains differentiation of adrenocortical cells and that impairment of this cascade leads to dedifferentiation and increased proliferation of adrenocortical cells.^{99,100} Adrenocortical carcinomas from some patients have been found to have a loss of heterozygosity (LOH) for the ACTH receptor gene, resulting in markedly decreased ACTH receptor messenger ribonucleic acid (mRNA) expression.¹⁰⁰ Growth of the tumors with LOH for the ACTH receptor gene also may be more aggressive than the other tumors. An activating mutation of G₁₂ that constitutively suppresses adenylyl cyclase activity has also been found in adrenocortical tumors.⁹⁹ Thus decreased ACTH receptor activity may be associated with tumorigenesis.

Interestingly, many patients with ACTH-independent macronodular adrenal hyperplasia (AIMAH)—a cause of ACTH-independent Cushing syndrome caused by inactivating mutations of the putative tumor-suppressor gene *ARMC5*¹⁰¹—exhibit increased glucocorticoid levels in response to noncorticotropin hormones that do not normally induce glucocorticoid release.^{102–107} These hormones include gastric inhibitory peptide, exogenous arginine and lysine vasopressin, LH, human chorionic gonadotropin (HCG), angiotensin II, catecholamines, leptin, and serotonin receptor agonists.^{102–107} Increased expression of the receptors for these ligands in the abnormal adrenal glands has been implicated as a possible explanation for the abnormal induction of glucocorticoid release by these noncorticotropin ligands.¹⁰⁷ However, receptors for some of these ligands are expressed in normal adrenal glands.¹⁰⁷ Thus the mechanism for this phenomenon remains to be fully elucidated.

Other Melanocortin Receptors

Murine studies reveal that melanocortin-3 receptor (MC3R), another of the five members of the melanocortin receptor family, regulates fat deposition, as mice with MC3R deficiency or partially inactive receptors have normal resting energy expenditure and increased fat mass and reduced fat-free mass, including decreased bone formation.^{72,108} The role of the MC3R in humans is less clear. More than 24 human MC3R variants have been identified without evidence of obesity.^{109,110} However, patients with these variants were not phenotyped for fat mass to assess whether they phenocopy the mouse model, which exhibits normal body weight but greater energy intake and

altered energy partitioning that is biased toward lipid-accumulating cells' altered partitioning.^{110–112} Several coding variants (p.D158Y, p.T280S, p.I183N), which are likely pathologic because of significantly decreased cyclic AMP production, as well as noncoding variants, demonstrate early-onset obesity in all affected individuals.⁹⁷ Homozygosity for a pair of single-nucleotide polymorphisms of the MC3R gene (p.T6K+ p.V81I) that result in production of partially inactive MC3Rs was found to be associated with pediatric-onset obesity in Caucasian American and African American children.¹¹³ Subjects homozygous for the double mutation had higher body mass index (BMI)-z, fat mass and percent fat mass, as well as waist circumference. That is despite finding no differences in energy intake, resting energy expenditure, total energy expenditure, respiratory quotient, physical activity.^{114–117} A mouse model carrying the human MC3R T6K+V81I variants showed that the double mutant exhibited greater fat mass and feeding efficiency with reduced fat-free mass.¹⁰⁸ These findings are similar to the MC3R knockout mouse.

The melanocortin-4 receptor (MC4R) is another member of the melanocortin receptor family and plays a role in controlling appetite and weight.¹¹⁸ The MC4R has baseline constitutive (i.e., ligand-independent) activity that can be inhibited by the inverse agonist agouti-related peptide (AgRP).^{118,119} Activation of the MC4R by its natural agonist α -MSH produces anorexigenic effects.^{118,120,121} More than 150 naturally occurring MC4R mutations have been identified, causing hyperphagic obesity, increased lean body mass, increased bone density, and increased linear growth.^{122–127} Patients with homozygous mutations appear to have more severe obesity than their heterozygous relatives, consistent with codominant inheritance.^{10,128}

MC4R mutations are thought to be the most common monogenic cause of human obesity. The prevalence of pathogenic MC4R mutations in obese populations varies widely, ranging from 0.5% to 5.8%, depending on the screening criteria and population.^{124,129–131} AgRP gene polymorphisms appear to be associated with anorexia nervosa.^{125,126}

Little is known about melanocortin-5 receptors (MC5Rs) in animals and humans. There is only weak evidence from a single linkage and association study of families in Quebec that suggests that MC5Rs may also play a role in regulating body weight and fat mass.¹²⁷

Another member of the melanocortin receptor family, the MC1R, controls skin and hair pigmentation.^{132,133} Activation of MC1Rs in skin and hair follicle melanocytes by the proopiomelanocortin (POMC)-derived peptides α -MSH and ACTH stimulates the synthesis of eumelanin, a brown-black pigment.^{134,135} Inhibition of MC1R baseline constitutive activity by agouti protein, or specific mutations, leads to release of pheomelanin, a red-yellow pigment, from the melanocytes.¹³⁵

Inactivating homozygous mutations of the POMC gene cause hypoadrenalism, red hair, fair skin, and early-onset obesity. Hypoadrenalism is characterized by glucocorticoid deficiency because of lack of ACTH production from the POMC precursor. Fair skin and red hair are caused by a lack of ACTH and α -MSH-induced melanocyte release of eumelanin that results from activation of MC1Rs. Of note, nonwhite patients with homozygous POMC mutations do not appear to have the fair skin and red hair phenotype.^{130,136} In white individuals, eumelanin synthesis appears to be dependent on POMC-derived peptides, whereas in darker individuals, other genes may control eumelanin synthesis.¹³⁷ Obesity is caused by lack of α -MSH-induced anorectic effects, which normally result when α -MSH activates MC4Rs.¹³⁸ Heterozygosity for POMC gene mutations has been associated with hyperphagia, early-onset obesity, and increased linear growth.^{138–140} Both homozygous^{141,142} and heterozygous¹⁴³ mutations of

prohormone convertase 1 cause obesity in humans. Prohormone convertase 1 acts on POMC, proinsulin, and proglucagon. Patients with prohormone convertase 1 deficiency also have neonatal enteropathy and postprandial hypoglycemia. The cause of enteropathy is unknown but hypothesized to be related to the processing of GLP-2 by prohormone convertase 1. GLP-2 is known to stimulate proliferation and repair of intestinal epithelium.¹⁴⁴

Vasopressin Receptors

Nephrogenic diabetes insipidus (NDI) results from decreased responsiveness of the renal tubule to arginine vasopressin (AVP), with resulting excessive loss of free water. NDI is characterized by polydipsia and polyuria that is not responsive to vasopressin and vasopressin analogs.¹⁴⁵ Vasopressin binds to the V2 vasopressin receptor (AVPR2), a G_s-coupled receptor, in the basolateral membrane of collecting duct principal cells in the kidney and activates translocation of aquaporin-2 (AQP2) water channels to the apical membrane, thereby inducing water permeability. X-linked NDI is caused by inactivating mutations of the V2 vasopressin receptor (AVPR2) gene located at Xq28 and accounts for about 90% of genetically determined NDI.^{146–149} More than 200 AVPR2 mutations have been described, including missense, nonsense, insertions, deletions, and complex rearrangements.¹⁵⁰ Mutations have been categorized into five classes based on mechanism, including abnormal transcription, mRNA processing, translation, aberrant folding and intracellular retention, loss of the G protein binding site, loss of the AVP binding site, and defects in intracellular trafficking.^{145,151,152} Some patients with X-linked NDI are responsive to high doses of desmopressin. Autosomal recessive NDI (ARNDI) is caused by loss-of-function mutations in the gene for the AQP2 water channel and accounts for about 10% of genetic forms of NDI.^{151,153} More than 50 known mutations cause ARNDI. Autosomal dominant forms of NDI are also caused by mutations in AQP2 that are functional, but fail to be transported to the apical membrane. Eleven mutations have been described accounting for less than 1% of genetic forms of NDI and generally have a milder phenotype than ARNDI or X-linked NDI.

Gain-of-function mutations in the V2 vasopressin receptor have also been reported.¹⁵⁴ Deoxyribonucleic acid (DNA) sequencing of two patients' V2R gene identified heterozygous missense mutations in both, with resultant changes in codon 137 from arginine to cysteine (p.R137C) or leucine (p.R137L). These mutations resulted in constitutive activation of the receptor and clinical features of inappropriate antidiuretic hormone secretion (SIADH), which was termed *nephrogenic syndrome of inappropriate antidiuresis (NSIAD)*.¹⁵⁴ To date, around 30 cases of NSIAD have been reported, most of which are described in males as the condition is X-linked. Patients can present in infancy but sometimes do not present until adulthood. There have been several reports of female patients with NSIAD, some diagnosed in infancy and others in adulthood.¹⁵⁵ Patients with the p.R137L mutation demonstrated the expected decrease in AVP levels with a water-loading test, but urine AQP2 levels remained inappropriately elevated.¹⁵⁶

The Glycoprotein Hormone Receptor Group

The glycoprotein hormones include TSH, FSH, LH, and HCG. These hormones share common α subunits that dimerize with hormone-specific β subunits. TSH, FSH, and LH bind to the extracellular amino-terminal domain of the TSH, FSH, and LH receptors, respectively.^{4,6,157,158} The effects of HCG are mediated by the LH receptor, which is also known as the *luteinizing hormone/choriogonadotropin receptor (LHCGR)*.¹⁵⁹

Glycoprotein hormone receptors have a large (350 to 400 residues) extracellular amino-terminal domain, also known as the *ectodomain*, that participates in ligand binding (see Fig. 3.3).^{7,159} The ectodomain includes leucine-rich repeats that are highly conserved among the glycoprotein hormone receptors.^{7,159} There is 39% to 46% similarity of the ectodomain and 68% to 72% similarity of the transmembrane or serpentine domain among the three glycoprotein hormone receptors.⁷

The glycoprotein hormone receptors are coupled to G_s, and hormone binding stimulates adenylyl cyclase, leading to increased intracellular cyclic AMP levels and protein kinase A (PKA) activation.¹⁵⁹ Mutations leading to endocrine dysfunction have been reported for each of the glycoprotein hormone receptors.

Luteinizing Hormone/Choriogonadotropin Receptors

Both inactivating and activating mutations of the LH receptor have been found in humans.¹⁵⁹ The LH receptor gene is located in chromosome 2p21 and consists of 11 exons.^{160,161} Exon 1 encodes a peptide that directs the LH receptor to the plasma membrane.¹⁵⁹ Exons 2 through 10 encode the ectodomain.¹⁵⁹ The last exon encodes the transmembrane domains that are also known as the *serpentine regions*.^{7,159,160} Single nonsense mutations, amino acid changes, and partial gene deletions have been described that generate LH receptors with decreased activity.¹⁵⁹ Single-amino-acid changes have also been found that lead to activation of G_s in the absence of ligand binding.¹⁵⁹

Development of LH resistance requires biallelic mutations that inactivate the LH receptor gene, as one normal receptor allele is capable of producing adequate receptor protein to ensure physiologic signaling.¹⁵⁹ In contrast, activating mutations of the LH receptor gene cause endocrine disorders in the heterozygous state.¹⁵⁹

In the fetus, LH receptors are primarily activated by HCG.¹⁵⁹ Leydig cells begin to express LH receptors shortly after testicular differentiation at 8 weeks of gestation.¹⁵⁹ Thereafter, androgen production, caused by activation of these receptors by HCG, plays an important role in the development of male genitalia and testicular descent.¹⁵⁹ Thus male infants, with inactivating mutations of the LH receptor, may present with abnormally developed genitalia—including micropenis, cryptorchidism, and an XY disorder of sexual differentiation.¹⁵⁹

Males with mutations that completely inactivate the LH receptor exhibit failure of fetal testicular Leydig cell differentiation. This phenotype, which is known as *type 1 Leydig cell hypoplasia*, includes female external genitalia with a blind-ending vagina, absence of Müllerian derivatives, and inguinal testes with absent or immature Leydig cells.^{162–169} In addition, patients have elevated serum LH levels, normal serum FSH levels, and decreased serum testosterone levels that do not increase in response to HCG administration.^{162–169} Mutations that lead to this phenotype include a nonsense mutation (Arg545Stop) that results in a receptor that is missing TM4-7, an p.Ala593Pro change, and a TM7 deletion (p.Leu608del, p.Val609) that decreases cell-surface expression of the LH receptor.^{167,168,170} These mutant receptors are unable to couple to G_s.^{167,168,170}

Males with mutations that do not completely inactivate the LH receptor present with type 2 Leydig cell hypoplasia, which is characterized by a small phallus and decreased virilization.¹⁶⁶ A mutation that leads to this phenotype includes the insertion of a charged lysine at position 625 of TM7 in place of hydrophobic isoleucine that disrupts signal transduction.¹⁷¹ Another mutation (p.Ser616Tyr, found in patients with mild Leydig cell hypoplasia) is associated with decreased

cell-surface expression of the LH receptor.^{168,171} Other deletion and nonsense mutations have also been found to cause mild Leydig cell hypoplasia.¹⁵⁹

Males with inactivating mutations of the LH receptor may also present with a phenotype intermediate in severity between type 1 and type 2 Leydig cell hypoplasia. A compound heterozygote patient with p.Ser616Tyr on one allele and an inactivating deletion (Δ exon 8) on the other allele, presented with Leydig cell hypoplasia, micropenis, and hypospadias.¹⁷² The Cys131Arg mutation has also been found in patients with Leydig cell hypoplasia, small phallus, and hypospadias.¹⁷³ This mutation is located in the leucine-rich repeat segment of the LH receptor extracellular domain and interferes with high-affinity ligand binding.¹⁷³

Deletion of exon 10 of the LH receptor gene leads to an LH receptor that binds LH and HCG normally.¹⁷⁴ Interestingly, whereas HCG binding can elicit normal transmembrane signaling, LH binding fails to activate the receptor.¹⁷⁴ Because HCG is the principal hormone in utero that activates the LH receptor, and second-messenger response of the mutant receptors to HCG is not impaired, it is not surprising that a male patient found to be homozygous for the mutation was born with normal male genitalia.^{72,174} Pubertal progression and later gonadal function, however, are dependent on LH activation of the LH receptor.^{72,174}

Because deletion of exon 10 of the LH receptor gene results in a mutant LH receptor, with diminished intracellular signaling in response to LH, it is also not surprising that the patient homozygous for this mutation was found to have delayed pubertal development, small testes, and hypergonadotropic hypogonadism, when evaluated at the age of 18 years.¹⁷⁴ Prolonged HCG therapy resulted in normalization of testicular testosterone production, increased testicular size, and the appearance of spermatozoa in semen.¹⁷⁴ Similarly, inactivating mutations of the LH β subunit cause abnormal pubertal development, severe testosterone deficiency, and azoospermia, but normal external genitalia in males. In females, inactivating mutations of the LH β subunit are associated with normal pubertal development and menarche followed by oligomenorrhea, enlarged multicystic ovaries and infertility.¹⁷⁵

Females with loss of function mutations of the LH receptor may be asymptomatic or present with amenorrhea or oligomenorrhea.¹⁵⁹ Females with complete inactivating LH receptor mutations commonly have primary amenorrhea, inability to ovulate, and decreased estrogen and progesterone levels, accompanied by elevated LH and FSH levels.^{168,176} Affected individuals may have signs of low estrogen levels, including a hypoplastic uterus, a thin-walled vagina, decreased vaginal secretions, and decreased bone mass,^{168,176} although pubertal breast development is normal.¹⁷⁷ Homozygous LH receptor mutations (p.N400S, and p.Ala449Thr) have been associated with empty follicle syndrome, a disorder in which no oocytes are retrieved during in vitro fertilization.¹⁷⁸

Mutations that constitutively activate LH receptors cause male-limited precocious puberty (MLPP), also known as *testotoxicosis*—which may be familial or sporadic.^{169,179,180} Boys with this condition develop GnRH-independent precocious puberty before the age of 4 years, when p.Asp578Gly is present, and as early as the first year of life, when the p.Asp578Tyr mutation is present.^{159,181–183} Patients with this condition may also have an enlarged phallus at birth.¹⁸¹

During the first 5 years of life, patients with MLPP have very low LH and FSH levels but have testosterone levels in pubertal range.¹⁸⁴ During adolescence and adult life, testosterone levels do not increase above age-appropriate concentrations and gonadotropin levels normalize.^{159,184–186} Thus adolescents

and adults with MLPP do not usually manifest signs of androgen excess (such as hirsutism or severe acne).^{159,184} Most mutations that cause MLPP are located in the TM6 and i3, regions that participate in receptor-Gs protein coupling.¹⁵⁹ A milder phenotype was reported in a patient with a heterozygous activating mutation (p.C617Y) in TM7.¹⁸⁷ This mutation was inherited from the patient's mother who was apparently unaffected. Somatic activating mutations cause sporadic Leydig cell adenomas.^{118,188,189}

Activating mutations of the LH receptor do not appear to cause clinical disturbances in females. In prepubertal girls, this may be caused by low or absent LH receptor expression or because of insufficient aromatase expression in prepubertal granulosa cells. During puberty, activation of LH receptors on ovarian theca cells leads to the production of androgens that are converted to estrogens by aromatase in granulosa cells.¹⁵⁹ LH, along with FSH, also plays a role in inducing the differentiation of follicles into Graafian follicles and triggers ovulation and release of the oocyte.¹⁵⁹ Detailed phenotyping of the carrier mother of an MLPP male with the p.Asp578Gly activating mutation of the LH receptor failed to reveal any abnormalities in her menstrual cycles or fertility. LH dynamics, androgen, and FSH levels, as well as response to GnRH agonists, were normal.¹⁸⁴

Follicle-Stimulating Hormone Receptors

Inactivating and activating FSH receptor mutations have been described,¹⁹⁰ but they are far less common than LH receptor mutations.¹⁹⁰ The FSH receptor gene is located in chromosome 2 at p21 and contains 10 exons.¹⁹¹ The last exon of the FSH receptor gene encodes the transmembrane and intracellular domains.¹⁹²

FSH is required in females for normal follicle maturation and regulation of estrogen production by ovarian granulosa cells.^{190,193,194} FSH is required in pubertal males for Sertoli cell proliferation, testicular growth, and the maintenance of spermatogenesis.^{190,195}

The first inactivating mutation of the FSH receptor was found in Finnish females with autosomal recessive hypergonadotropic ovarian dysgenesis (ODG). ODG is characterized by primary amenorrhea, infertility, and streak or hypoplastic ovaries in the presence of a 46XX karyotype and elevated gonadotropin levels.¹⁹⁶ Twenty-two out of 75 Finnish patients with ODG were found to be homozygous for a c.C566T point mutation in exon 7 of the FSH receptor gene.¹⁹⁷ This mutation leads to the production of an FSH receptor with an p.Ala189Val substitution in an area of the extracellular ligand-binding domain that is thought to play a role in turnover of the receptor or in directing the receptor to the plasma membrane.¹⁹⁷ The mutated receptor demonstrates normal ligand-binding affinity but has decreased binding capacity and impaired signal transduction, when studied in transfected mouse Sertoli cells.¹⁹⁷ Males homozygous for this mutation have variable impairment of spermatogenesis and low to low-normal testicular volume, but are not azoospermic and can be fertile.¹⁹⁸ The c.C556T point mutation is uncommon outside Finland, where the carrier frequency is 0.96%.¹⁹⁹ Other mutations that alter signal transduction but not receptor expression or binding include p.Ala189Val, p.Asn191Ile, p.Ala419Thr, and p.Phe591Ser. The p.Ala189Val mutation causes primary hypergonadotropic amenorrhea in women and no spermatogenesis in men in the homozygous state and secondary amenorrhea in the heterozygous state.^{190,200} The nearby Asn191Ile mutation also causes hypergonadotropic amenorrhea in the homozygous state, but no clinical phenotype in the heterozygous state.²⁰¹ The Ala419Thr mutation was identified in a heterozygous

woman with primary amenorrhea.²⁰² The Phe591Ser mutation causes primary amenorrhea and premature ovarian failure (POF) in the homozygous state and a predisposition to sex cord ovarian tumors in the heterozygous state.²⁰³ Primary amenorrhea and POF have been described in women with homozygous mutations that totally impaired receptor binding to FSH²⁰⁴ or that resulted in reduced expression of the FSH receptor on the cell surface.²⁰⁵

A more recently published series described 13 Chinese families with nonsyndromic premature ovarian insufficiency.²⁰⁶ They found three novel mutations and 11 previously described homozygous mutations and 3 previously described compound heterozygous mutations. Clinical manifestations ranged from primary amenorrhea to normal menarche with oligomenorrhea or secondary amenorrhea. It was the first study to identify a frameshift mutation (p.Lys140Argfs*16 in the ectodomain) and a missense mutation in the signal peptide of the FSH receptor (p.Gly15Asp). The other novel mutation was p.Pro504Ser in the transmembrane domain.

Compound heterozygosity for mutations that cause partial loss of FSH receptor function may cause endocrine dysfunction in women.^{200,207} Women may present with infertility, secondary amenorrhea, osteoporosis, and a history of normal or delayed onset of puberty, accompanied by elevated LH and FSH, low-normal plasma estradiol, low plasma inhibin B levels, slightly enlarged ovaries with immature follicles, and a small uterus.²⁰⁷ This may be caused by FSH receptor gene mutations that result in an p.Ile160Thr mutation in the extracellular domain that impairs cell-surface expression and an p.Arg573Cys mutation in e3 that interferes with signal transduction.²⁰⁷ Other women present with primary amenorrhea and very elevated gonadotropin, low plasma estradiol and inhibin B levels, normal-size ovaries with immature follicles, and a normal-size uterus.²⁰⁰ This condition is associated with an p.Asp224Val substitution in the extracellular domain, leading to impaired cell-surface expression and a p.Leu601Val substitution in e3 impairing signal transduction.²⁰⁰

Activating mutations of the FSH receptor have also been described. Surprisingly, a hypophysectomized male was found to be fertile and to have serum testosterone levels above 4.9 nmol/L (141 ng/dL) and normal testis volume, in spite of undetectable gonadotropin levels.²⁰¹ This patient was found to be heterozygous for a c.A1700G mutation in exon 10 of the FSH receptor gene that resulted in a p.Asp567Gly substitution in an area of the third intracytoplasmic loop that is highly conserved among FSH, LH, and TSH receptors.^{201,208,209} The same substitution in corresponding areas of the LH and TSH receptors also results in constitutively active receptors and is found in MLPP and thyroid adenomas, respectively.^{201,208,210} Other activating mutations have been identified to cause spontaneous ovarian hyperstimulation syndrome (OHSS). OHSS is a common complication of treatment protocols used to induce ova for in vitro fertilization and is characterized by multiple follicular cysts lined by luteinized cells, which can result in abdominal discomfort and distention, as well as ovarian enlargement and fluid sequestration. One such mutation is the p.Asp567Asn, which was found in a woman with recurrent spontaneous OHSS.²¹¹ The p.Thr449Ile and p.Thr449Ala mutations cause a conformational change that leads to loss of specificity for FSH, leading to sensitivity to HCG²¹² and TSH,²¹³ causing spontaneous OHSS during pregnancy or with hypothyroidism. The p.Ile545Thr mutation caused spontaneous OHSS in a woman during the first trimester of pregnancy, despite a normal HCG level.²¹⁴ This mutant receptor displayed detectable constitutive activity, as well as promiscuous activation by HCG and TSH.

Thyroid-Stimulating Hormone Receptors

The TSH receptor gene is located on chromosome 14 and contains 10 exons, with the first nine exons encoding the large extracellular domain and the 10th exon coding the remainder of the receptor.^{215–218} At low extracellular TSH concentrations, TSH receptor activation leads to stimulation of $G\alpha_s$ —which activates adenylyl cyclase, resulting in increased intracellular cyclic AMP levels.^{219,220} At higher extracellular TSH concentrations, activation of the TSH receptor also stimulates the G_q and G_{11} proteins—activating phospholipase C and resulting in the production of diacylglycerol and inositol phosphate.²²⁰

TSH receptors differ from the other glycoprotein hormone receptors in that they exist in two equally active forms.^{221,222} These are the single-chain and two-subunit forms of the TSH receptor (Fig. 3.4). The single-chain form of the TSH receptor is made up of three contiguous subunits: the A subunit, C peptide, and B subunit.^{222–224} The A subunit begins at the amino-terminal of the extracellular domain and contains most of the extracellular domain.^{222–224} The C peptide is connected to the carboxy-terminal of the A subunit and continues the extracellular domain.^{222–224} The C peptide contains a 50-amino-acid sequence that is only found in TSH receptors.^{222–224} The B subunit is connected to the C terminal of the C peptide and contains the TMs and the carboxy-terminal cytoplasmic portion of the receptor.^{222–224} The two-subunit form of the receptor is missing the C peptide, which is cleaved from the protein during intracellular processing and consists of the A and B subunits attached by disulfide bonds.^{225–228} It is surprising that both receptor forms are activated equally by TSH because the C peptide and nearby regions of the A and B subunits participate in signal transduction.^{221–223,229,230}

Missense mutations of the TSH receptor gene, leading to replacement of Ser-281 near the carboxy-terminus of the A subunit, with Ile, Thr, or Asn, result in a constitutively active TSH receptor that may cause *intrauterine* or congenital hyperthyroidism, or toxic adenomas.^{224,231–233} Activating somatic mutations that cause toxic adenomas have also been found in different transmembrane domains of the TSH receptor.^{234–241} More specifically, clusters of mutations are located in the i3 and TM6 regions—found to be involved with signal transduction in all glycoprotein hormone receptors.^{234–236,238–240} The prevalence of activating mutations of the TSH receptor in toxic adenomas has been estimated to range from 2.5% in Japan to 86% in Brazil.^{237,238,240,242–245}

Activating somatic mutations of the TSH receptor have also been found in multinodular goiters.²⁴⁶ Interestingly, different activating mutations have been found in separate nodules in the same individual.²⁴⁶ Some well-differentiated thyroid carcinomas have activating mutations of the TSH receptor.^{247–249} Somatic activating mutations of the *GNAS* gene encoding $G\alpha_s$ have also been found in some toxic adenomas and differentiated thyroid carcinomas.^{99,250,251} Activating germline mutations of the TSH receptor can cause sporadic or autosomal dominant inherited nonautoimmune hyperthyroidism that presents in utero, during infancy, during childhood, and in some cases in adulthood.^{209,233,252–260} These mutations have been found in the amino-terminal extracellular and transmembrane domains.^{209,233,253–261}

Patients with heterozygous mutations that lead to constitutively active TSH receptors typically develop hyperthyroidism.²²² In contrast, biallelic loss of function mutations in the TSH receptor genes cause hypothyroidism.²²² Most known loss-of-function TSH receptor mutations are located in the amino-terminal extracellular domain.²⁶² A spontaneous p.Asp410Asn substitution, near the carboxy-terminus of the C peptide, results in a TSH receptor, with normal ligand

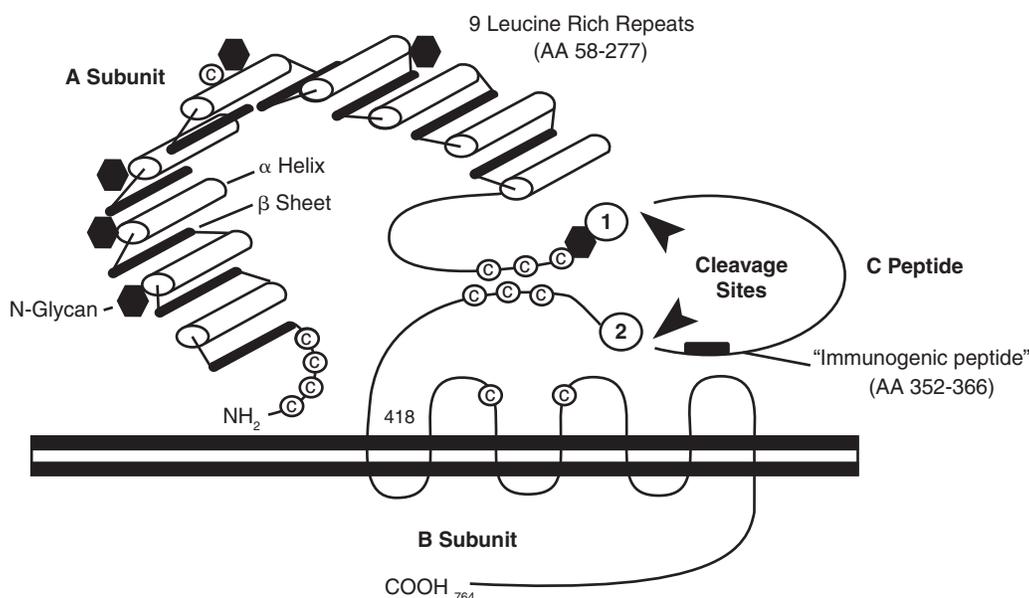


Fig. 3.4 The thyroid-stimulating hormone (TSH) receptor. There are two forms of TSH receptors. The single-chain form is made up of an A subunit, C peptide, and B subunit. Posttranslational cleavage of the C peptide from the single chain form results in the two-subunit form. This form consists of the A subunit joined to the B subunit by disulfide bonds between the carboxy-terminal cysteine residues of the A subunit and the amino-terminal cysteine residues of the B subunit. (From Rapoport, B., Chazenbalk, G. D., Jaume, J. C., McLachlan, S. M. (1998). The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. *Endocr Rev*, 19, 676. Copyright 1998, The Endocrine Society. With permission.)

binding affinity and impaired G_{α_s} -mediated signal transduction.²⁶³ Patients who are homozygous for this mutation present with compensated hypothyroidism.²⁶³

Patients homozygous or compound heterozygous for loss-of-function mutations of the TSH receptor present with the syndrome of resistance to TSH (RTSH). Loss-of-function mutations of the TSH receptor that cause RTSH have been identified in the amino-terminal extracellular domain, TM4, TM6,²⁶⁴ i2, e1, and e3.²⁶⁵ Clinical severity of RTSH may range from a euthyroid state accompanied by elevated TSH levels (fully compensated RTSH), to mild hypothyroidism unaccompanied by a goiter (partially compensated hypothyroidism), to congenital thyroid hypoplasia accompanied by profound hypothyroidism (uncompensated RTSH).^{76,263,266–270} In patients with uncompensated RTSH, a small bilobar thyroid gland is located at the normal site.⁷⁶ Loss-of-function mutations of the TSH receptor are a rare cause congenital hypothyroidism^{271, 272} more common in Japan and Taiwan ($\leq 7\%$ of children),^{273,274} where p.R450H is particularly frequent. Because expression of the sodium-iodide symporter is TSH dependent,¹⁷⁹ thyroid gland uptake of ¹²⁵Iodine and ^{99m}technetate is diminished or absent in patients with RTSH.^{76,275} In rare cases, iodine uptake is high-normal.²⁷⁶ These compound heterozygous mutations of the TSH receptor had some G_{α_s} activity and no G_{α_q} activity, suggesting that iodine uptake is solely controlled by G_{α_s} activity and not G_{α_q} activity. Some families have been found to have an autosomal-dominant form of RTSH that is not caused by a mutation of the TSH receptor.^{277,278}

Human Chorionic Gonadotropin and Thyroid-Stimulating Hormone Receptors during Pregnancy

Because of its structural similarity with TSH, at very high concentrations, HCG can activate the TSH receptor.²⁷⁹ During pregnancy, HCG activation of TSH receptors leads to elevation in thyroid hormones after the ninth week of gestation—and decreases in TSH levels between the 9th and 12th weeks of gestation.²⁸⁰ This phenomenon does not usually result in maternal hyperthyroidism (gestational thyrotoxicosis).^{280,281}

However, when HCG levels are abnormally elevated because of gestational trophoblastic disease caused by a molar pregnancy or choriocarcinoma, hyperthyroidism may occur.^{282–286} The prevalence of thyrotoxicosis in gestational trophoblastic disease correlates with HCG levels. In one study of 196 patients treated with chemotherapy for gestational trophoblastic neoplasia, the prevalence of thyrotoxicosis was 7%.²⁸⁷ Biochemical thyrotoxicosis only occurred in patients with HCG levels $>10^5$ and clinical thyrotoxicosis only occurred in patients with HCG levels greater than 10^6 . Serum TSH is consistently suppressed when HCG levels are above 4×10^5 mIU/mL.²⁸⁸

A mother and daughter were identified with recurrent gestational hyperthyroidism and normal serum HCG levels.^{211,213,214,289} These individuals were found to be heterozygous for a missense mutation in the TSH receptor gene, resulting in a p.Lys183Arg substitution in the extracellular domain of the receptor. It is believed that this substitution increases sensitivity of the receptor to activation by HCG, causing gestational hyperthyroidism.

The Gonadotropin-Releasing Hormone Receptor Group

Gonadotropin-Releasing Hormone Receptors

The GnRH receptor gene is located on 4q13 and includes three exons.^{290,291} Unlike glycoprotein hormone receptors, GnRH receptors lack an intracellular carboxy-terminal domain.^{292,293} In contrast to most GPCRs, the GnRH receptor is coupled to G_q/G_{11} and hence ligand-binding leads to stimulation of phospholipase C and not adenylyl cyclase.²⁹⁴ Phospholipase C cleaves phosphatidylinositol-4,5-diphosphate (PIP₂) to inositol 1,4,5-triphosphate (IP₃) and diacylglycerol, leading to increased protein kinase C activity.^{295,296}

Some patients with IHH are homozygous or compound heterozygous for loss-of-function mutations in the GnRH receptor gene.^{297–299} Unlike patients with Kallmann syndrome (KS), they have a normal sense of smell.^{297–299} GnRH receptor mutations were found in approximately 5% of patients with

normosmic congenital hypogonadotropic hypogonadism.³⁰⁰ GnRH receptor mutations that cause IHH result in decreased binding of GnRH or impaired GnRH receptor signal transduction, or decreased GnRH receptor cell membrane expression because of misrouting of GnRH receptor oligomers from the ER.^{47,48,297–299} Some mutations, including p.E90K, p.L266R, and p.S168R, that cause misfolding and retention, within the ER, exhibit a dominant negative effect because of retention of wild-type receptors.⁴⁸

Female patients with mutations that partially compromise GnRH receptor function may present with primary amenorrhea and infertility, associated with a normal breast development, normal or small uterus, and small ovaries with immature follicles.^{297,301} Males with the same mutations may present with incomplete hypogonadotropic hypogonadism (characterized by a delayed and incomplete puberty) or with complete hypogonadotropic hypogonadism (characterized by absent puberty).^{297,301}

Some patients with IHH caused by mutated GnRH receptors have partial or normal gonadotropin responses to exogenous GnRH.^{297,301} However, decreased amplitude in the pulsatile LH secretion can be observed in these patients.²⁹⁷ Females with a partial or normal gonadotropin response to exogenous GnRH are more likely than nonresponders to become fertile in response to pulsatile exogenous GnRH.^{297,302}

Activating mutations of the GnRH receptor have not been described in the germline or in pituitary adenomas.³⁰³

The Thyrotropin-Releasing Hormone and Secretagogue Receptor Group

Thyrotropin-Releasing Hormone Receptors

Like the GnRH receptor, TRH receptor activation leads to increased phospholipase C activity.³⁰⁴ To date, only inactivating mutations that cause endocrine dysfunction have been reported for the TRH receptor. One patient was identified with central hypothyroidism caused by mutated TRH receptors.³⁰⁵ He presented during the ninth year of life with short stature (−2.6 SD), accompanied by a delayed bone age (−4.1 SD), a low plasma thyroxine level, and a normal plasma TSH level. Exogenous TRH did not induce an increase in plasma TSH and prolactin levels. He was found to be compound heterozygous for TRH receptor gene mutations, resulting in receptors that failed to bind TRH or induce IP3 production. Another family was identified with complete resistance to TRH because of a nonsense mutation in the TRHR (p.R17X) producing a TRH receptor that lacked the entire transmembrane domain.³⁰⁶ The proband was homozygous and presented with short stature, growth failure, and fatigue at age 11 years. He had a low free T₄, with a low-normal TSH. TRH stimulation testing failed to stimulate TSH or prolactin. Surprisingly his 33-year-old sister who was also homozygous had escaped detection despite two normal pregnancies brought to term. She had no signs or symptoms of hypothyroidism but exhibited thyroid function tests similar to the proband. She breastfed normally. Both the proband and his sister had normal cognitive function. This report suggested that the TRH receptor is not essential for normal cognitive function or female fertility and lactation. The mouse model corroborates these findings.³⁰⁷

Other Class A Receptors That Transduce Hormone Action

Free Fatty Acid Receptor 1

At the time a new GPCR is discovered, the ligand for the newly discovered receptor is often unknown. Thus until a specific ligand is discovered, these GPCRs are known as orphan receptors. According to the Human Genome Organization (HUGO) Gene

Nomenclature Committee, these G protein-coupled orphan receptors should be named alphanumerically GPR followed by a number, until their ligand is known. Once a specific ligand is identified, a more specific name is given the receptor.

The ligands for GPR40 were unknown when the receptor was first discovered. The HUGO Gene Nomenclature Committee changed the name of the receptor to free fatty acid receptor 1 (FFAR1) when the ligands were identified as medium- and long-chain fatty acids. With rare exceptions that are clearly identified, this chapter follows HUGO Gene Nomenclature Committee recommendations (see www.gene.ucl.ac.uk/nomenclature/index.html for more information on receptor nomenclature).

FFAR1 is one of several GRCRs for lipid mediators. Lipid mediators are intercellular lipid messengers that include sphingosine 1-phosphate, sphingosylphosphorylcholine, dioleoyl phosphatidic acid, lysophosphatidic acid, eicosatetraenoic acid, bile acids, and free fatty acids.⁶⁷ FFAR1 is activated by medium- and long-chain fatty acids, whereas FFAR2 (formerly known as GPR43) and FFAR3 (formerly known as GPR41) are activated by shorter-chain fatty acids.⁶⁷ There is now evidence that FFAR1 activation by medium- and long-chain fatty acids has endocrine implications. FFAR1 is expressed in human pancreatic β-islet cells.³⁰⁸ FFAR1 is involved in cholecystokinin secretion from I cells in response to fatty acids.³⁰⁹ It has also been implicated in fatty acid stimulated GLP-1 and GIP secretion from L and K cells.³¹⁰ GPR120 is expressed in enteroendocrine cells and has a physiologic role in GLP-1 secretion.³¹¹ FFAR2 and FFAR3 are expressed in adipose tissue and FFAR3 has been implicated in leptin production.³¹¹

Fatty acid-induced stimulation of FFAR1 in β-islet cells leads to activation of the G_{αq}-phospholipase C second-messenger pathway, which in turn leads to release of calcium from the ER that augments insulin-mediated increases in intracellular calcium concentrations because of glucose-induced activation of voltage-gated calcium channels.^{312–315} Because an increased intracellular calcium concentration induces insulin release, FFAR1-mediated augmentation of glucose-mediated increases in the intracellular calcium concentration leads to amplification of glucose-stimulated insulin release.^{312–315}

A variant in FFAR1 (p.Gly180Ser), found in a Sicilian population, resulted in obesity, impaired glucose tolerance, and lipid stimulated insulin secretion.³¹⁶ Two other variants, p.Arg211His and p.Asp175Asn, are not associated with alterations in insulin release.^{316,317} TAK-875, an FFAR1 agonist, was shown to reduce hemoglobin A1c in patients with type 2 diabetes, in a phase 2 clinical trial.³¹⁸ Wild-type mice placed on an 8-week high-fat diet develop glucose intolerance, insulin resistance, hypertriglyceridemia, and hepatic steatosis—whereas FFAR1 knockout mice on the same diet do not develop these conditions.³¹⁹ The clinical relevance for patients is not yet clear. However, an Arg211His polymorphism in the FFAR1 gene may explain some of the variation in insulin secretory capacity found in Japanese men: Arg/Arg homozygotes had lower serum insulin levels, homeostasis model of insulin resistance, and homeostasis model of beta-cell function than His/His homozygotes.³²⁰

KISS1 Receptor/GPR54

Studies in animal models suggests that Kiss1-expressing neurons in the hypothalamus modulate GnRH expressing neurons to initiate puberty and modulate sex steroid feedback on GnRH release.^{321,322} Homozygous inactivating mutations in the gene encoding the KISS1 receptor (GPR54) were initially described in French and Saudi Arabian patients with IHH; in both cases the affected subjects came from consanguineous families.^{321,323–325} The Saudi patients carried a p.Leu148Ser

mutation, whereas the French patients carried a 155bp deletion. Leu148 is highly conserved among class A GPCRs.³²⁶ The mutation does not affect expression, ligand binding, or association with G_s , but impairs ligand-induced catalytic activation of G_s .³²⁶ At the same time, an African American patient with IHH was described who was compound heterozygous for inactivating GPR54 mutations.³²⁵ Since publication of these initial reports, additional patients have been described. A boy with a Jamaican father and a Turkish-Cypriot mother, and with cryptorchidism and micropenis at birth, and undetectable LH and FSH levels at 2 months of age, was found to have compound heterozygous GPR54 mutations.³²⁷ Another missense mutation (p.Leu102Pro) that exhibits complete inactivation of GPR54 signaling has been identified.³²⁸ Surprisingly, patients with this mutation exhibited spontaneous pulsatile LH and FSH secretion with normal frequency, and a blunted amplitude and family members had partial pubertal development.³²⁸ Biallelic loss of function mutations in GPR54 are a rare cause of normosmic IHH.^{321,327} A study of genetic causes of normosmic congenital hypogonadotropic hypogonadism showed that KISS1R mutations accounted for 2% of the variants.³⁰⁰

Unlike patients with KS, but similar to patients with GnRH mutations, patients with GPR54 mutations have an intact sense of smell. In contrast to patients with IHH caused by GnRH mutations, patients with GPR54 mutations increase serum gonadotropin levels in response to exogenous GnRH.

Ligands for GPR54 derive from a single precursor protein, kisspeptin-1.^{329,330} The longest derivative protein that acts as a ligand for GPR54 is metastatin, so called because it is a metastasis suppressor gene in melanoma cells.³²⁹ Metastatin consists of kisspeptin-1 69-121.^{329,330} However, shorter carboxy-terminal peptides derived from kisspeptin-1 bind and activate GPR54.³²⁹ Administration of metastatin to adult male volunteers increases LH, FSH, and testosterone levels.³³¹

An activating mutation in GPR54 was identified in a patient with central precocious puberty.³³² The adopted girl was found to have an Arg386Pro mutation, which led to prolonged activation of signaling in response to kisspeptin. Mutational analysis of 28 subjects with idiopathic central precocious puberty failed to find any variants in KISS1 or KISS1R.³³³ A larger study showed an association between several variants and central precocious puberty in Korean girls.³³⁴

Orexin Receptors

Orexins act on specific receptors that are located predominantly in the hypothalamus to control food intake and play a role in the regulation of sleep/wakefulness.³³⁵⁻³³⁷ There are two types of orexin receptors: the orexin-1 and the orexin-2 receptors.³³⁷ There are also two types of orexins, orexin A and orexin B, formed from the precursor peptide preproorexin.³³⁷ Orexins are also known as *hypocretins*, and orexin A is synonymous with hypocretin-1 and orexin B with hypocretin-2.^{336,337} Orexin A acts on orexin-1 and orexin-2 receptors, whereas orexin B only acts on orexin-2 receptors.^{335,338,339}

Like most class A GPCRs, orexin receptors couple with $G_{q/11}$ and G_i/G_o to activate phospholipase C and inhibit adenylyl cyclase, respectively.^{337,340-342} Surprisingly, evidence suggests that orexin receptors also couple with G_s —which increases adenylyl cyclase activity.³⁴² Orexins increase food intake and duration of wakefulness.^{335,336,343} Orexin A and activation of the orexin-1 receptor have greater orexigenic effects than orexin B and activation of the orexin-2 receptor.³⁴⁴ The orexin-2 receptor mediates the arousal effect of orexins.³⁴⁴ Most patients with narcolepsy with cataplexy have diminished levels of orexin A concentrations in cerebral spinal fluid and lack orexin-containing neurons.^{336,345-348} This is thought to be because

of postnatal cell death of orexin neurons in the hypothalamus.³⁴⁹ HLA DQB1*0602 is associated with narcolepsy with cataplexy and an autoimmune process has been suggested, but no autoantibodies have been identified. Thus far, no mutations in orexin receptors have been found in humans. A mutation in the orexin-2 receptor causes narcolepsy in dogs.³⁵⁰ There is one described mutation (p.Leu16ARG) in the *HCRT* gene in a child with early-onset narcolepsy with cataplexy.³⁴⁶ This mutation was shown to impair processing and trafficking of the mutant orexin, leading to undetectable orexin A concentrations in the cerebrospinal fluid.

Ghrelin Receptors

The ghrelin receptor is also known as the GH secretagogue receptor type 1a because activation of receptors in the hypothalamus and pituitary somatotrophs enhances GH secretion.³⁵¹ Ghrelin is a product of posttranslational modification of the ghrelin gene product proghrelin.³⁵² Ghrelin is mainly produced in the stomach.^{353,354} Activation of ghrelin receptors located in the hypothalamus induces growth hormone-releasing hormone (GHRH) secretion. At the level of pituitary somatotrophs, it stimulates GH secretion. The ghrelin receptor also stimulates the appetite (i.e., an orexigenic role).

Plasma ghrelin levels are elevated just before eating and decrease rapidly after eating.^{355,356} In addition, intravenous administration of ghrelin to humans increases appetite and food intake.³⁵⁷ Plasma ghrelin levels are elevated in individuals with Prader Willi syndrome.³⁵⁸ Thus hyperphagia in patients with Prader Willi syndrome may be caused at least in part by overactivation of ghrelin receptors by ghrelin. Screening of 184 extremely obese children and adolescents for mutations of the ghrelin receptor gene failed to identify a single mutation likely to cause obesity.³⁵⁹ In contrast, large-scale screening studies have identified mutations and single nucleotide polymorphisms (SNPs) scattered throughout the ghrelin receptor,³⁶⁰ and two SNPs and one mutation in the promoter region, associated with increased transcriptional activity, cosegregated with obesity.

Short individuals in two unrelated Moroccan kindreds were found to have a C to A transversion at position 611 in the first exon of the ghrelin receptor gene.³⁶¹ This transversion results in replacement of the apolar and neutral amino acid alanine at position 204 of the receptor by the polar and charged amino acid glutamate (p.Ala204Glu). This mutation interferes with normal constitutive activity of the receptor and decreases cell membrane expression of the receptor. Receptor activation by ghrelin, however, is preserved. Two-thirds of heterozygous individuals in the kindreds studied had short stature, with height more than or equal to 2 SDs below the mean. One heterozygous individual's height was -3.7 SDs below the mean. Before onset of GH therapy, the only individual in the kindreds homozygous for the mutation had a height -3.7 SD, and became overweight during puberty. The weight of the patients heterozygous for the mutation varied from underweight to overweight. Another patient who presented with severe short stature (-3 SD), vomiting, ketosis, hypoglycemia, and low BMI was identified to be a compound heterozygote for a p.W2X and an p.R237W mutation in the ghrelin receptor.³⁶² His serum insulin-like growth factor-1 (IGF-1) level was low at 44 ng/mL and he failed GH stimulation testing, but had a normal IGF-1 generation test. This patient had improvement in his height velocity and resolution of hypoglycemia after treatment with GH. Four more ghrelin receptor mutations were identified in a Japanese cohort with short stature (p.Q36del, p.P108L, p.C173R, and p.D246A).³⁶³ p.Q36del showed a minor reduction in activity. C173R led to intracellular retention. D246 caused impaired signaling, and P108L led to

reduced binding affinity to ghrelin. Two other mutations were identified in a cohort of patients with constitutional delay of growth and puberty in Brazil (Ser84Ile and Val182Ala).³⁶⁴ Both resulted in decreased basal activity. The patients were short at presentation (−2.4 and −2.3 SD) but reached a normal adult height without treatment.

Another product of posttranslational modification of proghrelin, obestatin, appears to play a role in controlling appetite and weight.³⁵² Activation of the obestatin receptor, previously known as *GPR39*, in rats results in decreased food intake and weight.

Melanin-Concentrating Hormone Receptors

Formerly known as *SLC-1* or *GPR24*, the type 1 melanin-concentrating hormone (MCH) receptor (MCHR1)—and the more recently discovered type 2 MCH receptor (MCHR2), formerly known as *SLT* or *GPR145*—may play a role in regulating feeding and energy metabolism in humans.^{365–367} When activated, MCHR1 couples with $G_{q/11}$ and $G_{i/o}$ to increase phospholipase C activity and inhibit adenylyl cyclase activity, respectively.^{365,366,368} MCHR2 couples with $G_{q/11}$, and MCH binding leads to increased phospholipase C activity.^{367,369}

Studies in rodents reveal that MCH is an orexigenic hormone, and treatment of rodents with MCHR1 antagonists decreases food intake, weight, and body fat.^{37,365,370–372} MCHR2 is not expressed in rodents.³⁷³ Deletion of the MCHR1 in mice prevents overeating in response to food cues under sated conditions.³⁷⁴ Two loss-of-function mutations were identified in MCHR1 in humans (R210H and P377S).³⁷⁵ Cells transfected with either mutant receptor failed to respond to MCH, despite normal cell-surface expression of the receptor, suggesting a receptor activation defect. These mutations were identified in two markedly underweight individuals and were not found in an obese cohort.^{375,376} Analysis of the *MCHR1* gene in more than 4000 obese German, Danish, French, and American children and adolescents revealed several SNPs and gene variations in the German children and adolescents that may be associated with obesity.³⁷⁶ Another study of 106 American subjects with early onset obesity failed to definitively identify MCHR1 and MCHR2 mutations as a cause of obesity.³⁷⁷

CLASS B RECEPTORS THAT TRANSDUCE HORMONE ACTION

Growth Hormone–Releasing Hormone Receptor

The GHRH receptor gene is located at 7p14.³⁷⁸ GHRH receptors interact with G_s to stimulate adenylyl cyclase, resulting in increased intracellular cyclic AMP levels that lead to somatotroph proliferation and GH secretion.³⁷⁹ Thus it is not surprising that activating mutations in G_{α_s} leading to constitutive activation of adenylyl cyclase have been found in some GH-secreting pituitary adenomas in humans.³⁸⁰

Many mutations in the GHRH receptor that cause isolated GH deficiency have been identified. These include six splice site mutations, two microdeletions, two nonsense mutations, one frameshift mutation, 10 missense mutations, and one mutation in the promoter.^{381–384} The first naturally occurring mutation in the GHRH receptor (p.D60G) was found in the little mouse, which has a dwarf phenotype.^{385,386} This mutation in a conserved amino acid in the extracellular domain impairs the ability to bind GHRH.³⁸⁷ The first human mutation in the GHRH receptor (p.Glu72X) was identified in a consanguineous Indian family.^{388,389} The same mutation was found in three apparently unrelated consanguineous kindreds from India, Pakistan, and Sri Lanka.^{378,390} A different mutation

(5′ splice site mutation in intron 1) was identified in a large Brazilian kindred of more than 100 individuals.³⁷⁹ Both mutations result in the production of markedly truncated proteins with no receptor activity.^{379,390} A frameshift mutation was identified in a patient with severe short stature and was the first documented case of early-onset anterior pituitary hypoplasia.³⁸³ In another family, two siblings with isolated GH deficiency were found to be compound heterozygous for inactivating GHRH receptor gene mutations.³⁹¹ Three more novel mutations were identified in families with severe short stature in the United Kingdom (p.W273S, p.R94L, and p.R162W).³⁸¹ The only mutation found in the promoter region of GHRH affects one of the Pit-1 binding sites.³⁹²

Studies of subjects in these large kindreds who are homozygous or compound heterozygous for inactivating GHRH receptor gene mutations have shown that affected children experience severe postnatal growth failure with proportionate short stature.^{378,379,390,391} Males have high-pitched voices and moderately delayed puberty.^{378,379,390} Unlike infants with complete GH deficiency, they do not have frontal bossing, microphallus, or hypoglycemia.^{378,390,393} Other features include a doll facies, reduced muscle mass, central adiposity, wrinkled thin skin, and delayed pigmentation of the hair in children and teens.³⁹⁴ Bone age is delayed with respect to chronological age, but advanced with respect to height age.³⁹⁰ Some patients have been found to have pituitary hypoplasia.^{378,390} Growth velocity increased with exogenous GH therapy.^{378,379,390,391} Remarkably, GH treatment of two siblings from Turkey, with the p.E72X mutation, allowed them to reach a normal adult height, despite pretreatment heights of −6.7 and −8.6 SD and initiation of GH around age 14 years.³⁹⁵

Further studies in the Brazilian cohort revealed that homozygotes had increased abdominal obesity, a higher low-density lipoprotein (LDL), and total cholesterol but normal carotid wall thickness and no evidence of premature atherosclerosis.³⁹⁶ Treatment of these patients with GH for 6 months improved body composition, reduced LDL and total cholesterol, and increased high-density lipoprotein (HDL). Surprisingly, this was associated with increased carotid intima-media thickness and atherosclerotic plaques.³⁹⁷ Reevaluation, 5 years after the discontinuation of GH, showed a return to baseline for these measures.³⁹⁸ Patients with a null mutation affecting the GHRH receptor also exhibited altered sleep patterns with abnormalities in both rapid eye movement and nonrapid eye movement sleep.³⁹⁹ Heterozygotes for the null mutation had normal adult heights and IGF-1 SD scores but exhibited reduced body weight, BMI, lean mass, fat mass, and increased insulin sensitivity.⁴⁰⁰

SNPs in the GHRH receptor have been shown to contribute to height-SDS variation,^{401,402} but mutations remain a rare cause of isolated GH deficiency.⁴⁰³

Gastric Inhibitory Polypeptide Receptors

The gastric inhibitory peptide receptor (*GIPR*) gene is located on the long arm of chromosome 19.⁴⁰⁴ Two functional isoforms exist in humans because of alternate splicing.⁴⁰⁵ GIPR activation induces G_{α_s} activation of adenylyl cyclase.^{405–407} Gastric inhibitory polypeptide (GIP) is also known as *glucose-dependent insulinotropic polypeptide* and is released by K cells in the small intestine in response to food. GIP has numerous physiologic actions, including stimulation of glucagon, somatostatin, and insulin release by pancreatic islet cells.^{408,409} Human mutations in the GIPR have not been identified to date. One study identified an SNP in the GIPR that is associated with insulin resistance in obese German children.⁴¹⁰ Another study identified an SNP in the GIPR that is associated with reduced fasting and induced C-peptide levels.⁴¹¹

The GIPR is implicated in food-dependent Cushing syndrome. Circulating cortisol levels in patients with food-dependent or GIP-dependent Cushing syndrome rise abnormally in response to food intake.^{102,103} GIP does **not** normally induce cortisol release from adrenocortical cells.^{412,413} These patients may have adrenal adenomas or nodular bilateral adrenal hyperplasia that overexpresses GIPRs that abnormally stimulate cortisol secretion when activated.^{412,414} Thus in these patients postprandial GIP release leads to activation of these abnormally expressed and functioning adrenal GIPRs, resulting in excessive adrenal cortisol secretion.^{412,413}

Parathyroid Hormone and Parathyroid Hormone-Related Peptide Receptors

Two types of PTH receptors have been identified. The type 1 PTH receptor (PTHr1) is activated by PTH and parathyroid hormone-related peptide (PTHrP) and mediates PTH effects in bone and kidney.⁴¹⁵ In spite of 51% homology to the PTHr1, the type 2 PTH receptor (PTHr2) is activated by PTH but not PTHrP.^{415–418} The PTHr2 is particularly abundant in the brain and pancreas but is also expressed in the growth plate; its natural ligand is TIP39.^{419–421} The function of the PTHr2 is largely unknown.^{415,416}

The PTHr1 has a large amino-terminal extracellular domain containing six conserved cysteine residues.⁴¹⁵ Ligand binding induces the PTHr1 to interact with G_s and G_q proteins, leading to activation of the adenylyl cyclase/PKA and phospholipase C/protein kinase C second-messenger pathways, respectively.^{422–428} Interestingly, mutations in i2 interfere with coupling of the PTHr1 to G_q, without interfering with coupling to G_s—whereas mutations in i3 disrupt coupling of the receptor to both G proteins.^{429,430} Binding of PTH to the PTHr1 leads to internalization of a portion of plasma membrane containing a ternary complex of activated receptor-G_s-adenylyl cyclase that exhibits sustained production of cyclic AMP.⁴³¹ In contrast, PTHrP binding to the PTHr1 receptor leads to formation of a complex that remains on the cell surface and generates cyclic AMP for only a short period of time.⁴³¹

Biallelic loss-of-function mutations in the *PTHr1* gene cause Blomstrand chondrodysplasia.⁴¹⁵ This lethal disorder is characterized by accelerated chondrocyte differentiation, resulting in short-limbed dwarfism, mandibular hypoplasia, lack of breast and nipple development, and severely impacted teeth.^{415,432} One patient with this rare condition was found to be homozygous for a point mutation that resulted in a p.Pro132Leu substitution in the amino-terminal domain that interferes with ligand binding.^{433,434} Another patient was found to be homozygous for a frameshift mutation that results in a truncated receptor lacking TM5-7, and contiguous intracellular and extracellular domains.⁴³⁵

A third patient was found to have a maternally inherited mutation that altered splicing of maternal mRNA, resulting in a PTHr1 with a deletion of residues 373 through 383 in TM5 (which also interferes with ligand binding).⁴³⁶ In spite of heterozygosity for the mutation, the patient was unable to produce normal PTHr1s, because for unknown reasons the paternal allele was not expressed.⁴³⁶ Heterozygosity for somatic or germline p.Arg150Cys missense mutations in the PTHr1 was identified in two out of six patients with enchondromatosis,⁴³⁷ a condition that is usually sporadic and which is attributed to a postzygotic somatic cell mutation. Enchondromas are benign cartilage tumors that develop in the metaphyses and may become incorporated into the diaphyses of long tubular bones, in close proximity to growth plate cartilage; there is an increased risk of malignant transformation to osteosarcoma.²⁰ Patients with multiple enchondromatosis

(OMIM ID: 166000) have Ollier disease (World Health Organization terminology), a disorder characterized by the presence multiple enchondromas with an asymmetric distribution of lesions that vary in size, number, and location. When multiple enchondromatosis occurs with soft tissue hemangiomas, the disorder is known as *Maffucci syndrome*. In vitro studies showed that the p.Arg150Cys mutation was mildly activating but led to stimulation of phospholipase C rather than adenylyl cyclase.⁴³⁷ A transgenic knockin mouse expressing the mutant PTHr1, under the control of the collagen type 2 promoter, showed development of tumors that are similar to those observed in human enchondromatosis.⁴³⁷ The clinical significance of these observations are uncertain, as most cases of enchondromatosis are caused by mutations in the *IDH1* and *IDH2* genes.^{438,439}

Biallelic loss of function mutations in PTHr1 are also the cause for Eiken syndrome,⁴⁴⁰ which is characterized by a skeletal dysplasia with severely retarded ossification, principally of the epiphyses, pelvis, hands, and feet, as well as abnormal modeling of the bones. Duchatelet et al.⁴⁴¹ mapped Eiken syndrome to chromosome 3p near the *PTHr1* gene. Affected individuals were homozygous for a nonsense mutation in the carboxy-terminal cytoplasmic tail of the *PTHr1* gene (p.R485X). In a 7-year-old boy with Eiken syndrome, who was born to unaffected first-cousin parents, Moirangthem et al.⁴⁴² identified a homozygous missense mutation at a conserved residue in the *PTHr1* gene (p.E35K). Finally, nonsyndromic primary failure of tooth eruption (PFE) is caused by heterozygous mutations in the *PTHr1* gene.⁴⁴³ Three distinct mutations, namely c.1050-3C > G, c.543+1G > A, and c.463G > T, were identified in 15 affected individuals from four multiplex pedigrees. All mutations truncate the mature protein and therefore should lead to a functionless receptor.

Some cases of Jansen metaphyseal chondrodysplasia have been found to be caused by constitutively activating mutations of the *PTHr1* gene.^{444–446} This autosomal-dominant disorder is characterized by short-limbed dwarfism caused by impaired terminal chondrocyte differentiation and delayed mineralization, accompanied by hypercalcemia.^{444,445} Interestingly, constitutive activation appears to result predominantly in excessive G_s activity because adenylyl cyclase activity is increased and phospholipase C activity is unchanged in COS-7 cells expressing mutated receptors.^{444–446}

Other Class B Receptors That Transduce Hormone Action

Other class B receptors that transduce hormone action include glucagon-like peptide-1, glucagon, calcitonin, and corticotrophin-releasing factor receptors.⁴⁴⁷ Class B receptors usually couple with heterotrimeric G_s proteins, leading to the activation of adenylyl cyclase—which in turn leads to elevated intracellular cyclic AMP levels.^{447,448} (See chapter on Diabetes Mellitus, for a discussion of the role of GLP1 in promoting insulin secretion and the use of GLP1 analogues or inhibitors of GLP1 breakdown in therapy.)

CLASS C RECEPTORS THAT TRANSDUCE HORMONE ACTION

Calcium-Sensing Receptors

The calcium-sensing receptor (CaSR) is located on the long arm of chromosome 3 (3q21.1).⁴⁴⁹ The CaSR has a large amino-terminal domain that contains nine potential glycosylation sites.⁴⁵⁰ Binding of ionized calcium to the CaSR leads to activation of phospholipase C via activation of G_{q/11} proteins.^{71,450,451}

The CaSR is an integral component of a feedback system that uses PTH and renal tubular calcium reabsorption to keep the serum concentrations of ionized calcium within a narrow physiologic range.⁴⁵² Increased extracellular ionized calcium concentrations activate CaSRs in parathyroid chief and renal tubular epithelial cells, leading to decreased PTH release and renal tubular calcium reabsorption.^{450,453} When ionized calcium concentrations fall, CaSR activation decreases—leading to increased PTH release and enhanced renal tubular calcium reabsorption.^{450,453}

The CaSR also binds magnesium, and thus PTH secretion can be inhibited by elevated serum concentrations of magnesium with consequent hypocalcemia.⁴⁵⁴ The CaSR may participate in magnesium homeostasis by altering reabsorption of magnesium in the thick ascending limb of Henle in the kidneys.^{455,456} It is probable that increased peritubular levels of magnesium activate renal CaSRs, leading to inhibition of reabsorption of magnesium from the thick ascending limb of Henle—which in turn leads to increased renal excretion of magnesium.^{455,456}

Both loss of function and gain of function mutations in the CaSR have been described in patients with hypocalcemia and hypercalcemia, respectively.⁴⁵⁷ Familial (benign) hypocalciuric hypercalcemia type 1 (FHH1) and neonatal severe hyperparathyroidism (NSHPT) are caused by loss-of-function mutations of the *CaSR* gene.^{458,459} Most of these mutations are located in the amino-terminal extracellular domain.^{460,461} With few exceptions, individuals heterozygous for loss-of-function mutations have FHH1, a benign condition characterized by very low urinary calcium excretion, mild hypercalcemia, normal or slightly elevated serum PTH levels, and few if any symptoms of hypercalcemia or hyperparathyroidism. In contrast, individuals homozygous for such mutations will develop NSHPT, a life-threatening condition characterized by severe hypercalcemia, markedly elevated serum PTH levels and skeletal defects.^{462,463} Therefore children of consanguineous FHH parents are at risk for NSHPT.^{460,464,465} Occasionally, infants with NSHPT are heterozygous for *CaSR* gene mutations that encode a dominant negative receptor protein.⁴⁶⁶ In most cases, FHH1 is transmitted in an autosomal dominant manner, but autosomal recessive inheritance has been described in one kindred in which the CaSR mutation was only weakly inactivating.⁴⁶⁷

Decreased CaSR function impedes calcium ion suppression of PTH release and renal tubular calcium reabsorption.⁴⁵⁹ Thus FHH1 is characterized by mild hypercalcemia that is accompanied by inappropriately normal or elevated serum PTH levels and by relatively low urinary calcium excretion.^{462,468,469} Individuals with FHH1 may also have hypermagnesemia as a result of decreased peritubular inhibition of magnesium reabsorption from the thick ascending loops of the kidneys by the CaSR.⁴⁵⁶ In addition to FHH1, there are two other variants, FBH type 2 (FBH2) and FBH type 3 (FBH3), that are caused by heterozygous loss of function mutations in *GNA11*⁴⁷⁰ and *AP2S1*,⁴⁷¹ respectively.

FHH1 is caused by heterozygous loss-of-function mutations of the *CaSR* gene on 3q21.1.⁴⁷² Two other chromosomal loci have been identified in patients with FBH who do not have *CaSR* gene mutations. FHH2 has been mapped to *GNA11*, which encodes the α subunit of G11, located 19p13.3 and is biochemically and clinically similar to FHH1.^{472,473} FBH3, which is also known as the Oklahoma variant (FHH_{OK}), is caused by mutations in the *AP2S1* gene located at 19q13.^{472,474} Adults with FHH3 have hypophosphatemia, elevated serum PTH levels, and osteomalacia, in addition to the clinical and biochemical findings found in individuals with FHH1 and FHH2.^{472,475} NSHPT is characterized by severe hypercalcemia accompanied by elevated circulating PTH levels,

undermineralization of bone, rib cage deformity, and multiple long-bone and rib fractures.⁴⁶²

Activating mutations of the *CaSR* gene cause autosomal-dominant hypocalcemia type 1 (ADH1), as increased CaSR function leads to increased calcium ion suppression of PTH release and suppression of renal tubular calcium reabsorption.^{451,472, 476–480} ADH1 is characterized by hypocalcemia and hypomagnesemia, accompanied by inappropriately normal or increased urinary calcium excretion and inappropriately normal or low serum PTH levels.^{451,478–480} Patients with ADH1 may be asymptomatic or may present with tetany, muscle cramps, or seizures during infancy or childhood.^{459,478–480} Similar to inactivating mutations, most activating mutations are located in the amino-terminal extracellular domain.^{476,478,479,481} Treatment of patients with ADH1 with activated forms of vitamin D (e.g., calcitriol) is complicated, as normalization of serum calcium levels is associated with worsening of hypercalciuria, hence further increasing the risk of nephrolithiasis, in nephrocalcinosis, and in renal impairment.^{478,479} In contrast, urinary calcium excretion is not excessive in patients with ADH2, who have activating mutations in *GNA11*.^{470,482,483} *GNA11* encodes the α subunit of G11, the principle G protein that couples to CaSR in parathyroid cells but not in the kidney.

Some patients with activating mutations of the *CaSR* gene will develop Bartter syndrome type V, which similar to other types of Bartter syndrome, is characterized by hypokalemic metabolic alkalosis and by hyperaldosteronism caused by elevated renin levels.^{484,485} Patients with Bartter syndrome type V, unlike patients with other types of Bartter syndrome, may also have symptomatic hypocalcemia and are at risk for developing nephrocalcinosis because of hypercalciuria.^{484,485} Evidence from in vitro functional expression studies suggests that patients with mild or moderate heterozygous gain-of-function mutations of the CaSR develop ADH1, whereas those with severe heterozygous gain-of-function mutations of the CaSR will also develop Bartter syndrome type V.^{484–486}

Some single-amino-acid polymorphisms of the *CaSR* gene appear to predict whole-blood ionized and serum total calcium levels and may increase the risk for bone and mineral metabolism disorders in individuals with other genetic and environmental risk factors for these disorders.^{487–489} Individuals heterozygous or homozygous for a Gln1011Glu *CaSR* gene polymorphism tend to have higher calcium levels than individuals with the polymorphism.^{489,490} The 15.4% of 387 healthy young Canadian women, with at least one *CaSR* gene allele with an Ala986Ser polymorphism, were found to have higher total calcium levels than the remainder of the women without the polymorphism.⁴⁸⁸

Another study of 377 unrelated healthy Italian adult males and females found that 24% of study subjects were heterozygous or homozygous for the p.Ala986Ser polymorphism and confirmed the finding that individuals without the polymorphism have lower whole-blood ionized calcium levels than individuals with the polymorphism.⁴⁸⁹ The p.Ala986Ser polymorphism has also been associated with Paget disease and primary hyperparathyroidism.^{490–492} Individuals with a less common Arg990Gly polymorphism tend to have lower whole-blood ionized calcium levels than individuals without the polymorphism.⁴⁸⁹ The p.Arg990Gly polymorphism has been found to be associated with hypercalciuria and nephrolithiasis.^{492,493}

Autoantibodies against the CaSR that interfere with binding of calcium to the receptor may cause autoimmune hypocalciuric hypercalcemia.⁴⁹⁴ These patients have primary hyperparathyroidism with the clinical and biochemical features of patients with FHH1.⁴⁹⁴ Conversely, autoantibodies that activate the CaSR are a cause of autoimmune-acquired

hypoparathyroidism.⁴⁹⁵ Both conditions may occur in association with other autoimmune conditions (such as autoimmune thyroiditis), with celiac disease in patients with autoimmune hypocalciuric hypercalcemia, and with autoimmune thyroiditis and autoimmune polyglandular syndrome types 1 and 2 in patients with autoimmune-acquired hypoparathyroidism.^{494–496} Autoantibodies that activate the CaSR were found in approximately one-third of individuals with acquired hypoparathyroidism.⁴⁹⁶

G PROTEIN GENE DISORDERS

A growing number of human disorders are associated with somatic or germline mutations in genes that encode the subunits of G proteins and lead to either a gain of function or a loss of function in the signaling protein.⁴⁹⁷ Here, we will limit discussion to disorders associated with mutations of the *GNAS* gene that encodes $G\alpha_s$, as this is the most common G protein to cause endocrine disorders.

Inactivating Mutations of the *GNAS* Gene

Pseudohypoparathyroidism type 1a (PHP1a; Albright hereditary osteodystrophy [AHO]) and pseudopseudohypoparathyroidism (PPHP) are caused by heterozygous inactivating mutations of the *GNAS* gene that encodes $G\alpha_s$.^{498,499} PHP1a is characterized by end organ resistance to PTH with consequent hypocalcemia, hyperphosphatemia, and elevated circulating PTH levels. In addition, patients also manifest resistance to other hormones whose receptors couple to Gs, such as GHRH, TSH, gonadotropin, calcitonin, and hypothalamic neurotransmitters. PHP1a patients also have neurocognitive impairment and obesity that reflect the effect of the $G\alpha_s$ in the brain. In addition, patients manifest a constellation of developmental defects that have been termed *Albright hereditary osteodystrophy* and which include heterotopic ossifications, short stature, craniofacial anomalies, and brachydactyly D/E of the hands and feet, characterized by shortened fingers and short fourth and fifth metacarpals.^{500–503} Patients with the associated condition PPHP share the same features of AHO as PHP1a, but do not have hormone resistance.^{500,501,504} The distinction between these two manifestations of the same gene defect is not stochastic but results from a complex mechanism of genomic imprinting that controls transcription of the *GNAS* gene. Hence, patients with a *GNAS* mutation on a maternal allele will develop a more severe form of $G\alpha_s$ deficiency with hormone resistance (i.e., PHP1a), whereas patients with identical mutations on the paternal *GNAS* allele will have a milder condition with normal hormone responsiveness (i.e., PPHP).^{500–504}

Although most cells express $G\alpha_s$ from both parental alleles, in some cells $G\alpha_s$ expression is suppressed from the paternal *GNAS* allele. Thus patients with PHP1a develop hormone resistance that is limited to the thyroid, pituitary somatotrophs, and proximal renal tubule cells because in these cells, $G\alpha_s$ is derived principally from the maternal *GNAS* allele.⁵⁰⁵ Thus patients with maternally inherited inactivating *GNAS* gene mutations express very little $G\alpha_s$ in these cells and develop hormone resistance.⁵⁰⁵ Because $G\alpha_s$ is not expressed from the paternal allele in imprinted tissues, PPHP patients with paternally inherited inactivating *GNAS* gene mutations do not experience a deficit in $G\alpha_s$ protein in these cells, as they will have a normal amount of $G\alpha_s$ protein that is produced from the wild-type maternal *GNAS* allele.⁵⁰⁵ Patients with either PHP1a or PPHP express only 50% of the normal amount of $G\alpha_s$ protein in cells in which $G\alpha_s$ transcription is not controlled by the imprinting mechanism, which leads to haploinsufficiency, and likely accounts for the similar features of AHO that occur in these two conditions. Subjects with paternally inherited *GNAS*

mutations have variable features of AHO without hormonal resistance and have been described to have PPHP, progressive osseous heteroplasia (POH),⁵⁰⁶ or osteoma cutis,^{507–509} based on the clinical phenotype. The basis for these distinctions is unknown.^{498,499}

Subjects with PHP type 1b (PHP1b; MIM 603233) lack typical features of AHO, but may have mild brachydactyly. PTH resistance is the principal manifestation of hormone resistance, but some patients have slightly elevated serum levels of TSH and normal serum concentrations of thyroid hormones as evidence of partial TSH resistance. It was initially thought that PHP1b is caused by inactivating mutations in the *PTHRI* gene.⁵¹⁰ However, no deleterious *PTHRI* gene mutations have been found in patients with PHP1b.^{511,512}

Epigenetic defects in imprinting of *GNAS* are the cause of PHP1b.^{498,499} There are three alternative first exons for *GNAS* (i.e., NESP55, XL α s, and exon A/B) that splice onto exons 2 through 13 and are associated with differentially methylated regions (DMRs). Most relevantly, the exon A/B DMR is methylated in the maternal germ cells and seems to be the principal control element for transcription from exon 1.^{505,513} Loss of methylation in the DMR upstream of exon A/B of the maternal allele is a consistent finding in patients with PHP1b, and this epigenetic defect accounts for decreased $G\alpha_s$ expression from the affected allele.^{514–516} Most cases of autosomal dominant PHP1b are caused by microdeletions on the maternal allele that include exons 3 to 5 or 4 to 6 of the gene encoding syntaxin-16 (*STX16*).⁵¹⁴ Three other maternally inherited microdeletions involving NESP55 and AS (delNESP55/ASdel3-4) or AS (delAS3-4) alone⁵¹⁷ have been identified, and these deletions produce a more global disruption of methylation that includes three *GNAS* DMRs (A/B, XL/AS, and NESP55). The genetic basis for most cases of sporadic PHP1b remains unknown and does not appear to be associated with the *GNAS* locus.⁵¹⁸ These patients have global epigenetic defects in methylation that affect all three DMRs. In some cases, partial⁵¹⁸ or complete⁵¹⁹ paternal uniparental disomy (UPD) for chromosome 20 has been identified, in which two normal copies of *GNAS* are both derived from the father. Paternal UPD would predict a near complete deficiency of $G\alpha_s$ in imprinted cells and tissues in which $G\alpha_s$ is not transcribed from the paternal allele.

$G\alpha_s$ is not expressed in the renal proximal tubules of these patients because of lack of a *GNAS* allele with a maternal epigenotype. In contrast, both of the *GNAS* alleles with paternal epigenotypes are normally expressed in nonimprinted cells in which expression of $G\alpha_s$ from the paternal allele is not suppressed.⁵²⁰

Activating Mutations of the *GNAS* Gene

When a GPCR is activated by a ligand, the activated receptor interacts with the heterotrimeric G protein and allows release of bound GDP from the $G\alpha$ subunit with replacement by GTP. The GTP-bound $G\alpha$ dissociates from the $G\beta\gamma$ dimer, and both complexes are free to associate with downstream signal generating molecules.^{9,10,14} The generation of second messengers is terminated by an intrinsic GTPase within the $G\alpha$ subunit that acts as a timer; hydrolysis of GTP to GDP inactivates $G\alpha$ and increases its affinity for $G\beta\gamma$ —leading to reassociation of an inactive heterotrimeric G protein that is ready for another cycle of receptor-induced activation.^{9,10,14} For $G\alpha_s$, the amino acids Arg201²¹⁴ and Gln227²⁴⁰ are critical to GTPase activity.⁵²⁰ *GNAS* mutations that result in substitutions of these amino acid residues lead to abrogation of GTPase activity and therefore prolong the active state of $G\alpha_s$, thereby leading to constitutive (i.e., receptor ligand-independent) stimulation of adenylyl cyclase.⁵²⁰ Somatic mutations of these residues that

disrupt GTPase activity are present in approximately 40% of GH-secreting and some ACTH-secreting and nonsecreting pituitary tumors; in some parathyroid, ovarian, testicular, thyroid, and adrenal tumors; and in some intramuscular myxomas.^{99,521,522}

More widespread mosaic Arg201²¹⁴ Gα_s mutations that decrease GTPase activity cause fibrous dysplasia or (when tissue distribution of the mutation is very widespread) McCune-Albright syndrome, which is characterized by the triad of café-au-lait spots, polyostotic fibrous dysplasia, and primary endocrine hyperfunction, particularly gonadotropin-independent precocious puberty.^{523–527}

Patients with McCune-Albright syndrome may also have excessive GH production, hyperthyroidism, and hypercortisolism, as well as nodularity of the pituitary, thyroid, and adrenal glands because of the growth-promoting effects of excessive cyclic AMP in these tissues.^{528,529} Hypophosphatemia, which is not uncommon in patients with McCune-Albright syndrome, appears to be caused by excessive production of the phosphatonin fibroblast growth factor (FGF)-23 by fibrous dysplasia skeletal lesions.^{530,531} Patients with McCune-Albright syndrome may also have non-endocrine problems, such as hepatobiliary abnormalities, cardiomyopathy, optic neuropathy, and sudden death.^{532–534}

CYTOKINE RECEPTORS

Cytokines are molecules produced by one cell that act on another cell.⁵³⁵ Thus the term *cytokine* can apply not only to molecules with immunologic functions, but also to hormones. Therefore GH, prolactin, and leptin are classified as type 1 cytokines.⁵³⁶ These and other type 1 cytokines (including interleukins [ILs] 2–9, 11–13, and 15; erythropoietin; thrombopoietin; and granulocyte-colony-stimulating factor) are characterized by a four α-helical bundle structure and signaling via type 1 cytokine receptors.⁵³⁶ Type 2 cytokines include the interferons and IL-10 and do not include hormones.⁵³⁶

Type 1 cytokines are divided into long-chain and short-chain cytokines.⁵³⁶ Prolactin, leptin, and GH belong to the long-chain subclass of type 1 cytokines because their helixes are 25 amino acids in length.⁵³⁶ The short-chain type 1 cytokines, including IL-2 and stem cell factor, have helixes of approximately 15 amino acids in length.⁵³⁶

Structure and Function of Type 1 Cytokine Receptors

All type 1 cytokine receptors have four conserved cysteine residues, fibronectin type 2 modules, a Trp-Ser-X-Trp-Ser motif in the extracellular domain, and a proline-rich Box 1/Box 2 region in the cytoplasmic domain.^{536,537} With the exception of stem cell factor, type 1 cytokine receptors do not contain catalytic domains, such as kinases.⁵³⁶

Type 1 cytokine receptors for long-chain type 1 cytokines require homodimerization for activation.^{536,538} First, the ligand binds a monomeric receptor.^{536,538} Then, the ligand interacts with a second receptor to induce receptor dimerization and activation.^{536,538} Activated receptors then stimulate members of the Janus family of tyrosine kinases (Jak kinases) to phosphorylate tyrosine residues on both the kinase itself and the cytoplasmic region of the receptors.^{536,539} Signal transducers and activators of transcription (STATs) then dock on the phosphorylated cytoplasmic receptor domains or Jak kinases via an SH2 domain, and are tyrosine phosphorylated.⁵³⁶ The phosphorylated STATs then dissociate from the receptors or Jak kinases, form homodimers or heterodimers, and translocate to the nucleus.^{536,540,541} In the nucleus, the STAT dimers bind and alter the activity of regulatory regions of target DNA.^{536,540,542}

There are four Jak kinases.^{540,543} Jak3 is only expressed in lymphohematopoietic cells, whereas Jak1, Jak2, and Tyk2 are expressed in every cell.^{536,544,545} There are seven STATs (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6), which have different SH2 domain sequences that confer different receptor specificities.^{536,539–541}

Cytokine Receptors That Transduce Hormone Action

The actions of GH, prolactin, and leptin are mediated via specific type 1 cytokine receptors.⁵³⁶ Mutations of the growth hormone receptor (GHR) and the leptin receptor have been identified as the bases of specific endocrine disorders (Table 3.3).

Growth Hormone Receptors

The *GHR* gene is located on the short arm of chromosome 5 (5p13.1-p12), and 9 of the 13 exons of the gene encode the receptor.^{546–549} A secretion signal sequence is encoded by exon 2, the amino-terminal extracellular ligand binding domain is encoded by exons 3 through 7, the single transmembrane domain is encoded by exon 9, and the carboxy-terminal cytoplasmic domain is encoded by exons 9 and 10.^{546–549} Growth hormone binding protein (GHBP) is produced by proteolytic cleavage of the extracellular domain of the GHR from the rest of the receptor.⁵⁵⁰ Approximately 50% of circulating GH is bound to GHBP.⁵⁵⁰

Binding of GH to its receptor induces receptor dimerization and association with JAK2, a member of the Janus kinase family, which results in self-phosphorylation of JAK2 and a cascade of phosphorylation of cellular proteins, including Stat1, Stat3, and Stat5.^{551–555} The most critical of these proteins is STAT5b, which couples GH binding to the activation of gene expression that leads to the intracellular effects of GH, including synthesis of IGF-1, insulin-like growth factor binding protein 3 (IGFBP3), and acid labile subunit (ALS). The phosphorylated STATs translocate to the nucleus, where they regulate GH-responsive genes.^{552–555} In particular, GH indirectly controls growth by regulating production of IGF-1—which has direct effects on cell proliferation and hypertrophy.⁵⁵⁶ Jak2 also activates the mitogen activated protein (MAP) kinase and insulin receptor substrate pathways.^{557–559} However, the extent to which these pathways contribute to GH action is as yet unknown.⁵⁴⁹

Patients are considered to have growth hormone insensitivity (GHI) if they do not exhibit appropriate growth and metabolic responses to physiologic levels of GH.⁵⁵⁰ The phenotype of GHI is variable and ranges from isolated moderate postnatal growth failure to severe postnatal growth failure, accompanied by the classic features of Laron syndrome in Ecuadorian patients with GHR deficiency.^{550,560–564} Features of GHR deficiency include frontal temporal hairline recession, prominent forehead, decreased vertical dimension of face, hypoplastic

TABLE 3.3 Cytokine Receptors and Clinical Conditions Associated With Receptor Mutations

Receptor	Germline Mutation	Endocrine Disorder
Growth hormone receptor	Some inactivating (heterozygous) Inactivating (homozygous, compound heterozygous)	Partial growth hormone insensitivity with mild to moderate growth failure Growth hormone insensitivity/ Laron syndrome with moderate to severe postnatal growth failure
Leptin receptor	Inactivating (homozygous)	Obesity and hypogonadotropic hypogonadism

nasal bridge, shallow orbits, blue sclera, small phallus before puberty, crowded permanent teeth, absent third molars, small hands and feet, hypoplastic fingernails, hypomuscularity, delayed age of onset for walking, high-pitched voice, increased total and LDL cholesterol, and fasting hypoglycemia.^{550,564} All patients with GHI have normal or elevated circulating GH levels, markedly decreased circulating IGF-1 levels, and a delayed bone age.⁵⁵⁰

Patients homozygous or compound heterozygous for deletion of exons 5 and 6—or homozygous or compound heterozygous for numerous nonsense, missense, frameshift, and splice-point mutations throughout the *GHR* gene—have been found to have GHI characterized by severe postnatal growth failure and usually low or absent circulating GHBP levels.^{550,565–574} Patients homozygous or compound heterozygous for the p.Arg274Thr or the p.Gly223Gly splice mutations that result in a truncated receptor that cannot be anchored to the plasma membrane (or that result in the p.Asp152His missense mutation that interferes with GHR dimerization) have normal circulating GHBP levels.⁵⁵⁰

Patients heterozygous for mutations that alter the GHR have dimerization complexes that consist of two wild-type receptors, a wild-type receptor and a mutant receptor, and two mutant receptors. Thus heterozygosity for loss-of-function *GHR* gene mutations may have a dominant negative effect because the wild-type receptor/mutant receptor dimers may not be able to function normally.⁵⁷⁵ As expected from this phenomenon, some patients with moderate to severe growth failure have been found to be heterozygous for loss-of-function point or splice mutations of the *GHR* gene that alter the cytoplasmic or extracellular domains.^{550,576–579} In some cases, nonsense-mediated mRNA decay can lead to degradation of the aberrant mRNA and prevent a potential dominant negative effect.⁵⁸⁰

Some patients with severe short stature and GHI do not have GHR mutations. Rather, they have defects in GHR-mediated intracellular signaling—including impaired STAT activation.⁵⁸¹ Some patients with GHI were found to have STAT5b mutations.^{582–586} Unlike patients with GHR mutations, patients with STAT5b mutations also exhibited severe neurocognitive delay, chronic lung disease (lung fibrosis and/or lymphoid,

interstitial pneumonia, severe eczema, T-cell lymphopenia, and abnormal T-cell function.^{583,587} Patients with GHI can now be successfully treated with recombinant IGF-1,⁵⁸⁸ but this does not result in a normal adult height, in contrast to GH deficiency in which GH therapy can achieve normal adult height.

LEPTIN RECEPTORS

The leptin receptor (*LEPR*, also known as *Ob-R*) gene is located at 1p31. There are five isoforms of *LEPR* because of alternative splicing of the *LEPR* gene transcript (Fig. 3.5).⁵⁸⁹ Only the Ob-Rb isoform contains both the Jak kinase binding and STAT motifs necessary to maximally transduce the effects of leptin.⁵⁸⁹ The Ob-Ra, Ob-Rc, and Ob-Rd isoforms contain intact extracellular and transmembrane but are missing the STAT motif from their cytoplasmic domains.⁵⁸⁹ The Ob-Re isoform is missing the transmembrane and cytoplasmic domains.⁵⁸⁹ Thus the Ob-Rb isoform is thought to be the main isoform involved in mediating the effects of leptin.⁵⁸⁹

Three sisters from a consanguineous kindred were found to be homozygous for a splice mutation in the *LEPR* gene that resulted in expression of an 831-amino-acid protein (Ob-Rhd) that lacks transmembrane and cytoplasmic domains.⁵⁹⁰ They had been hyperphagic and morbidly obese since birth.⁵⁹⁰ They were found to have elevated circulating leptin levels, decreased TSH and GH secretion, and failure of pubertal development caused by hypogonadotropic hypogonadism.⁵⁹⁰ Heterozygous carriers of the mutation are not morbidly obese and do not have delayed or absent puberty.⁵⁹⁰

Nonsense or missense *LEPR* mutations were identified in 3% of a selected cohort of 300 subjects with early-onset obesity. Individuals with mutations had hyperphagia, severe obesity beginning in the first year of life with a mean BMISDS of +5.1, high rate of childhood infections, altered immune function, with moderately reduced CD4 counts (988 vs. 1100 cells/mL³), and delayed puberty because of hypogonadotropic hypogonadism. Childhood growth was normal but final height was at -1.7 to -2 SD because of the lack of a pubertal growth spurt. Importantly, circulating leptin levels were within the range predicted by the elevated fat mass, and clinical features

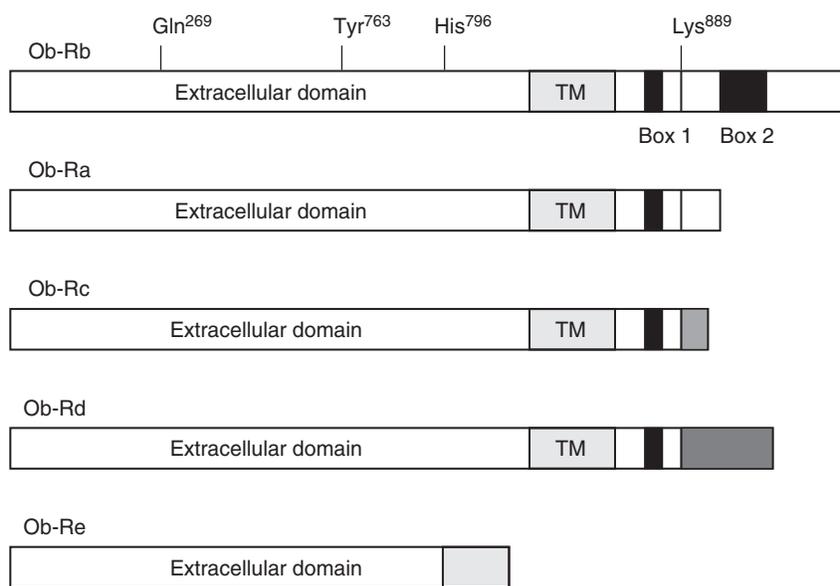


Fig. 3.5 Leptin receptor isoforms. There are five leptin receptor isoforms. Box 1 represents the Jak kinase binding motif, and box 2 represents the signal transducers and activators of transcription (STAT) motif. The Ob-Rb isoform is the only isoform that contains Jak kinase binding and STAT motifs, and it is thus thought to be the main isoform involved in mediating the effects of leptin. The Ob-Ra, Ob-Rc, and Ob-Rd isoforms are missing the STAT motif. The Ob-Re isoform is missing the transmembrane (TM) and cytoplasmic domains. (From Chen, D., & Garg, A. (1999). Monogenic disorders of obesity and body fat distribution. *J Lipid Res*, 40, 1737. With permission.)

were less severe than those of subjects with congenital leptin deficiency.⁵⁹¹ Heterozygous family members had an average BMISDS of +0.6 similar to that of family members with normal leptin receptors. Functional characterization of these missense mutations revealed defects causing intracellular retention, misfolding, or failure to signal to downstream pathways.⁵⁹² Additional novel mutations have been reported that cause similar phenotypes.^{593,594}

RECEPTOR TYROSINE KINASES

The receptor tyrosine kinase (RTK) superfamily consists of 15 receptor tyrosine kinase families (Fig. 3.6).⁵⁹⁵ With one exception, these families consist of receptors with one membrane-spanning domain (see Fig. 3.6).⁵⁹⁵ The single-membrane-spanning receptors typically contain an amino-terminal extracellular portion, a transmembrane helix, a juxtamembrane region, a tyrosine kinase (TK) domain, and a carboxy-terminal region (see Fig. 3.6).⁵⁹⁵ These receptors require dimerization to be maximally activated.⁵⁹⁵⁻⁵⁹⁷ Receptors belonging to the insulin RTK family differ from other RTKs, as they contain two membrane-spanning polypeptide chains, linked by disulfide bonds, to two intervening extracellular peptide chains and thus do not dimerize (see Fig. 3.6).⁵⁹¹

Activation of RTKs leads to phosphorylation of tyrosine residues in the activation loop (A-loop) in the TK domain(s), resulting in activation of the TK(s).^{591,598} Activation of the TK(s), in turn, induces the transfer of phosphate from adenosine triphosphate (ATP) to tyrosine residues in the cytosolic portion of the receptor and in cytosolic proteins that serve as docking sites for second messengers.⁵⁹¹

There is a growing body of evidence suggesting that members of the receptor TK superfamily can directly and indirectly interact with heterotrimeric G proteins. The insulin, IGF-1, and IGF-2 receptors appear to directly interact with G_{i/o} and G_{q/11}, G_{i/o}, and G_i, respectively.⁵⁹⁹ The FGF receptors (FGFRs) appear to directly and indirectly interact with G_s.⁵⁹⁹ Congenital alteration of function of receptors in the insulin and the FGF RTK families leads to endocrine disorders (Table 3.4).

INSULIN RECEPTOR TYROSINE KINASE FAMILY

The insulin RTK family includes the insulin receptor (INSR) and the IGF-1 receptor (IGF1R).⁵⁹⁹ These receptors are

TABLE 3.4 Receptor Tyrosine Kinases and Clinical Conditions Associated with Receptor Mutations

Receptor	Germ-Line Mutation	Endocrine Disorder
Insulin receptor	Inactivating (heterozygous)	Some cases of type A syndrome
	Inactivating (homozygous, compound heterozygous)	Rabson-Mendenhall, Donohue (leprechaunism), and some cases of type A syndromes
IGF-1 receptor	Gene deletion (heterozygous)	Pre- and postnatal growth failure
FGFR1	Inactivating mutation (heterozygous)	Kallmann syndrome, missing teeth, cleft palate
FGFR2	Inactivating mutation (heterozygous)	Apert, Pfeiffer, Crouzon syndromes
FGFR3	Activating mutations (heterozygous)	Achondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans, thanatophoric dysplasia types I and II, and platyspondylic lethal skeletal dysplasias (San Diego types)

FGFR, Fibroblast growth factor receptor; *IGF-1*, insulin-like growth factor-1.

heterotetramers consisting of two α and β subunits in an $\alpha\beta\alpha$ configuration (Fig. 3.7).⁶⁰⁰⁻⁶⁰² The cysteine-rich extracellular α subunits are linked by disulfide bonds, and each α subunit is linked to a plasma membrane-spanning and cytosolic β subunit by disulfide bonds.^{602,603} Each β subunit contains a TK domain and a carboxy-terminal region that contain tyrosine residues.⁵⁹¹

Both insulin and IGF-1 can bind INSRs and IGF1Rs. However, insulin has greater affinity for the INSR and IGF-1 has greater affinity for the IGF1R. Ligand binding alters the conformation of the receptor, resulting in *trans*-autophosphorylation of the carboxy-terminal tyrosine residues on one β subunit by the TK on the other β subunit.^{604,605} The phosphorylated tyrosine residues create motifs that can be bound by Src homology 2 (SH2)-domain-containing proteins, including Shc, Grb-2,

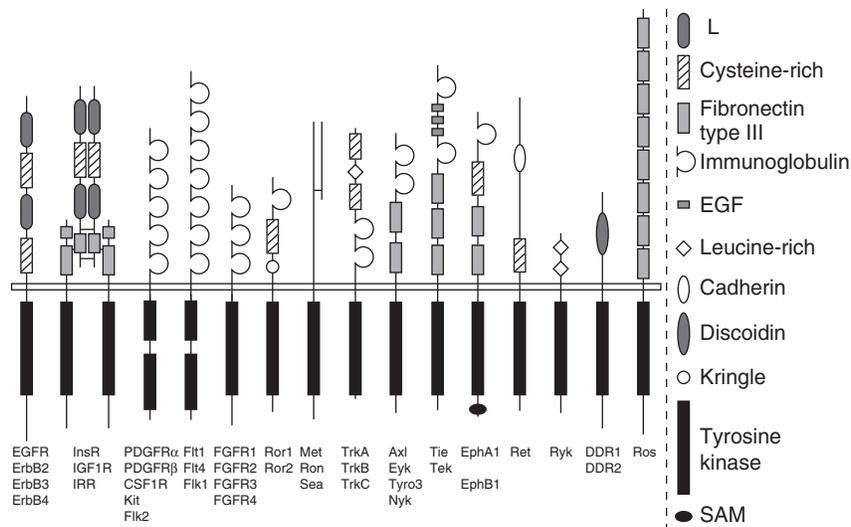


Fig. 3.6 The 15 tyrosine kinase receptor families. Each family has a characteristic extracellular portion and a cytoplasmic portion that contains a tyrosine kinase domain. (From Hubbard, S. R. (1999). Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol*, 71, 344. With permission.)

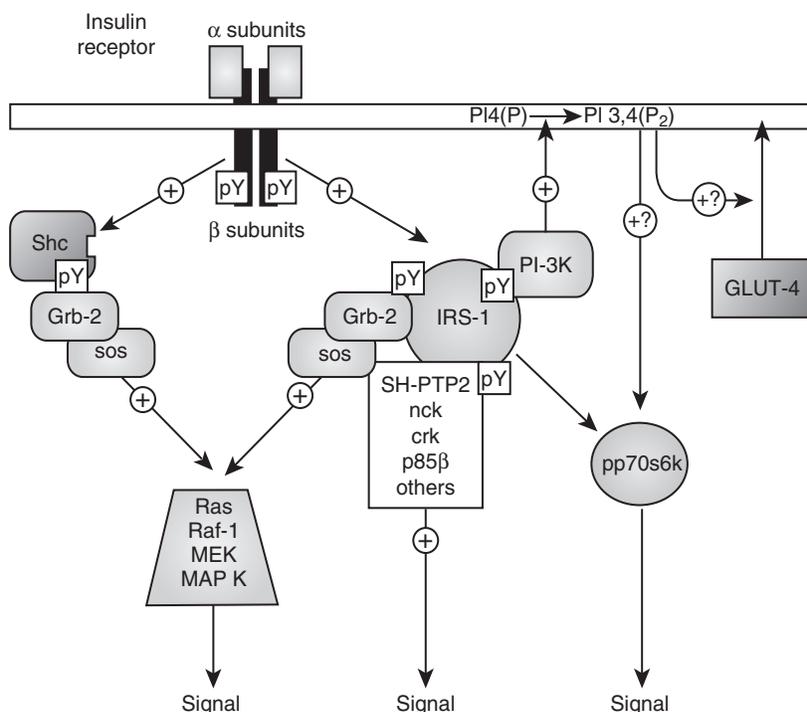


Fig. 3.7 Insulin receptor signaling. Insulin receptor substrate proteins, SH2-domain-containing proteins (including Grb-2 and Shc), and other proteins (including SOS) interact to activate the Ras/Raf-1/MAPK cascade, the PI-3K/PKB cascades, and other enzymes—including SH-PTP2 (SHP2) and p70 (s6k). (Modified from White, M. F. (1997). The insulin signaling system and the IRS proteins. *Diabetologia*, 40, S10. With permission.)

SHP2, nck, phosphatidylinositol-3-kinase (PI3K), and Crk.^{606–610} The receptor TK also phosphorylates tyrosine residues in insulin receptor substrate proteins (IRS), including IRS-1 and IRS-2, that bind INSRs and IGF1Rs.^{610–613} When phosphorylated, these tyrosine residues create motifs that are bound by SH2-domain-containing proteins.^{606,607,609,610,613}

Thus insulin receptor substrates can serve as docking proteins—allowing SH2-domain-containing proteins to indirectly interact with INSRs and IGF1Rs, when steric constraints do not permit direct interactions between the proteins and the receptors.^{606,607,610} Ultimately, IRSs, SH2-domain-containing proteins, and other proteins (including mSOS) interact to activate the Ras/Raf/MAPKK/MAPK and PI3K/protein kinase B (PKB) cascades (see Fig. 3.7).^{609,610,613}

Activation of the Ras/Raf/MAPKK/MAPK cascade increases mitogenesis and proliferation, and activation of the PI3K/PKB cascade increases glucose uptake and glycogen synthesis.^{602,614–619} Evidence suggests that IGF-1 has a greater effect on cell growth than on glucose metabolism because activation of the IGF1R stimulates the Ras/MAPK cascade more than INSR activation.^{602,618} Conversely, it appears that insulin has a greater effect on glucose metabolism because INSR activation stimulates the PI3K/PKB cascade more than IGF1R activation.^{602,619}

THE INSULIN RECEPTOR

The *INSR* gene is located on 19p and contains 22 exons.⁶²⁰ The $\alpha\beta$ half-receptor precursors are derived from proteolysis of a single proreceptor comprised of α and β subunits in tandem and disulfide linkage of these subunits.^{610,620,621} These $\alpha\beta$ half-receptor precursors then join to form a single $\alpha\beta\alpha$ heterotetrameric insulin receptor.⁶²¹ Interestingly, $\alpha\beta$ half-receptor precursors encoded by one allele may combine with $\alpha\beta$

half-receptor precursors encoded by the other allele to form a single insulin receptor.⁶²² This phenomenon explains how heterozygous mutations resulting in impaired β subunit TK activity can have a dominant negative effect because activation of the INSR requires *trans*-autophosphorylation of one β subunit by the other β subunit.⁶²²

Mutations in the insulin receptor lead to Donohue syndrome (leprechaunism), Rabson-Mendenhall syndrome, or type A insulin resistance syndrome. Patients with leprechaunism or Donohue syndrome are severely insulin resistant.^{623,624} They present during infancy with severe intrauterine and postnatal growth retardation, lipoatrophy, and acanthosis nigricans.^{623,625} They also have dysmorphic features that include globular eyes, micrognathia, and large ears.^{623,625} Affected male infants commonly have penile enlargement, whereas affected female infants often have clitoromegaly and hirsutism.^{623,625} In spite of hyperinsulinemia associated with glucose intolerance or diabetes mellitus, the major glucose metabolism problem for these patients is fasting hypoglycemia.^{623,625} Many patients with this condition do not survive past the first year of life.^{623,625} Unlike patients with Rabson-Mendenhall syndrome, patients with leprechaunism do not present with diabetic ketoacidosis.⁶²⁶

Patients with Rabson-Mendenhall syndrome present during childhood with severe insulin resistance.^{625–627} Although patients with this disorder may present initially with fasting hypoglycemia, eventually they develop severe diabetic ketoacidosis that is refractory to insulin therapy.⁶²⁶ Patients with this condition also have acanthosis nigricans, accelerated linear growth, dystrophic nails, premature and dysplastic dentition, coarse facial features, and pineal hyperplasia.^{625–628}

Patients with type A insulin resistance syndrome have acanthosis nigricans and severe inherited insulin resistance in the absence of INSR autoantibodies.^{629–631} Patients with this

syndrome tend to be lean and develop glucose intolerance.^{628,631} Females with this syndrome also exhibit signs of ovarian hyperandrogenism, including hirsutism, severe acne, clitoromegaly, oligomenorrhea, and infertility.^{629–631}

Patients with type B insulin resistance syndrome are distinguished from patients with type A insulin resistance syndrome by the presence of anti-INSR antibodies in the plasma that block insulin binding.^{629–632} Patients with type B insulin resistance syndrome present during adulthood with acanthosis nigricans, ovarian hyperandrogenism, and severe insulin resistance in association with signs of autoimmune disease—including alopecia areata, vitiligo, primary biliary cirrhosis, arthritis, and nephritis.^{629–632} Surprisingly, these patients may present with fasting hypoglycemia that may or may not be accompanied by postprandial hyperglycemia.^{629–632} Hodgkin disease and ataxia-telangiectasia are also associated with this syndrome.⁶³¹ The term *HAIR-AN* (hyperandrogenism, insulin resistance, and acanthosis nigricans) has also been used to describe women with features of types A and B insulin resistance syndromes in association with obesity.⁶³¹ However, this term is imprecise because many women who have been labeled as having *HAIR-AN* may actually have type A or B insulin resistance syndrome or severe polycystic ovary syndrome.⁶³¹

Mutations in the *INSR* have been found in all patients with leprechaunism and Rabson-Mendenhall syndrome, and in 10% to 15% of patients with type A insulin resistance syndrome.^{624,631,633} These mutations are divided into five classes.^{628,631,634} Class I mutations are frameshift or nonsense mutations that prematurely terminate translation and thus interfere with *INSR* synthesis. Class II mutations interfere with posttranslational processing and intracellular trafficking of the *INSR*. Class III mutations decrease insulin binding to the *INSR*. Class IV mutations are point mutations usually located on the intracellular region of the β subunit that decrease *INSR* TK activity. Class V mutations increase *INSR* degradation by increasing insulin-induced endocytosis and degradation of the receptors.

Patients with Rabson-Mendenhall syndrome and leprechaunism are homozygous or compound heterozygous for these mutations.^{623,624,626,631,633–645} Some patients with type A syndrome have been found to be heterozygous for dominant negative β -subunit mutations that reduce TK activity by 75%.^{634,639,646–650} Other patients with type A syndrome have been found to be homozygous or compound heterozygous for α -subunit mutations that interfere with receptor trafficking to the plasma membrane, β -subunit mutations that interfere with TK activity, or mutations that interfere with proreceptor cleavage into α and β subunits. Still other patients have been found to have decreased *INSR* mRNA levels that may be caused by a loss-of-function mutation in the *INSR* gene promoter.^{628,651–655} Interestingly, one patient with leprechaunism with parents with type A syndrome has been described.⁶³⁹ The proband was found to be homozygous for an *INSR* mutation that decreases TK activity, and the parents were found to be heterozygous for the mutation.⁶³⁹

THE INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR

The growth-promoting effects of IGF-1 are mediated by IGF1Rs. IGF1R $\alpha\beta$ subunits are encoded by a single gene.⁶²¹ Like the insulin receptor, an $\alpha\beta$ half-receptor precursor is produced that then joins with a half-receptor precursor that may be encoded from the other allele to form a complete heterotetrameric IGF1R.⁶²¹ The IGF1R has 100-fold less affinity for insulin than for IGF-1.⁶⁵⁶

Patients who are heterozygous for a ring chromosome 15, which results in deletion of the *IGF1R* gene, have intrauterine growth retardation (IUGR) and postnatal growth failure. Other associated features include delayed bone age, mental

retardation, cardiac abnormalities, cryptorchidism, and dysmorphic features that include microcephaly, triangular face, frontal bossing, hypertelorism, and brachydactyly.^{657,658} Similarly, IUGR and postnatal growth failure are commonly found in patients heterozygous for deletion of distal 15q that results in deletion of the *IGF1R* gene. Patients with deletion of distal 15q often have microcephaly, triangular facies, hypertelorism, high-arched palate, micrognathia, cystic kidneys, and lung hypoplasia or dysplasia.^{656,659–661} However, the ring chromosome and deleted area of distal 15q may lead to loss of other genes—and it is unknown to what extent absence of the *IGF1R* gene contributes to the complex phenotype of these patients.^{656,658}

Complete loss of IGF1R function is lethal in the mouse model.^{662,663} Nevertheless, genetic screening of 42 short children, with a history of IUGR (approximately 10% of infants with IUGR remain small), revealed a girl who was compound heterozygous for missense mutations in IGF1R (p.R108Q and p.K115N).⁶⁶⁴ Fibroblasts cultured from the patient had decreased IGF-1 receptor function compared with that in control fibroblasts. Moreover, in a second cohort of 50 children with short stature who had elevated circulating IGF-1 concentrations, Abuzzahab et al.⁶⁶⁴ identified one boy with a heterozygous nonsense mutation (R59X) in IGF1R that reduced the number of IGF-1 receptors on fibroblasts. Children with IGF1R mutations have severe IUGR, microcephaly, and short stature, with biallelic mutations having a more severe phenotype.^{664–667}

In addition to defective *INSR* function, some patients with leprechaunism and Rabson-Mendenhall syndrome are resistant to the glucose lowering or growth promotion of IGF-1 and have abnormal IGF1R function—resulting in decreased ligand binding or altered intracellular signaling.^{656,668–672} No deleterious *IGF1R* gene mutation has been identified in patients with these syndromes, and many patients with leprechaunism and Rabson-Mendenhall syndrome have normally functioning IGF1Rs and no evidence of IGF-1 resistance.^{656,673}

Until recently, no activating mutations of the IGF1R had been found. Microduplication of 15q26.3, which includes the *IGF1R*, is believed to be the cause of an overgrowth phenotype. Recently, a heterozygous activating mutation of the IGF1R was discovered in a patient with extreme tall stature at +3.06 SD, 6'8".⁶⁷⁴ The proband had a birth length at +1.2 SD. His growth spurt started at age 16 years and he continued growing until age 20 years. He had very low levels of IGF-1 (-3 SD), with a delayed and prolonged growth spurt associated with decreased testosterone and SHBG levels in the setting of normal LH and FSH levels. His mother and father were at +1.1 SD and -0.3 SD in height. The variant was studied in mouse fibroblasts that lack the IGF1R and showed hyperstimulation of genes known to be regulated by the IGF1R compared with wild-type IGF1R. It also led to 50- to 70-fold increases in androgen receptor expression. The patient developed a meningioma of the sphenoid that was resected at age 40 years.

THE FIBROBLAST GROWTH FACTOR RECEPTOR FAMILY

There are four members of the FGFR TK family.⁵⁹¹ These are FGFR1, FGFR2, FGFR3, and FGFR4. These receptors consist of a single polypeptide chain that contains an amino-terminal extracellular region, a transmembrane region, and a cytosolic region (see Fig. 3.6).⁵⁹¹ The extracellular region contains three immunoglobulin-like domains: IgI, IgII, and IgIII (see Fig. 3.6).⁶⁷⁵ The cytosolic region contains a TK domain split into two segments (TK1 and TK2) by an intervening amino acid segment.⁶⁷⁵

FGFs are a family of growth factors involved in angiogenesis, wound healing, and embryonic development. The FGFs are heparin-binding proteins and interactions with cell-surface-associated heparan sulfate proteoglycans have been shown to be essential for FGF signal transduction. FGFs are key players in the processes of proliferation and differentiation of wide variety of cells and tissues. In humans, 22 members of the FGF family have been identified, all of which are structurally related signaling molecules.^{675,676} As monomers, FGFs can only bind a single FGFR—forming an inactive 1:1 complex.⁵⁹⁶ FGFR activation by dimerization occurs when two or more FGF molecules in 1:1 complexes are linked by heparan sulfate proteoglycans.⁵⁹⁶

Activation of FGFRs increases receptor TK activity.⁶⁷⁵ Increased TK activity leads to autophosphorylation of a tyrosine residue in the carboxy-terminal region, resulting in a binding site for the SH2 domain of phospholipase C γ (PLC γ).^{677,678} Once PLC γ is bound to this site, it is phosphorylated and activated.^{677,678} In chondrocytes, activation of FGFR3 also induces activation of STAT1.⁶⁷⁹

Fibroblast Growth Factor Receptor 1

Inactivating mutations of the *FGFR1* gene are a cause of autosomal-dominant KS.⁶⁸⁰ Individuals with KS have anosmia and isolated hypogonadotropic hypogonadism.^{681,682} The *FGFR1*, which is located on 8p12, plays a role in olfactory and GnRH neuronal migration from the nasal placode to the olfactory bulb, and in the subsequent migration of the GnRH neurons to the hypothalamus.⁶⁸⁰ Before identification of these *FGFR1* gene mutations, X-linked KS was thought to be caused only by inactivating mutations of the *KAL1* gene.^{681,683,684} *KAL1* encodes anosmin-1, the ligand for the *FGFR1* receptor.⁶⁸⁵ Like *FGFR1*s, anosmin-1 plays a role in olfactory and GnRH neuronal migration to the nasal placode, and in the subsequent migration of GnRH neurons to the hypothalamus.^{681,685,686}

There is a high penetrance for anosmia and signs of hypogonadotropic hypogonadism (including lack of puberty, microphallus, and cryptorchidism) in the 10% of KS patients with X-linked KS because of *KAL1* gene mutations.⁶⁸¹ Female carriers of *KAL1* gene mutations do not have anosmia or isolated hypogonadotropic hypogonadism.⁶⁸¹ In contrast to patients with KS caused by *KAL1* gene mutations, the approximately 10% of KS patients with *FGFR1* gene mutations (even within the same kindred) exhibit variable phenotypes, ranging from anosmia and complete hypogonadotropic hypogonadism (characterized by cryptorchidism and microphallus in males and absent pubertal development in both genders) to anosmia or delayed puberty.^{680,687,688}

It has also been noted that in most kindreds with *FGFR1* gene mutations, females present with more mild KS phenotypes than males.^{680,688} Female carriers may even be asymptomatic.^{680,688} Because the *KAL1* gene is located on the X chromosome, females may produce more anosmin-1 than males.⁶⁸⁰ Thus a possible explanation for milder KS phenotypes in females with *FGFR1* gene mutations may be that the increased anosmin-1 levels in females may lead to increased anosmin-1-induced activation of the mutant *FGFR1*s that may partially compensate for the mutation.⁶⁸⁰ Interestingly, missing teeth and cleft palate are not an uncommon finding in individuals with KS resulting from *FGFR1* gene mutations, whereas unilateral renal agenesis and bilateral synkinesia are associated with KS arising from *KAL1* gene mutations.⁶⁸⁷

Fibroblast Growth Factor Receptors 2–4

A diverse group of skeletal disorders are caused by activating mutations in the *FGFR1* gene, as well as in the related genes encoding *FGFR2* and *FGFR3*. In general, gain-of-function

mutations in *FGFR1* and *FGFR2* cause most of the syndromes involving craniosynostosis, whereas the dwarfing syndromes are largely associated with *FGFR3* mutations. Osteoglophonic dysplasia is a “crossover” disorder that has skeletal phenotypes usually associated with *FGFR1*, *FGFR2*, and *FGFR3* mutations.⁶⁸⁹ Osteoglophonic dysplasia is caused by missense mutations in highly conserved residues comprising the ligand-binding and transmembrane membranes of *FGFR1*, thus defining novel roles for this receptor as a negative regulator of long bone growth.⁶⁸⁹ Gain-of-function mutations in genes encoding the other FGF receptors cause related skeletal disorders: Pfeiffer syndrome (activating mutations of *FGFR1* and *FGFR2*), Crouzon syndrome (gain-of-function *FGFR2* mutations), Crouzon syndrome with acanthosis nigricans (an *FGFR3* mutation), Apert syndrome (*FGFR2* mutations), and craniosynostosis (gain-of-function *FGFR3* mutations).⁶⁷⁵ Several autosomal-dominant short-limb dwarfism syndromes—including achondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN), hypochondroplasia, and three types of platyspondylic lethal skeletal dysplasias (PLSD) (thanatophoric dysplasia I [TDI], thanatophoric dysplasia II [TDII], and San Diego types [PLSD-SD])—are often caused by heterozygous constitutively activating *FGFR3* gene mutations.^{690–693}

Individuals with achondroplasia have activating mutations in the transmembrane domain of *FGFR3*, with the p.Gly380Asn found in more than 95% of achondroplastic patients.^{691,694–696} From 40% to 70% of individuals with hypochondroplasia have an activating p.Asn540Lys mutation in the TK1 domain.^{691,697–700} All individuals with TDII have an activating p.Lys650Glu mutation in the activating loop of the TK2 domain, and more than 90% of individuals with TDI and PLSD-SD have *FGFR3* mutations.^{691,693} Patients with SADDAN have an activating mutation in the same codon as patients with TDII.⁷⁰¹ Instead of the p.Lys650Glu mutation associated with TDII, patients with SADDAN have a Lys650Met mutation.⁷⁰¹ However, unlike patients with TDII patients with SADDAN do not have craniosynostosis and a cloverleaf skull—and often survive past childhood.⁷⁰¹

The *FGFR3* gene is primarily expressed in endochondral growth plates of long bones, brain, and skin pre- and postnatally.^{702,703} Constitutional activation of *FGFR3*s in chondrocytes leads to growth arrest and apoptosis.^{679,704,705} In addition, constitutive activation of *FGFR3*s is also postulated to alter neuronal migration because patients with SADDAN, TDI, and TDII have neurologic abnormalities that may include developmental delay, paucity of white matter, polymicrogyria, dysplastic temporal cortex, dysplasia of nuclei, and neuronal heterotopia.^{701,706–708} Furthermore, constitutive activation of *FGFR3*s in skin fibroblasts and keratinocytes is thought to cause the acanthosis nigricans seen in patients with SADDAN and Crouzon syndrome with acanthosis nigricans.⁷⁰⁹ However, it is not yet known why some activating *FGFR3* mutations affect the skeletal system, central nervous system, and the skin, whereas other activating *FGFR3* mutations only affect the skeletal system.⁷⁰¹

Loss-of-function mutations in *FGFR3* also have been associated with human disease. An uncommon syndrome characterized by camptodactyly, tall stature, scoliosis, and hearing loss (CATSHL syndrome) has been associated with a heterozygous missense mutation (p.R621H) in the TK domain and partial loss of *FGFR3* function.⁷⁰⁹ These findings indicate that abnormal *FGFR3* signaling can cause human anomalies by promoting, as well as inhibiting, endochondral bone growth.⁷¹⁰

NUCLEAR RECEPTORS

Using a phylogenetic tree based on the evolution of two highly conserved nuclear receptor domains (the DNA-binding C domain and the ligand-binding E domain), Laudet divided

nuclear receptors into six related subfamilies. Subfamily 0 contains receptors, such as the embryonic gonad (EGON) and DAX1 receptors, that do not have a conserved C or the E domain (Fig. 3.8).^{711,712} Subfamily 1 includes the peroxisome proliferator-activated retinoic acid, thyroid hormone, and vitamin D₃ receptors. Subfamily 2 includes the hepatocyte nuclear factor-4 α (HNF4A) and retinoid X receptors (RXRs). Subfamily 3 contains the steroid receptors. Evidence suggests that subfamily 3 (which includes the glucocorticoid, androgen, progesterone, and mineralocorticoid receptors) rapidly evolved from a common steroid receptor gene about 500 million years ago.⁷¹³

Subfamilies 4 to 6 contain several nuclear receptors, namely NR4A1-3, NR5A1-2, and NR6A1.⁷¹⁴⁻⁷¹⁶ The nomenclature is as follows: nuclear receptor subfamily 4, group A, and members 1 for NR4A1. NR5A1 is also known as *steroidogenic factor-1* or *SF-1*.⁷¹⁵

General Structure of the Nuclear Receptors

Nuclear receptors are made up of four domains: A/B, C, D, and E (Fig. 3.9).⁷¹¹ Supporting the notion that nuclear receptor subfamilies are derived from a common ancestral orphan receptor, the C and E domains are highly conserved among the subfamilies.⁷¹¹ Mutations of several nuclear receptors are associated with endocrine disorders (Table 3.5).

The A/B domain is located at the amino-terminal and contains the activation function 1 (AF-1)/ τ 1 domain.⁷¹⁷ The AF-1/ τ 1 domain regulates gene transcription by interacting with proteins (such as the Ada and TFIID complexes) that induce transcription.^{718,719} This transactivation function of the AF-1/ τ 1 domain is not dependent on binding of the nuclear hormone receptor to its ligand and is not specific in its choice of DNA target sequences.^{711,720-722} Thus specificity of action of the nuclear hormone receptor is determined by the function of other nuclear hormone receptor domains.

The C domain has characteristics that help to confer specificity of action on each nuclear hormone receptor. This domain consists of two zinc-finger motifs responsible for the DNA-binding activity of the receptor and the selection of dimerization partners.^{723,724} Each zinc-finger module consists of a zinc ion surrounded by the sulfurs of four cysteine residues, resulting in a tertiary structure containing helices.^{723,724} The P-box lies near the cysteines of the first zinc finger and contains the three to four amino acids responsible for specificity of binding to response elements.^{724,725} The D-box consists of a loop of five amino acids attached to the first two cysteines of the second zinc finger that provides the interface for nuclear receptor dimerization.⁷²⁴

The D “hinge” domain contains nuclear localization signals and contributes to the function of the adjacent C and E domains.⁷¹¹ Thus the amino-terminal portion of the domain contributes to DNA binding and heterodimerization and the carboxy-terminal portion contributes to ligand binding.⁷²⁵⁻⁷²⁸ The nuclear localization signal plays a particularly important role in the function of glucocorticoids and mineralocorticoid receptors because these receptors bind their ligand in the cytoplasm and must then localize to the nucleus to alter gene transcription.⁷¹⁷

The E domain is known as the *ligand-binding domain* (LBD) or the *hormone-binding domain*. In addition to ligand binding, the E domain has effects on dimerization and transactivation.⁷¹¹ The LBD consists of 11 to 12 α helices (named H1 through H12) and contains a ligand-binding pocket that is made up of portions of some of the different helices.⁷²⁹⁻⁷³³ For example, the thyroid receptor (TR) LBD has a ligand-binding cavity that includes components from H2, H7, H8, H11, and H12.⁷³³ The contribution of different parts of LBD to the ligand-binding pocket accounts for the finding that mutation of single-amino-acid molecules in different helices of the LBD can interfere with ligand binding.⁷¹⁷

Unlike the AF-1/ τ 1 transcriptional activating factor, the E domain activation factor 2 (AF2-AD) requires ligand binding to function (see Fig. 3.9).⁷³⁰⁻⁷³⁷ Often, when the receptor is not bound by its ligand, corepressor complexes simultaneously bind the LBD and transcriptional machinery consisting of protein complexes that place transcription factors on nucleosome binding sites (see Fig. 3.9).⁷³⁰⁻⁷³⁷ The corepressor complexes then suppress gene transcription by using histone deacetylases to compact the nucleosomes into inaccessible structures (see Fig. 3.9).⁷³⁸⁻⁷⁴¹ Ligand binding induces structural rearrangements in the E domain that lead to release of these corepressor complexes from the transcriptional machinery and the LBD, and exposure of the transcriptional machinery and the LXXLL motif of the AF2-AD to coactivator complexes (see Fig. 3.9).⁷³⁰⁻⁷³⁷ These coactivator complexes have histone acetyltransferase activity that acts to relax nucleosome structures, enabling transcription factors to access nucleosome binding sites (see Fig. 3.9).⁷⁴²

Most nuclear receptors are capable of binding their hormone response element and repress transcription when they are not bound by their ligand.⁷⁴³ However, in the absence of ligand, steroid receptors are bound to a complex of heat-shock proteins instead of their response element and do not appear to repress transcription.⁷⁴⁴

Agonists and antagonists have different effects on the interaction between the ligand binding pocket and AF2-AD. For example, when 17 β -estradiol binds to the estrogen receptor, the position of the AF2-AD containing H12 is altered so that coactivators can access the LBD-binding coactivator binding site.⁷²⁹ However, when the estrogen antagonist raloxifene binds at the same site, the coactivator binding site on H12 remains blocked by other portions of H12.⁷²⁹

Although some nuclear receptors are fully active when bound as monomers to DNA, the hormone receptors in the nuclear receptor superfamily are most active when bound as homodimers or heterodimers (see Fig. 3.9).⁷¹¹ RXRs, HNF 4, and the steroid hormone receptors can bind DNA as homodimers or heterodimers.^{711,745,746} The α isoform of the estrogen receptor (ESR1) is particularly promiscuous, and is able to heterodimerize with HNF4A and retinoic acid receptors, the β isoform of the estrogen receptor (ESR2), RXR, and the thyroid hormone receptors.^{745,746} As a homodimer, RXR binds the dINSRect repeat 1 (DR1).⁷⁴⁷ It can also join the thyroid, vitamin D₃, and peroxisome proliferator-activated receptors to form heterodimers.^{711,748-750}

Interestingly, it has also been suggested that some steroid hormones also act on transmembrane receptors—and these interactions may be responsible for the acute cellular effects of steroids.⁷⁵¹ Progesterone has been shown to interact with the G protein-coupled uterine oxytocin, nicotinic acetylcholine, γ -aminobutyric acid_A, *N*-methyl-D aspartate, and sperm cell membrane progesterone receptors.⁷⁵²⁻⁷⁵⁷ Cell membrane estrogen and glucocorticoids receptors have also been identified.⁷⁵⁸⁻⁷⁶¹

SUBFAMILY 1 NUCLEAR RECEPTORS: THYROID HORMONE, VITAMIN D₃, AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

Thyroid Hormone Receptors

The two thyroid hormone receptor (THR) isoforms—thyroid hormone receptor α (THRA) and thyroid hormone receptor β (THRB)—are encoded by different *c-erbA* genes on chromosomes 17 and 3, respectively.^{728,762} Alternate splicing leads to expression of TR α 1, TR α 2, TR β 1, and TR β 2 with different tissue distributions. TR α 2 does not bind thyroid hormone and its function is not understood. TR α 1 is the predominant subtype in cardiac and skeletal muscle, bone, gastrointestinal tract, and the central nervous system. TR β 1 is the predominant

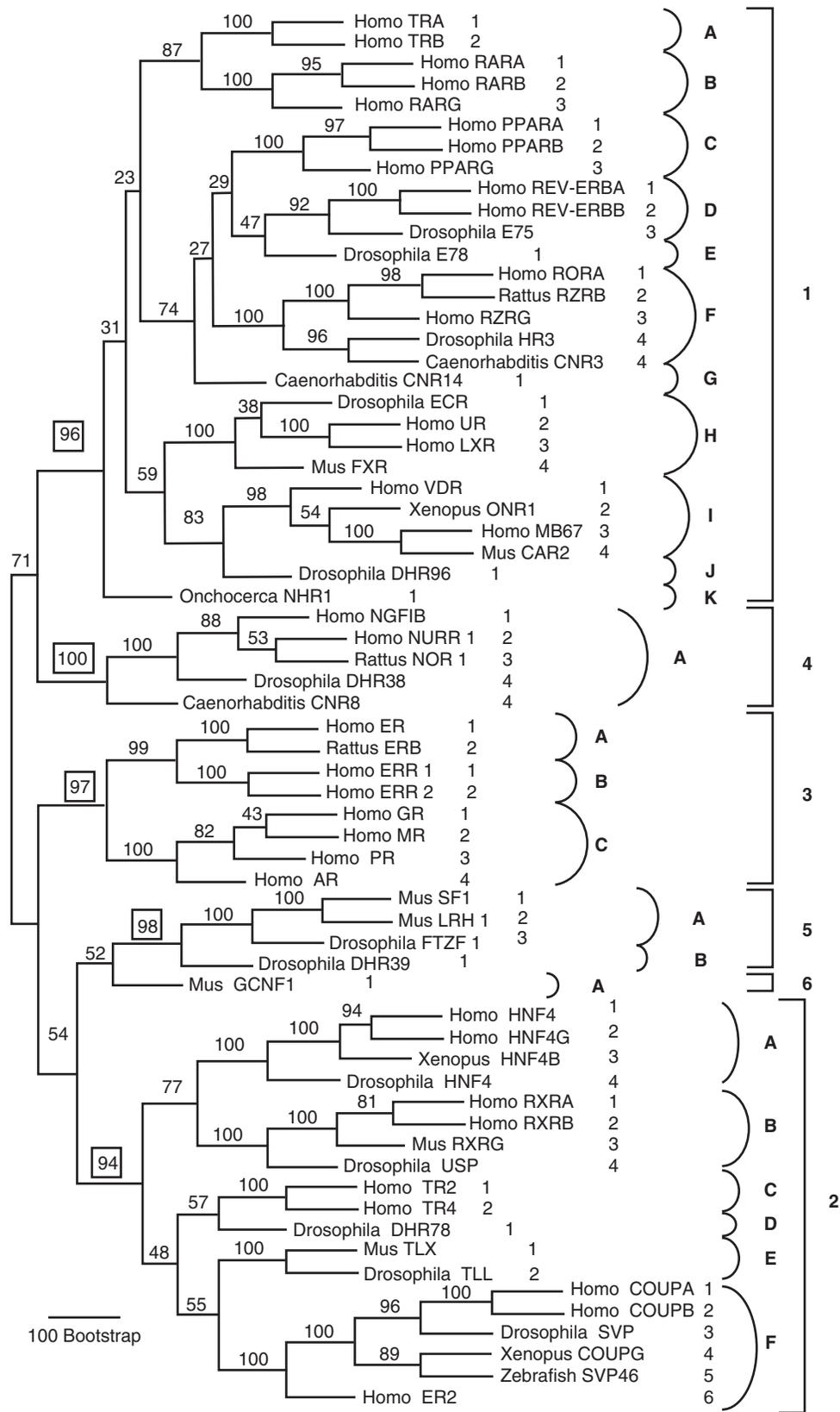


Fig. 3.8 Phylogenetic tree of nuclear receptors based on the evolution of the highly conserved C and E domains. Numbers at the right side of the figure represent subfamilies, and capital letters represent groups of more closely related receptors. The small numbers to the right of the receptor names are used in combination with the subfamily letters and group letters in a proposed nuclear receptor nomenclature. This nomenclature proposes that nuclear receptors should be named NR, followed by subfamily number, group letter, and individual receptor number. Thus the mineralocorticoid receptor is named NR3C2 and the peroxisome proliferator-activating receptor γ receptor is named NR1C3 according to this nomenclature. Numbers to the left of the receptor names represent bootstrap values. Values that define subfamilies with more than one member are boxed. (From the Nuclear Receptors Nomenclature Committee (1999). A unified nomenclature system for the nuclear receptor subfamily. *Cell*, 97, 161. With permission.)

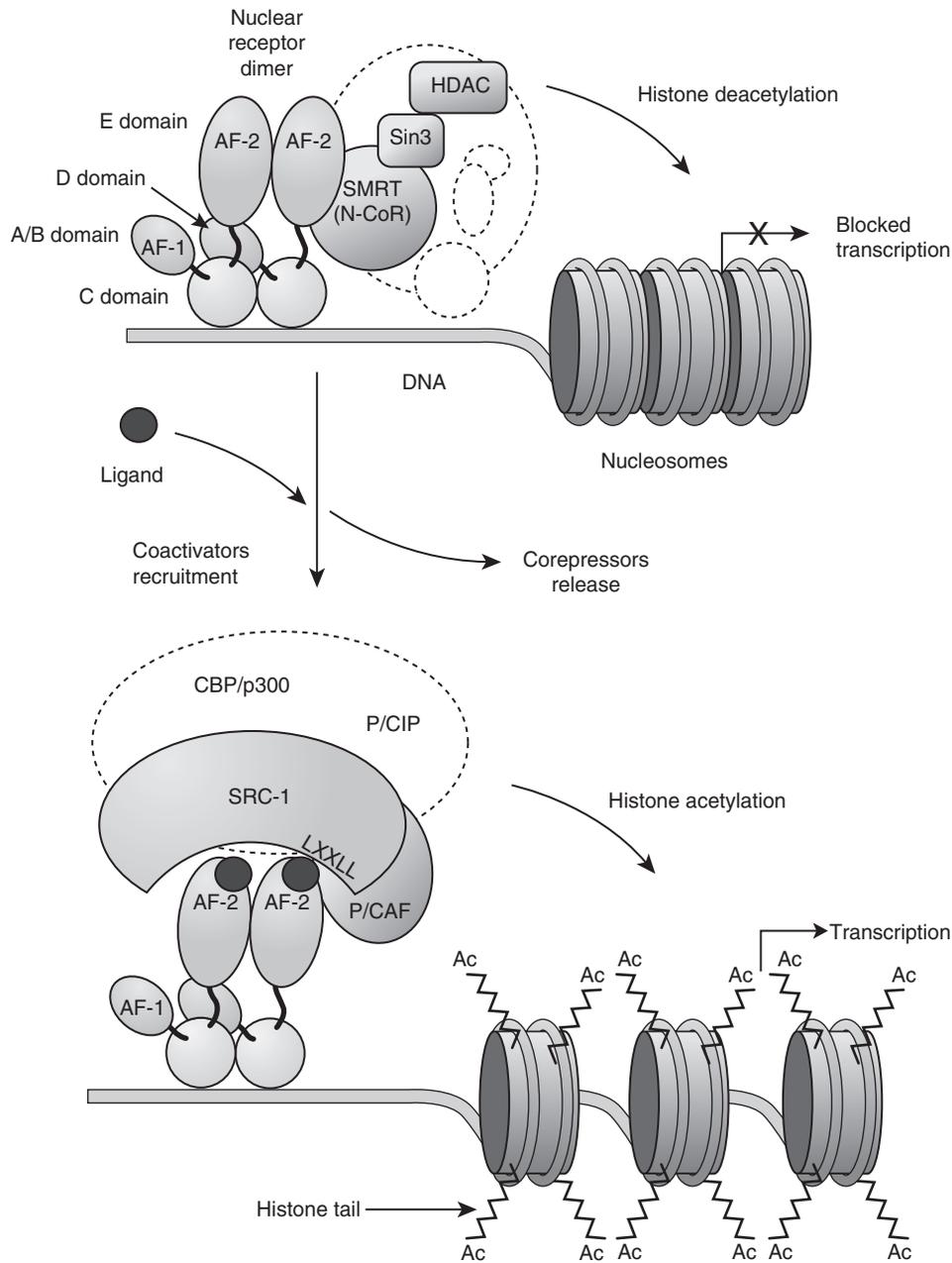


Fig. 3.9 Ligand-induced activation of transcription by nuclear receptors. Often, corepressors, including silencing mediator of retinoid and thyroid hormone receptors (SMRT) and nuclear receptor corepressor (*N-CoR*), bind a nuclear receptor that is not bound by its ligand. These corepressors then associate with Sin3, which in turn associates with a histone deacetylase (*HDAC*). Then, *HDAC* represses transcription by deacetylating histone tails—resulting in compaction of the nucleosomes into structures that are inaccessible to transcription factors. Ligand binding induces structural changes in the E domain that result in release of the corepressor/Sin3/*HDAC* complexes from the receptor and binding of coactivator complexes that may include steroid receptor coactivator 1 (*SRC-1*), p300/cyclic adenosine monophosphate responsive element binding protein (*CBP*), p300/*CBP*-associated factor (*P/CAF*), or p300/*CBP* cointegrator-associated protein (*pCIP*) to the LXXLL motif of the AF2-AD. Then the coactivator complexes induce transcription by acetylating (*Ac*) the histone tails—resulting in decompaction of the nucleosomes into structures that are accessible to transcription factors. Dashed lines are used to represent coactivator and corepressor complexes because their composition *in vivo* is yet unknown. (Modified from Robyr, D., Wolffe, A. P., & Wahli, W. (2000). Nuclear hormone receptor coregulators in action: diversity for shared tasks. *Mol Endocrinol*, 14, 339. Copyright 2000, The Endocrine Society. With permission.)

subtype in kidney and liver, whereas TR β 2 is expressed in hypothalamus and pituitary, as well as retina and cochlea. THR α s that are not occupied by the thyroid hormone triiodothyronine (T_3) exist as homodimers or heterodimers, with RXRs that are attached to DNA thyroid hormone response elements in association with corepressor proteins.⁷⁶³ Thyroid hormone binding induces the release of the corepressors from the THR.⁷⁶³ A coactivator, steroid receptor coactivator-1 (*SRC-1*), is then able to attach to the THR—enabling activation of transcription.⁷⁶³

Generalized resistance to thyroid hormones (GRTH) can be autosomal recessive or autosomal dominant, as the presence of a single normal THRB allele is sufficient for normal receptor function. Autosomal dominant GRTH is caused by the presence of an abnormal THRB that interferes with the function of the normal receptor in a dominant-negative fashion.^{762,764,765} The prevalence of dominant GRTH, based on newborn screening, was reported to be 1:40,000 live births.⁷⁶⁶ Patients with this syndrome have an impaired receptor response to T_3 .⁷⁶²

TABLE 3.5 Nuclear Receptors and Clinical Conditions Associated With Receptor Mutations

Receptor	Germ-Line Mutation	Endocrine Disorder
Thyroid Hormone Receptor β (TR β)	Inactivating mutations (heterozygous and homozygous)	Generalized resistance to thyroid hormones
Vitamin D ₃ receptor	Inactivating mutations (homozygous)	Vitamin D ₃ resistance
PPAR γ 2	Inactivating mutations (heterozygous)	Obesity or early-onset type 2 diabetes mellitus
HNF-4	Inactivating mutations (heterozygous)	Maturity-onset diabetes of the young (MODY) type 1 Hyperinsulinemic hypoglycemia in the newborn period followed in later life with MODY1 in some patients
Glucocorticoid receptor	Inactivating mutations (heterozygous)	Glucocorticoid resistance
Androgen receptor	Inactivating mutations (X-linked recessive)	Androgen insensitivity syndrome, Kennedy disease
Estrogen receptor α (ER α)	Inactivating mutations (homozygous)	Tall stature and incomplete epiphyseal fusion
Mineralocorticoid receptor	Inactivating mutations (heterozygous, homozygous)	Pseudohypoaldosteronism type 1
	Activating mutations (homozygous)	Syndrome of apparent mineralocorticoid excess
DAX1	Inactivating mutations (X-linked recessive)	X-linked adrenal hypoplasia congenita

DAX1, Dosage-sensitive sex-reversal adrenal hypoplasia congenital critical region on the X chromosome gene 1; *HNF-4*, hepatocyte nuclear factor-4; *PPAR γ 2*, peroxisome proliferator-activating receptor γ 2.

They have elevated T₃, reverse T₃, and thyroxine (T₄) levels with slightly high TSH levels.^{762,764,765} Consistent with the TSH elevation and goiter, serum thyroglobulin levels tend to be elevated and radioiodine uptake high. Unlike autoimmune thyrotoxicosis, the T₃:T₄ ratio is normal. The clinical manifestations are variable including some hyperthyroid and some hypothyroid symptoms. Findings include goiter in 65% to 85% of those affected, hyperactivity in 33% to 68%, tachycardia in 33% to 75%, failure to thrive, raised metabolic rate, low bone density, hearing defects, learning disabilities, developmental delay, and delayed bone age.^{762,764,765,767} GRTH can be detected by newborn screening if the program measures T₄, in addition to or in lieu of TSH only, as T₄ would be elevated and TSH would be elevated. The combination of findings normally associated with hyperthyroidism and hypothyroidism is caused by differential expression of TR α versus TR β . The hypothalamus and pituitary express TR β and this leads to elevated TSH levels. Cardiac tissues express TR α and this leads to tachycardia as elevated T₄ and T₃ act on the normal TR α receptor.

Mutations have been found in the D and E domains of THR β s of GRTH patients.^{762,768,769} These mutations alter ligand binding or transactivation.^{762,769,770} However, most mutant THR β s retain the ability to repress transactivation of target genes through interactions with corepressors.^{769,770} Some of the GRTH mutant receptors continue to associate with corepressors and are unable to bind the coactivator SRC-1 even when bound by T₃.^{770,771} Thus mutant THR β s have a dominant negative effect in the heterozygous state because they are able to interfere with the function of wild-type receptors by repressing transcription of target DNA.^{762,769,770}

Patients who carry biallelic loss-of-function mutations of THR β demonstrate more severe clinical abnormalities than patients with heterozygous dominant negative mutations. One patient with a deletion of both THR β alleles presented with deaf mutism, dysmorphic features, and stippled epiphyses.⁷⁷² Another patient homozygous for a THR β mutant ("kindred S receptor"), with an amino acid deletion in the ligand-binding domain, presented with mental retardation, very delayed bone age, and very elevated T₃ and T₄ levels.^{768,773} Heterozygous carriers of the kindred S receptor mutation have milder clinical manifestations of GRTH because this mutant THR β retains corepressor activity and thus has dominant negative effects.^{768,773,774}

Mutations in THRA were not identified until recently.⁷⁷⁵⁻⁷⁷⁷ The first described proband is heterozygous for a nonsense mutation (p.E403X) that inhibits the wild-type receptor in a dominant negative fashion.⁷⁷⁵ The patient had hypothyroid features (growth retardation, developmental delay, skeletal dysplasia, and constipation) that reflect the distribution of

target tissues in which THRA is expressed. This is associated with borderline low or normal T₄ and free T₄, low reverse T₃, borderline high or normal T₃ and free T₃, a low T₄:T₃ ratio, and normal TSH. After thyroxine treatment, normalizing T₄ levels, T₃ levels became elevated. It has been suggested that this may be related to increased type 1 deiodinase activity (which converts T₄ to T₃) and reduced type 3 deiodinase activity (which converts T₄ to reverse T₃ and T₃ to T₂).⁷⁷⁸ The mutant receptor failed to bind radiolabeled T₃ and failed to activate a thyroid hormone responsive reporter gene and inhibited the activity of the wild-type receptor. The second report described a one base insertion in a father and a daughter, causing a frameshift and premature termination at codon 406.⁷⁷⁶ Analyses of the mutant receptor in vitro, after expression in cultured cells, revealed that the mutant receptor fails to respond to stimulation by T₃ and exerts a strong dominant negative effect on the wild-type receptor. This and other subsequent reports^{777,779} identified more clinical features, such as macrocephaly, dysmorphic facies (round, flat face with coarse features) increased birth length and weight, anemia, and slightly elevated cholesterol. Several reports have recently documented different mutations, including missense mutations with partially active receptors.⁷⁸⁰⁻⁷⁸³ These studies demonstrated that the severity of clinical findings correlated with the degree of receptor impairment. The p.A263V mutation exhibited impaired transcription at low T₃ concentrations, but was active at higher T₃ concentrations and was associated with a milder phenotype. Another mutation (p.N359Y), which affects both TR α 1 and TR α 2, was associated with additional clinical features, namely, micrognathia, clavicular agenesis, metacarpal fusion, syndactyly, hyperparathyroidism, and chronic diarrhea.⁷⁸¹

Vitamin D Receptor

Severe rickets, hypocalcemia, secondary hyperparathyroidism, and increased 1,25-dihydroxyvitamin D (calcitriol) levels occur in patients with the autosomal recessive syndrome of "vitamin D resistance."⁷⁸⁴ These patients have defective vitamin D receptors (VDRs). Mutations causing this syndrome have been found in the zinc fingers of the DNA-binding domain (C domain), leading to decreased or abolished receptor binding to regulatory elements of target genes.⁷⁸⁵ Causative mutations have also been found that lead to the production of receptors that have decreased or abolished ability to bind calcitriol and heterodimerize with RXRs, which are required for the VDR to maximally transactivate target genes.^{785,786}

Less severe mutations in the VDR are associated with decreased gastrointestinal calcium absorption and bone

mineral density, even during childhood, and an increased risk for osteoporosis and fractures.^{787–792} However, it has not been possible to replicate these findings in some ethnic groups.^{793–797} Thus other factors (such as estrogen receptor genotype, dietary calcium, and age) probably contribute to the effects of VDR polymorphisms on bone mineral metabolism.^{790,792,798,799} Certain VDR polymorphisms are associated with decreased pre- and postnatal linear growth.^{800,801}

Other important associations have been found with VDR polymorphisms. Homozygous polymorphisms have been reported to be associated with primary hyperparathyroidism.⁸⁰² In addition, the presence of certain VDR alleles is associated with increased risk for the development of early-onset periodontal disease.⁸⁰³ Conversely, absence of such alleles has been associated with familial calcium nephrolithiasis.⁸⁰⁴ Absence of certain alleles may be a risk factor for the development of sarcoidosis.⁸⁰⁵ However, the presence of these alleles is associated with hypercalciuria and nephrolithiasis and increased risk in women for the development of metastatic breast cancer.^{806,807} VDR polymorphisms have also been associated with increased susceptibility to psoriasis, tuberculosis, leprosy, and other infections.^{808–812}

Peroxisome Proliferator-Activating Receptor γ

Peroxisome proliferator-activating receptor γ (PPAR γ) has a role in regulating adipocyte differentiation and metabolism. This formerly orphan receptor was adopted by the ligand prostaglandin J₂. Mutations in the gene encoding PPAR γ cause familial partial lipodystrophy type 3 (FPLD3).⁸¹³

Patients with FPLD3 exhibit fat loss in the arms and legs, severe insulin resistance, early-onset type 2 diabetes mellitus, and severe hypertriglyceridemia. Some heterozygous mutations have a dominant negative effect on the wild-type receptor (V290M and P467L).^{712,814} These mutations lead to amino acid substitutions that disturb the orientation of H12 in the E domain, leading to decreased ligand-dependent transactivation by AF-2/AD and coactivator recruitment.⁷¹² Other mutations are point mutations that cause FPLD3 simply because of haploinsufficiency without a dominant negative effect.^{815–822} In addition, a frameshift mutation that causes a similar phenotype was identified.⁸²³ Another mutation in PPAR γ expanded the phenotype to include muscular, immune, and hematologic features.⁸²⁴ This mutation is thought to induce a conformational change affecting transcriptional activation by the receptor.

A missense mutation leading to a p.Pro115Gln substitution near a site of serine phosphorylation at position 114 that suppresses transcriptional activation in PPAR γ 2 was found in some morbidly obese patients.⁸²⁵ The p.Pro115Gln substitution interferes with phosphorylation of the serine at position 114, leading to increased transcriptional activation by PPAR γ 2—which in turn leads to increased adipocyte differentiation and triglyceride accumulation.⁸²⁵

SUBFAMILY 2 NUCLEAR RECEPTORS: HEPATOCYTE NUCLEAR FACTOR AND RETINOID X RECEPTORS

This subfamily includes the HNF receptors and the RXRs. RXRs form heterodimers with other nuclear receptors (including the estrogen, vitamin D, and thyroid hormone receptors) and with PPAR γ (RXRs are discussed elsewhere in this chapter).

Hepatocyte Nuclear Factor Receptors

Alteration of another orphan nuclear receptor, HNF4A, also causes an endocrine disorder. Mutations of the *HNF4A* gene on chromosome 20 that alter the ligand-binding domain

(E domain) or the DNA-binding domain (C domain) have been found in patients with a form of monogenic diabetes, also known as maturity-onset diabetes of the young type 1 (MODY1).^{826–829} Patients with MODY usually develop diabetes mellitus by the end of the third decade of life. They have a defect in glucose-mediated stimulation of insulin secretion.^{827,830} Several studies have now described a biphasic phenotype of neonatal hyperinsulinism and macrosomia, with later onset of diabetes in patients with *HNF4A* mutations.^{831–835} In most cases, the hyperinsulinism is transient but can be persistent, requiring diazoxide treatment.^{833,834}

Patients who are heterozygous for the p.R76W *HNF4A* mutation also develop a Fanconi-Bickel like syndrome in addition to neonatal hyperinsulinism and macrosomia.^{836,837} The patients manifest the typical proximal tubulopathy of Fanconi-Bickel syndrome, including generalized aminoaciduria, low-molecular-weight proteinuria, glycosuria, hyperphosphaturia, and hypouricemia, plus additional features, including nephrocalcinosis, renal impairment, hypercalciuria with relative hypocalcemia, and hypermagnesaemia.⁸³⁶ The Fanconi-Bickel like phenotype has been recapitulated in a mouse model in which genetic ablation of *Hnf4a* was targeted to the kidney, indicating that *Hnf4a* is required for the formation of differentiated proximal tubules and that loss of *Hnf4a* decreased the expression of proximal tubule-specific genes.⁸³⁸ The p.R76W mutation occurs in the DNA-binding domain of HNF4A, and has been hypothesized to cause defective interaction with major regulatory genes; it remains unknown why patients with other HNF4A mutations do not develop the same renal phenotype.

Carriers of a glycine-to-serine substitution in codon 115 in the DNA-binding domain (C domain) appear to be at increased risk for developing low-insulin diabetes mellitus.⁸³⁹ Hepatocyte nuclear factors 3 (HNF-3 α , -3 β , and -3 γ) are also regulators of the early-onset type 2 diabetes genes *HNF1A*, *HNF4A*, and *IPF-1/PDX-1*—which are associated with MODY types 3, 1, and 4, respectively.^{840–843}

SUBFAMILY 3 NUCLEAR RECEPTORS: THE STEROID RECEPTORS AND GLUCOCORTICOID, ANDROGEN, ESTROGEN, AND MINERALOCORTICOID RECEPTORS

Glucocorticoid Receptors

Glucocorticoids strongly influence cardiovascular tone; have actions on liver, muscle, and adipose tissue; and exert potent antiinflammatory and immunosuppressive effects. Glucocorticoids are important in growth and development, as well as behavior and cognition. They influence multiple cellular processes, including proliferation, differentiation, and apoptosis. All of these actions are mediated by the glucocorticoid receptor (GR), which is a ligand activated transcription factor influencing transcription of approximately 20% of the genome. The gene for the glucocorticoid receptor is known as *NR3C1*. *NR3C1* is on chromosome 5 and consists of 10 exons. Multiple isoforms of the GR exist because of alternative splicing, insertions, deletions, and alternative translation initiation. The two main forms are hGR α and hGR β . hGR β does not bind natural or synthetic glucocorticoids; is expressed in few cell types, such as neutrophils and epithelial cells; and may exert inhibitory effects on the main glucocorticoid receptor hGR α . hGR α is ubiquitously expressed in all tissue but the suprachiasmatic nuclei of the hypothalamus.

Mutations in glucocorticoid receptors cause primary generalized glucocorticoid resistance (PGGR) or primary generalized glucocorticoid hypersensitivity (PGGH).⁸⁴⁴ PGGR is clinically characterized by the presence of elevated plasma cortisol and

ACTH levels, accompanied by the effects of hyperaldosteronism and hyperandrogenism in the absence of striae or central fat deposition.^{728,844–846} ACTH excess leads to adrenocortical hyperplasia, increased cortisol secretion, and increased production of adrenal steroids with androgenic (androstenedione, dehydroepiandrosterone [DHEA], DHEA-S) and mineralocorticoid activity (cortisol, deoxycorticosterone and corticosterone). The clinical spectrum is broad, ranging from asymptomatic to severe hyperandrogenism (characterized by severe acne, hirsutism, irregular menses, and infertility), mineralocorticoid excess (characterized by hypokalemic alkalosis and hypertension), and fatigue.^{845–847} Girls may present with ambiguous genitalia and both girls and boys may present with isosexual precocity.⁸⁴⁸ Clinical manifestations of glucocorticoid deficiency are infrequent and largely limited to fatigue.^{847,849,850} However, there were reports of childhood hypoglycemia.^{851,852}

Both homozygous^{850,851,853,854} and heterozygous^{854–858} mutations have been described. Heterozygous mutations that cause PGGR generally do so by exerting a dominant negative effect on the wild-type receptor.⁸⁴⁴

ACTH-secreting pituitary macroadenomas can also be caused by a frameshift mutation in the *GR* gene that interferes with signal transduction.⁸⁵⁷ Patients with this mutation manifest the symptoms of glucocorticoid resistance. The tumor develops as a result of impaired negative feedback regulation by glucocorticoids on the hypothalamic-pituitary axis.

PGGH was described in a 43-year-old female who presented with visceral obesity, hypertension, hyperlipidemia, and type 2 diabetes, and was found to have a mutation in the *GR* (D401H) that demonstrated increased transactivation of glucocorticoid responsive genes.⁸⁵⁹ The patient had evidence of glucocorticoid resistance in the hypothalamus–pituitary gland–adrenal gland axis and hypersensitivity at the vasculature, adipose tissue, and liver. This was associated with an elevated ACTH and am cortisol but normal urine free cortisol.

Androgen Receptors

The human androgen receptor (*AR*) gene is located on the X chromosome. Known disorders characterized by *AR* dysfunction caused by *AR* gene mutations are only expressed in patients with a 46 XY karyotype.⁸⁶⁰ These mutations may be transmitted from an asymptomatic carrier mother or can be de novo.

More than 1000 mutations have been described in the *AR* gene, with more than 500 mutations causing androgen insensitivity syndrome (AIS).⁸⁶¹ The phenotype of this syndrome can vary in severity and has been divided into mild, partial, and complete forms (MAIS, PAIS, and CAIS).⁸⁶² CAIS is characterized by intraabdominal testes, absence of müllerian structures, absence of androgen-induced body hair, such as pubic and axillary hair, and a female appearance.^{860, 863, 864} PAIS refers to individuals with ambiguous external genitalia with an enlarged clitoris or microphallus and patients with Reifenstein syndrome.⁸⁶³ Reifenstein syndrome is characterized by severe hypospadias with scrotal development and severe gynecomastia.⁸⁶³ MAIS refers to patients with *AR* mutations who are otherwise normal phenotypic males who present with adolescent gynecomastia or later infertility.

The phenotypic heterogeneity of AIS is caused by the varied locations of the mutations causing AIS. Functional consequences of each mutation causing AIS relate to the function of the domain in which the mutation is located. However, the degree of impairment of mutated receptor function in *in vitro* studies does not always correlate with the phenotypic severity of the syndrome.⁸⁶⁵

Mutations in exons that code for the *AR* hormone-binding domain decrease hormone-binding affinity.⁸⁶⁶ However, these

mutations do not abolish the hormone-binding capability of the receptor.⁸⁶⁶ Thus patients with these mutations usually present with PAIS or occasionally with CAIS.^{866–869} Patients with mutations in the hormone-binding domain do not appear to respond to treatment with high doses of testosterone.⁸⁶⁶ Mutations in the DNA-binding domain lead to failure of target gene regulation. Thus patients with these mutations usually manifest the CAIS.^{870, 871}

CAIS is also caused by a point mutation that results in a premature termination codon (p.Gln340ter), with apparent initiation of translation that is downstream of the termination codon. *In vitro* studies showed that the truncated *AR* is expressed at reduced levels.⁸⁷² Numerous other mutations have been described that cause truncation or deletion of the *AR* and complete AIS.^{873–876} Two patients with ambiguous genitalia and partial virilization were found to be mosaic for mutant *ARs*.^{877, 878}

Some patients with Reifenstein syndrome have been found to have a mutation in the DNA-binding domain that abolishes receptor dimerization.⁸⁷⁹ Other patients have been found to have a mutation in a different area of the DNA-binding domain that does not affect receptor dimerization, or to have mutations in the hormone-binding domain in the E domain.^{880–883} The Ala596Thr mutation in the D-box area of the DNA-binding domain has been associated with an increased risk of breast cancer.⁸⁸⁴

AIS is also a feature of Kennedy disease, which is an X-linked recessive condition causing spinal and muscular atrophy.⁸⁶⁷ This condition is caused by extension of a poly-CAG segment in the *AR* gene exon that codes for the N-terminus of the *AR*, leading to an increased number of glutamine residues in the A/B domain.^{885,886} *ARs* with a polyQ region increased to 48 glutamine residues accumulate abnormally in transfected cells because of misfolding and aberrant proteolytic processing.⁸⁸⁷ Because polyQ extension does not completely abolish transactivation, patients with Kennedy disease exhibit a mild partial AIS phenotype consisting of normal virilization accompanied by testicular atrophy, gynecomastia, and infertility.

Gain-of-function mutations in the *AR* have been described in prostate, breast, testicular, liver, and laryngeal cancers.⁸⁶¹

Estrogen Receptors

Two major full-length ESR isoforms have been identified in mammals. Estrogen receptor α (ESR1) was discovered first and mediates most of the known actions of estrogens.⁷²⁸ ESR1 is expressed primarily in the uterus, ovaries, testes, epididymis, adrenal cortices, and kidneys.⁸⁸⁸ Estrogen receptor β (ESR2) was discovered in 1996.⁸⁸⁹ ESR1 and ESR2 share 95% and 50% homology in the DNA-binding domain (DBD) and LBD, respectively.⁸⁹⁰ There is little homology in the amino-terminal between the two isoforms.⁸⁹⁰ ESR2 is expressed primarily in the uterus, ovaries, testes, prostate, bladder, lung, and brain.⁸⁸⁸ Although ESR2 has a high affinity for estrogens, it has less transactivating ability than ESR1 and has not yet been found to be involved in any pathologic condition.^{728,891,892}

There is evidence supporting the existence of other functional ESRs.⁸⁹³ Some of these putative ESRs localize to the cell membrane instead of, or in addition to, the nucleus. A 46-kDa amino-terminal truncated product of ESR1, named ER46, localizes to the cell membrane and mediates estrogen actions that are initiated at the cell membrane.⁸⁹⁴ Another of these putative ESRs has been named ER-X and is postulated to be a GPCR that localizes to the cell membrane.⁸⁹⁵ Another putative receptor is the aptly named heterodimeric putative estrogen receptor (pER), which has been found on the cell and nuclear membranes.⁸⁹⁶ The pER acts as a serine phosphatase.⁸⁹⁶ Five other

estrogen-binding proteins have also been identified, and at least three of them localize to the cell membrane.⁸⁹⁷⁻⁹⁰⁰

It had been thought that androgens provide the principal signals for closure of the epiphyses during puberty. In 1994, however, extensive studies of a 28-year-old man, with incomplete closure of epiphyses and tall stature, demonstrated that he was homozygous for a premature termination mutation of codon 157 in exon 2 of the *ESR1* gene. The patient had continued linear growth despite otherwise normal pubertal development, demonstrating that the ESR mediates epiphyseal closure.⁹⁰¹ Expression of this gene leads to the production of a nonfunctional ESR1 lacking both the DNA- and hormone-binding domains. He was also found to have increased estradiol levels, impaired glucose tolerance with hyperinsulinemia, and decreased bone density.

Further strengthening the association between ESR1 and epiphyseal closure is the observation that women with ESR1-positive breast cancer and a mutation in the B domain (B' allele) of ESR1 have an increased incidence of spontaneous abortion and tall stature.⁹⁰² These associations were not found in female carriers of the allele without breast cancer or with ESR1-negative breast cancer. Thus a second (as yet undiscovered) mutation is likely to play a role in the development of tall stature and spontaneous abortions in female carriers with ESR1-positive breast cancer.

Mineralocorticoid Receptors

Mineralocorticoid resistance is also known as *pseudohypoaldosteronism* (PHA). Both sporadic and familial cases with either autosomal-dominant or autosomal-recessive cases have been reported.⁹⁰³⁻⁹⁰⁷ Clinical presentation of patients with PHA ranges from asymptomatic salt wasting; to growth failure; to chronic failure to thrive, lethargy, and emesis; to life-threatening dehydration accompanied by severe salt wasting.^{904-906,908-910} Patients with the severe forms of PHA typically present within a year of birth and may even present in utero with polyhydramnios because of polyuria.⁹¹¹ Biochemically, the condition is characterized by urinary salt wasting, hyponatremia, elevated plasma potassium, aldosterone, and renin activity, and urinary aldosterone metabolism that are unresponsive to the mineralocorticoid treatment.⁹¹²⁻⁹¹⁴

Two forms of PHA are recognized (types I and II). Autosomal recessive (generalized) PHAI results from mutations in the epithelial sodium channel (ENaC), whereas autosomal dominant (renal) PHAI is caused by mutations in the mineralocorticoid receptor (MR). PHAII is the result of mutations in a family of serine-threonine kinases known as *WNK1* and *WNK4*, which are downstream of aldosterone action.⁹¹⁵ PHAI is characterized by renal tubular mineralocorticoid resistance, whereas PHAII results from mineralocorticoid resistance in the kidney, intestine, or salivary or sweat gland and is also known as *Gordon syndrome*.⁹¹⁶ Patients with these conditions present with hyperkalemia that only responds to treatment with nonchloride ions, such as bicarbonate or sulfate, which increase delivery of sodium to the distal tubule.⁹¹⁷ In general, patients with autosomal dominant PHAI have a mild salt-wasting syndrome and improve with age, whereas patients with autosomal recessive PHAI have severe salt wasting and hyperkalemia, as well as increased sweat and salivary sodium and frequent respiratory tract infections that fails to improve with age.⁹¹⁸

More than 50 different mutations of the MR causing autosomal dominant PHAI have been described (all heterozygous).⁹¹⁸⁻⁹²⁹ There is clear heterogeneity of clinical manifestations within families. Haploinsufficiency caused by mRNA or protein degradation is clearly sufficient to cause autosomal dominant PHAI.^{924,927,930} Yet some mutations have been shown to exert dominant negative effects on the wild-type receptor.⁹²⁷

Genetic disorders that are associated with increased activity of the MR cause severe early-onset hypertension. One such condition, characterized by excessive MR action, is termed the *syndrome of apparent mineralocorticoid excess*.⁹³⁰ Patients with this autosomal-recessive condition can exhibit pre- and postnatal growth failure, hypervolemic hypertension, medullary nephrocalcinosis, and hypokalemic metabolic alkalosis accompanied by hyporeninemic hypoaldosteronism.⁹³¹⁻⁹³⁴ Patients may also be asymptomatic and exhibit only biochemical abnormalities.⁹³⁵ Patients with this syndrome also have increased serum and urinary cortisol-to-cortisone ratios.⁹³⁴ This syndrome is caused by mutations in the 11 β -hydroxysteroid dehydrogenase type 2 (*HSD11B2*) gene that reduce enzymatic activity of the protein.^{933,934,936-938} The 11 β -HSD2 converts the active glucocorticoid cortisol to inactive cortisone; in contrast the type I isoform, encoded by the separate *HSD11B1* gene, has both 11- β dehydrogenase activity and 11-oxoreductase activity and can also convert cortisone to cortisol. The type I isoform is ubiquitously expressed, whereas the type II isoform has more restricted expression and is highly expressed in the kidney, where it is necessary to protect the renal MR from normally higher serum concentrations of cortisol, allowing aldosterone to regulate sodium homeostasis. Thus decreased activity of 11 β -HSD2 increases cortisol levels in kidney cells expressing MR tissues, leading to increased binding and activation of MRs by cortisol.^{933,934}

A transient form of mineralocorticoid resistance, probably because of abnormal maturation of aldosterone receptor function, also exists.⁹³⁹ This variant of PHA is known as *the syndrome of early-childhood hyperkalemia*. Children with this disorder present with failure to thrive or linear growth failure accompanied by hyperkalemia and metabolic acidosis. This condition resolves spontaneously by the second half of the first decade of life.

SUBFAMILY 0 NUCLEAR RECEPTORS: DAX1

Subfamily 0 nuclear receptors include DAX1.⁹⁴⁰ DAX1 plays a role in the regulation of steroid, mullerian-inhibiting substance, and gonadotropin production.

DAX1

The dosage-sensitive sex-reversal adrenal hypoplasia congenital critical region on the X chromosome gene 1 (*DAX1*) is an orphan nuclear receptor because its ligand has not yet been identified.⁹⁴¹ It has homologies in the E domain to other orphan receptors, including RXRs.⁹⁴² However, DAX1 has an unusual DNA-binding C domain that contains a tract of amino acid repeats instead of zinc-finger motifs.⁹⁴² DAX1 inhibits SF-1-mediated transcription. SF-1 is another orphan nuclear receptor that regulates transcription of adrenal and gonadal steroid hydroxylases, mullerian-inhibiting substance, and gonadotropin genes.^{943,944}

DAX1 gene mutations have been identified that cause X-linked adrenal hypoplasia congenita.⁹⁴² Patients with this condition have congenital adrenal insufficiency and are therefore deficient in glucocorticoid, mineralocorticoid, and androgen production.^{942,945} Some 40% of affected male patients present in the first 2 months of life with a salt-wasting adrenal crisis, and the remainder present later in childhood with mineralocorticoid insufficiency, resembling aldosterone synthase deficiency or PHA. Those who present with adrenal crisis can have hyperkalemia, hyponatremia, hypotension, or cardiac arrest, because of both mineralocorticoid and glucocorticoid deficiency in the setting of normal 17-hydroxyprogesterone levels. Patients may have normal cortisol levels. Imaging of

the adrenal by any modality at this age is not helpful, as the condition affects the definitive zone but not the fetal zone of the adrenal. The fetal zone is quite large at birth and does not atrophy until 6 to 12 months of age. Imaging cannot differentiate the fetal from the definitive zones and reports a normal adrenal gland in the first few months of life. Imaging after the first year of life would be expected to show a hypoplastic adrenal. Gonadotropin deficiency and azoospermia also occur in these patients but this does not present until puberty.^{940,945} Patients have findings consistent with hypogonadotropic hypogonadism (because of a combined hypothalamic/pituitary defect) and may have absent pubertal development or puberty that arrests at around Prader stage 3. Female carriers may have delayed puberty.⁹⁴⁰ All mutations that have been found to cause X-linked congenital adrenal hypoplasia are either located in or prevent transcription of the area of the E domain that inhibits SF-1-mediated transcription.^{941,946–948} Thus DAX1 mutations may cause X-linked congenital adrenal hypoplasia by altering SF-1 regulation of gonado- and adrenogenesis.⁹⁴⁶ DAX1 deletion may also occur in the setting of the contiguous gene deletion syndrome, resulting in complex glycerol kinase deficiency (cGKD) if individuals have deletions extending from the GK gene into the Duchene muscular dystrophy (DMD) gene, or involving a significant extension telomeric from DAX1.⁹⁴⁹

Duplications of Xp that include DAX1 in genetic males with XY karyotype lead to sex reversal. DAX1 overexpression represses all aspects of male differentiation beginning with the urogenital ridge progressing to a bipotential gonad, under the influence of WT1 and SF1, to differentiation into a testis under the influence of SF1, SOX9, SRY, and AMH and leads to female external genitalia with variable female internal organs from absent ovaries, uterus, and cervix to normal ovaries with rudimentary müllerian structures. Duplications in this region of the X chromosome are also associated with developmental delay, low-set ears, cleft palate, clinodactyly, hypotonia, thin upper lip, simian creases, and cardiac abnormalities.

SUMMARY

Understanding of receptors that transduce or influence hormone action has increased dramatically. As molecular biology techniques improve, it is expected that knowledge of receptor action will continue to increase at a rapid pace. It is likely that subtle defects in receptor function (such as regulatory or promoter region mutations that increase or decrease receptor gene expression, or mutations in second messenger proteins) will be found that cause endocrine disorders. It is also likely that new receptors will be discovered that transduce or influence hormone action and that endocrine roles will be found for receptors that were not previously thought to mediate or alter hormone action.

REFERENCES

1. Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol Pharmacol*. 2003; 64(6):1271–1276.
2. Milligan G, Bond RA, Lee M. Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol Sci*. 1995;16(1):10–13.
3. Landomiel F, De Pascali F, Raynaud P, Jean-Alphonse F, Yvinec R, Pellissier LP, et al. Biased signaling and allosteric modulation at the FSHR. *Front Endocrinol (Lausanne)*. 2019;10:148.
4. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *Embo J*. 1999;18(7):1723–1729.
5. Vassart G, Costagliola S. G protein-coupled receptors: mutations and endocrine diseases. *Nat Rev Endocrinol*. 2011;7(6):362–372.
6. Horn F, Weare J, Beukers MW, Horsch S, Bairoch A, Chen W, et al. GPCRDB: an information system for G protein-coupled receptors. *Nucleic Acids Res*. 1998;26(1):275–279.
7. Vassart G, Pardo L, Costagliola S. A molecular dissection of the glycoprotein hormone receptors. *Trends Biochem Sci*. 2004;29(3):119–126.
8. Baldwin JM. The probable arrangement of the helices in G protein-coupled receptors. *Embo J*. 1993;12(4):1693–1703.
9. Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, et al. Differential signal transduction by five splice variants of the PACAP receptor. *Nature*. 1993;365(6442):170–175.
10. Wess J. G-protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *Faseb J*. 1997;11(5):346–354.
11. Syrovatkin V, Alegre KO, Dey R, Huang XY. Regulation, signaling, and physiological functions of G-proteins. *J Mol Biol*. 2016;428(19):3850–3868.
12. Wang W, Qiao Y, Li Z. New insights into modes of GPCR activation. *Trends Pharmacol Sci*. 2018;39(4):367–386.
13. Smrcka AV. G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. *Cell Mol Life Sci*. 2008;65(14):2191–2214.
14. Daaka Y, Luttrell LM, Ahn S, Della Rocca GJ, Ferguson SS, Caron MG, et al. Essential role for G protein-coupled receptor endocytosis in the activation of mitogen-activated protein kinase. *J Biol Chem*. 1998;273(2):685–688.
15. Eason MG, Liggett SB. Identification of a Gs coupling domain in the amino terminus of the third intracellular loop of the alpha 2A-adrenergic receptor. Evidence for distinct structural determinants that confer Gs versus Gi coupling. *J Biol Chem*. 1995;270(42):24753–24760.
16. Fields TA, Casey PJ. Signalling functions and biochemical properties of pertussis toxin-resistant G-proteins. *Biochem J*. 1997;321(Pt 3):561–571.
17. Limbird LE. Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. *Faseb J*. 1988;2(11):2686–2695.
18. Rivkees SA, Barbhuiya H, Ilzerman AP. Identification of the adenine binding site of the human A1 adenosine receptor. *J Biol Chem*. 1999;274(6):3617–3621.
19. Reppert SM, Weaver DR, Stehle JH, Rivkees SA. Molecular cloning and characterization of a rat A1-adenosine receptor that is widely expressed in brain and spinal cord. *Mol Endocrinol*. 1991;5(8):1037–1048.
20. Thompson MD, Burnham WM, Cole DE. The G protein-coupled receptors: pharmacogenetics and disease. *Crit Rev Clin Lab Sci*. 2005;42(4):311–392.
21. Lu D, Yan H, Othman T, Turner CP, Woolf T, Rivkees SA. Cytoskeletal protein 4.1G binds to the third intracellular loop of the A1 adenosine receptor and inhibits receptor action. *Biochem J*. 2004;377(Pt 1):51–59.
22. Lu D, Yan H, Othman T, Rivkees SA. Cytoskeletal protein 4.1G is a binding partner of the metabotropic glutamate receptor subtype 1 alpha. *J Neurosci Res*. 2004;78(1):49–55.
23. Levitzki A, Marbach I, Bar-Sinai A. The signal transduction between beta-receptors and adenylyl cyclase. *Life Sci*. 1993; 52(26):2093–2100.
24. Csaba Z, Dournaud P. Cellular biology of somatostatin receptors. *Neuropeptides*. 2001;35(1):1–23.
25. Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC, Patel YC. Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *J Biol Chem*. 2000;275(11):7862–7869.
26. Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC. Receptors for dopamine and somatostatin: formation of heterooligomers with enhanced functional activity. *Science*. 2000; 288(5463):154–157.
27. Pfeiffer M, Koch T, Schroder H, Klutzny M, Kirscht S, Kreienkamp HJ, et al. Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst(3) receptor function by heterodimerization with sst(2A). *J Biol Chem*. 2001;276(17):14027–14036.
28. Born W, Fischer JA, Muff R. Receptors for calcitonin gene-related peptide, adrenomedullin, and amylin: the contributions of novel receptor-activity-modifying proteins. *Receptors Channels*. 2002; 8(3-4):201–209.

29. Born W, Muff R, Fischer JA. Functional interaction of G protein-coupled receptors of the adrenomedullin peptide family with accessory receptor-activity-modifying proteins (RAMP). *Microsc Res Tech.* 2002;57(1):14–22.
30. Lerner UH. Deletions of genes encoding calcitonin/alpha-CGRP, amylin and calcitonin receptor have given new and unexpected insights into the function of calcitonin receptors and calcitonin receptor-like receptors in bone. *J Musculoskelet Neuronal Interact.* 2006;6(1):87–95.
31. Christopoulos A, Christopoulos G, Morfis M, Udawela M, Laburthe M, Couvineau A, et al. Novel receptor partners and function of receptor activity-modifying proteins. *J Biol Chem.* 2003;278(5):3293–3297.
32. Angers S, Salahpour A, Bouvier M. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. *Annu Rev Pharmacol Toxicol.* 2002;42:409–435.
33. McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature.* 1998;393(6683):333–339.
34. Metherell LA, Chapple JP, Cooray S, David A, Becker C, Ruschendorf F, et al. Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nat Genet.* 2005;37(2):166–170.
35. Webb TR, Chan L, Cooray SN, Cheetham ME, Chapple JP, Clark AJ. Distinct melanocortin 2 receptor accessory protein domains are required for melanocortin 2 receptor interaction and promotion of receptor trafficking. *Endocrinology.* 2009;150(2):720–726.
36. Novoselova TV, Hussain M, King PJ, Guasti L, Metherell LA, Charalambous M, et al. MRAP deficiency impairs adrenal progenitor cell differentiation and gland zonation. *FASEB J.* 2018, fj201701274RR.
37. Cooray SN, Chung TT, Mazhar K, Szidonya L, Clark AJ. Bioluminescence resonance energy transfer reveals the adrenocorticotropin (ACTH)-induced conformational change of the activated ACTH receptor complex in living cells. *Endocrinology.* 2011;152(2):495–502.
38. Clark AJ, Weber A. Adrenocorticotropin insensitivity syndromes. *Endocr Rev.* 1998;19(6):828–843.
39. Asai M, Ramachandrapa S, Joachim M, Shen Y, Zhang R, Nuthalapati N, et al. Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity. *Science.* 2013;341(6143):275–278.
40. Biebermann H, Krude H, Elsner A, Chubanov V, Gudermann T, Gruters A. Autosomal-dominant mode of inheritance of a melanocortin-4 receptor mutation in a patient with severe early-onset obesity is due to a dominant-negative effect caused by receptor dimerization. *Diabetes.* 2003;52(12):2984–2988.
41. Tarnow P, Rediger A, Brumm H, Ambrugger P, Rettenbacher E, Widhalm K, et al. A heterozygous mutation in the third transmembrane domain causes a dominant-negative effect on signalling capability of the MC4R. *Obes Facts.* 2008;1(3):155–162.
42. Morello JP, Salahpour A, Petaja-Repo UE, Laperriere A, Lonergan M, Arthus MF, et al. Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. *Biochemistry.* 2001;40(23):6766–6775.
43. Zhu X, Wess J. Truncated V2 vasopressin receptors as negative regulators of wild-type V2 receptor function. *Biochemistry.* 1998;37(45):15773–15784.
44. Oksche A, Rosenthal W. The molecular basis of nephrogenic diabetes insipidus. *J Mol Med.* 1998;76(5):326–337.
45. Calebiro D, de Filippis T, Lucchi S, Covino C, Panigone S, Beck-Peccoz P, et al. Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. *Hum Mol Genet.* 2005;14(20):2991–3002.
46. Ulloa-Aguirre A, Janovick JA, Leanos-Miranda A, Conn PM. Misrouted cell surface GnRH receptors as a disease aetiology for congenital isolated hypogonadotropic hypogonadism. *Hum Reprod Update.* 2004;10(2):177–192.
47. Leanos-Miranda A, Ulloa-Aguirre A, Ji TH, Janovick JA, Conn PM. Dominant-negative action of disease-causing gonadotropin-releasing hormone receptor (GnRHR) mutants: a trait that potentially coevolved with decreased plasma membrane expression of GnRHR in humans. *J Clin Endocrinol Metab.* 2003;88(7):3360–3367.
48. Brothers SP, Cornea A, Janovick JA, Conn PM. Human loss-of-function gonadotropin-releasing hormone receptor mutants retain wild-type receptors in the endoplasmic reticulum: molecular basis of the dominant-negative effect. *Mol Endocrinol.* 2004;18(7):1787–1797.
49. Seifert R, Wenzel-Seifert K. Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedeberg's Arch Pharmacol.* 2002;366(5):381–416.
50. Tiberi M, Caron MG. High agonist-independent activity is a distinguishing feature of the dopamine D1B receptor subtype. *J Biol Chem.* 1994;269(45):27925–27931.
51. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev.* 2001;53(1):1–24.
52. Mason D, Hassan A, Chacko S, Thompson P. Acute and chronic regulation of pituitary receptors for vasopressin and corticotropin releasing hormone. *Arch Physiol Biochem.* 2002;110(1-2):74–89.
53. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. beta-Arrestin: a protein that regulates beta-adrenergic receptor function. *Science.* 1990;248(4962):1547–1550.
54. Mukherjee S, Palczewski K, Gurevich V, Benovic JL, Banga JP, Hunzicker-Dunn M. A direct role for arrestins in desensitization of the luteinizing hormone/choriogonadotropin receptor in porcine ovarian follicular membranes. *Proc Natl Acad Sci U S A.* 1999;96(2):493–498.
55. Bouvier M, Hausdorff WP, De Blasi A, O'Dowd BF, Kobilka BK, Caron MG, et al. Removal of phosphorylation sites from the beta 2-adrenergic receptor delays onset of agonist-promoted desensitization. *Nature.* 1988;333(6171):370–373.
56. Hunzicker-Dunn M, Gurevich VV, Casanova JE, Mukherjee S. ARF6: a newly appreciated player in G protein-coupled receptor desensitization. *FEBS Lett.* 2002;521(1-3):3–8.
57. Troispoux C, Guillou F, Elalouf JM, Firsov D, Iacovelli L, De Blasi A, et al. Involvement of G protein-coupled receptor kinases and arrestins in desensitization to follicle-stimulating hormone action. *Mol Endocrinol.* 1999;13(9):1599–1614.
58. Lazari MF, Liu X, Nakamura K, Benovic JL, Ascoli M. Role of G protein-coupled receptor kinases on the agonist-induced phosphorylation and internalization of the follitropin receptor. *Mol Endocrinol.* 1999;13(6):866–878.
59. Nakamura K, Lazari MF, Li S, Korgaonkar C, Ascoli M. Role of the rate of internalization of the agonist-receptor complex on the agonist-induced down-regulation of the lutropin/choriogonadotropin receptor. *Mol Endocrinol.* 1999;13(8):1295–1304.
60. Nakamura K, Liu X, Ascoli M. Seven non-contiguous intracellular residues of the lutropin/choriogonadotropin receptor dictate the rate of agonist-induced internalization and its sensitivity to non-visual arrestins. *J Biol Chem.* 2000;275(1):241–247.
61. Frenzel R, Voigt C, Paschke R. The human thyrotropin receptor is predominantly internalized by beta-arrestin 2. *Endocrinology.* 2006;147(6):3114–3122.
62. Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS. Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *J Biol Chem.* 2000;275(22):17201–17210.
63. Lohse MJ. Molecular mechanisms of membrane receptor desensitization. *Biochim Biophys Acta.* 1993;1179(2):171–188.
64. Morris AJ, Malbon CC. Physiological regulation of G protein-linked signaling. *Physiol Rev.* 1999;79(4):1373–1430.
65. Scarce-Levie K, Lieberman MD, Elliott HH, Conklin BR. Engineered G protein coupled receptors reveal independent regulation of internalization, desensitization and acute signaling. *BMC Biol.* 2005;3:3.
66. Barak LS, Oakley RH, Laporte SA, Caron MG. Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. *Proc Natl Acad Sci U S A.* 2001;98(1):93–98.
67. Im DS. Discovery of new G protein-coupled receptors for lipid mediators. *J Lipid Res.* 2004;45(3):410–418.
68. Krasnopurov VG, Bittner MA, Beavis R, Kuang Y, Salnikow KV, Chepurny OG, et al. alpha-Latrotoxin stimulates exocytosis by

- the interaction with a neuronal G-protein-coupled receptor. *Neuron*. 1997;18(6):925–937.
69. O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, et al. The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron*. 1993;11(1):41–52.
 70. Silve C, Petrel C, Leroy C, Bruel H, Mallet E, Rognan D, et al. Delineating a Ca²⁺ binding pocket within the venus flytrap module of the human calcium-sensing receptor. *J Biol Chem*. 2005;280(45):37917–37923.
 71. Ray K. Calcium-sensing receptor: trafficking, endocytosis, recycling, and importance of interacting proteins. *Prog Mol Biol Transl Sci*. 2015;132:127–150.
 72. Tao YX. Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. *Pharmacol Ther*. 2006;111(3):949–973.
 73. Magenis RE, Smith L, Nadeau JH, Johnson KR, Mountjoy KG, Cone RD. Mapping of the ACTH, MSH, and neural (MC3 and MC4) melanocortin receptors in the mouse and human. *Mamm Genome*. 1994;5(8):503–508.
 74. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*. 1997;18(3):378–403.
 75. Miller WL. Molecular biology of steroid hormone synthesis. *Endocr Rev*. 1988;9(3):295–318.
 76. Tiosano D, Pannain S, Vassart G, Parma J, Gershoni-Baruch R, Mandel H, et al. The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. *Thyroid*. 1999;9(9):887–894.
 77. Clark AJ, McLoughlin L, Grossman A. Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. *Lancet*. 1993;341(8843):461–462.
 78. Tsigos C, Arai K, Hung W, Chrousos GP. Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene [see comments]. *J Clin Invest*. 1993;92(5):2458–2461.
 79. Weber A, Toppari J, Harvey RD, Klann RC, Shaw NJ, Ricker AT, et al. Adrenocorticotropin receptor gene mutations in familial glucocorticoid deficiency: relationships with clinical features in four families. *J Clin Endocrinol Metab*. 1995;80(1):65–71.
 80. Tsigos C, Arai K, Latronico AC, DiGeorge AM, Rapaport R, Chrousos GP. A novel mutation of the adrenocorticotropin receptor (ACTH-R) gene in a family with the syndrome of isolated glucocorticoid deficiency, but no ACTH-R abnormalities in two families with the triple A syndrome. *J Clin Endocrinol Metab*. 1995;80(7):2186–2189.
 81. Naville D, Barjhoux L, Jaillard C, Faury D, Despert F, Esteva B, et al. Demonstration by transfection studies that mutations in the adrenocorticotropin receptor gene are one cause of the hereditary syndrome of glucocorticoid deficiency. *J Clin Endocrinol Metab*. 1996;81(4):1442–1448.
 82. Slavotinek AM, Hurst JA, Dunger D, Wilkie AO. ACTH receptor mutation in a girl with familial glucocorticoid deficiency. *Clin Genet*. 1998;53(1):57–62.
 83. Chung TT, Chan LF, Metherell LA, Clark AJ. Phenotypic characteristics of familial glucocorticoid deficiency (FGD) type 1 and 2. *Clin Endocrinol (Oxf)*. 2010;72(5):589–594.
 84. Tullio-Pelet A, Salomon R, Hadj-Rabia S, Mugnier C, de Laet MH, Chauouchi B, et al. Mutant WD-repeat protein in triple-A syndrome. *Nat Genet*. 2000;26(3):332–335.
 85. Cronshaw JM, Matunis MJ. The nuclear pore complex protein ALADIN is mislocalized in triple A syndrome. *Proc Natl Acad Sci U S A*. 2003;100(10):5823–5827.
 86. Juhlen R, Idkowiak J, Taylor AE, Kind B, Arlt W, Huebner A, et al. Role of ALADIN in human adrenocortical cells for oxidative stress response and steroidogenesis. *PLoS One*. 2015;10(4):e0124582.
 87. Shepard TH, Landing BH, Mason DG. Familial Addison's disease. *Am J Dis Child*. 1959;97:154–162.
 88. Migeon CJ, Kenny EM, Kowarski A, Snipes CA, Spaulding JS, Finkelstein JW, et al. The syndrome of congenital adrenocortical unresponsiveness to ACTH. Report of six cases. *Pediatr Res*. 1968;2(6):501–513.
 89. Monteleone JA, Monteleone PL. Hereditary adrenocortical unresponsiveness to ACTH—another case. *Pediatrics*. 1970;46(2):321–322.
 90. Spark RF, Etkorn JR. Absent aldosterone response to ACTH in familial glucocorticoid deficiency. *N Engl J Med*. 1977;297(17):917–920.
 91. Davidai G, Kahana L, Hochberg Z. Glomerulosa failure in congenital adrenocortical unresponsiveness to ACTH. *Clin Endocrinol (Oxf)*. 1984;20(5):515–520.
 92. Kelch RP, Kaplan SL, Biglieri EG, Daniels GH, Epstein CJ, Grumbach MM. Hereditary adrenocortical unresponsiveness to adrenocorticotropin hormone. *J Pediatr*. 1972;81(4):726–736.
 93. Turan S, Hughes C, Atay Z, Guran T, Haliloglu B, Clark AJ, et al. An atypical case of familial glucocorticoid deficiency without pigmentation caused by coexistent homozygous mutations in MC2R (T152K) and MC1R (R160W). *J Clin Endocrinol Metab*. 2012;97(5):E771–E774.
 94. Lacy DE, Nathavitharana KA, Tarlow MJ. Neonatal hepatitis and congenital insensitivity to adrenocorticotropin (ACTH). *J Pediatr Gastroenterol Nutr*. 1993;17(4):438–440.
 95. Allgrove J, Clayden GS, Grant DB, Macaulay JC. Familial glucocorticoid deficiency with achalasia of the cardia and deficient tear production. *Lancet*. 1978;1(8077):1284–1286.
 96. Petrykowski W, Burmeister P, Bohm N. Familial glucocorticoid insufficiency (author's transl.). *Klin Padiatr*. 1975;187(3):198–215.
 97. Modan-Moses D, Ben-Zeev B, Hoffmann C, Falik-Zaccai TC, Bental YA, Pinhas-Hamiel O, et al. Unusual presentation of familial glucocorticoid deficiency with a novel MRAP mutation. *J Clin Endocrinol Metab*. 2006;91(10):3713–3717.
 98. Clark AJ, Cammas FM, Watt A, Kapas S, Weber A. Familial glucocorticoid deficiency: one syndrome, but more than one gene. *J Mol Med*. 1997;75(6):394–399.
 99. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, et al. Two G protein oncogenes in human endocrine tumors. *Science*. 1990;249(4969):655–659.
 100. Reincke M, Mora P, Beuschlein F, Arlt W, Chrousos GP, Allolio B. Deletion of the adrenocorticotropin receptor gene in human adrenocortical tumors: implications for tumorigenesis. *J Clin Endocrinol Metab*. 1997;82(9):3054–3058.
 101. Assie G, Libe R, Espiard S, Rizk-Rabin M, Guimier A, Luscap W, et al. ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. *N Engl J Med*. 2013;369(22):2105–2114.
 102. Lacroix A, Bolte E, Tremblay J, Dupre J, Poitras P, Fournier H, et al. Gastric inhibitory polypeptide-dependent cortisol hypersecretion—a new cause of Cushing's syndrome [see comments]. *N Engl J Med*. 1992;327(14):974–980.
 103. Reznik Y, Allali-Zerah V, Chayvialle JA, Leroyer R, Leymarie P, Travert G, et al. Food-dependent Cushing's syndrome mediated by aberrant adrenal sensitivity to gastric inhibitory polypeptide [see comments]. *N Engl J Med*. 1992;327(14):981–986.
 104. Lacroix A, N'Diaye N, Mircescu H, Hamet P, Tremblay J. Abnormal expression and function of hormone receptors in adrenal Cushing's syndrome. *Endocr Res*. 1998;24(3-4):835–843.
 105. Arnaldi G, Gasc JM, de Keyser Y, Raffin-Sanson ML, Perraudin V, Kuhn JM, et al. Variable expression of the V1 vasopressin receptor modulates the phenotypic response of steroid-secreting adrenocortical tumors. *J Clin Endocrinol Metab*. 1998;83(6):2029–2035.
 106. Daidoh H, Morita H, Hanafusa J, Mune T, Murase H, Sato M, et al. In vivo and in vitro effects of AVP and V1a receptor antagonist on Cushing's syndrome due to ACTH-independent bilateral macronodular adrenocortical hyperplasia. *Clin Endocrinol (Oxf)*. 1998;49(3):403–409.
 107. Bourdeau I, Stratakis CA. Cyclic AMP-dependent signaling aberrations in macronodular adrenal disease. *Ann N Y Acad Sci*. 2002;968:240–255.
 108. Lee B, Koo J, Yun Jun J, Gavrilova O, Lee Y, Seo AY, et al. A mouse model for a partially inactive obesity-associated human MC3R variant. *Nat Commun*. 2016;7: 10522.
 109. Tao YX. Mutations in the melanocortin-3 receptor (MC3R) gene: impact on human obesity or adiposity. *Curr Opin Investig Drugs*. 2010;11(10):1092–1096.
 110. Yang F, Tao YX. Functional characterization of nine novel naturally occurring human melanocortin-3 receptor mutations. *Biochim Biophys Acta*. 2012;1822(11):1752–1765.
 111. Butler AA, Kesterson RA, Khong K, Cullen MJ, Pellemounter MA, Dekoning J, et al. A unique metabolic syndrome causes obesity in

- the melanocortin-3 receptor-deficient mouse. *Endocrinology*. 2000;141(9):3518–3521.
112. Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan XM, Yu H, et al. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet*. 2000;26(1):97–102.
 113. Feng N, Young SF, Aguilera G, Puricelli E, Adler-Wailes DC, Sebring NG, et al. Co-occurrence of two partially inactivating polymorphisms of MC3R is associated with pediatric-onset obesity. *Diabetes*. 2005;54(9):2663–2667.
 114. Santoro N, Perrone L, Cirillo G, Raimondo P, Amato A, Brienza C, et al. Effect of the melanocortin-3 receptor C17A and G241A variants on weight loss in childhood obesity. *Am J Clin Nutr*. 2007;85(4):950–953.
 115. Lee YS, Poh LK, Kek BL, Loke KY. The role of melanocortin 3 receptor gene in childhood obesity. *Diabetes*. 2007;56(10):2622–2630.
 116. Savastano DM, Tanofsky-Krass M, Han JC, Ning C, Sorg RA, Roza CA, et al. Energy intake and energy expenditure among children with polymorphisms of the melanocortin-3 receptor. *Am J Clin Nutr*. 2009;90(4):912–920.
 117. Obregon AM, Diaz E, Santos JL. Effect of the melanocortin-3 receptor Thr6Lys and Val81Ile genetic variants on body composition and substrate oxidation in Chilean obese children. *J Physiol Biochem*. 2012;68(1):71–76.
 118. Smith MA, Hisadome K, Al-Qassab H, Heffron H, Withers DJ, Ashford ML. Melanocortins and agouti-related protein modulate the excitability of two arcuate nucleus neuron populations by alteration of resting potassium conductances. *J Physiol*. 2007;578(Pt 2):425–438.
 119. Siegrist W, Drozd R, Cotti R, Willard DH, Wilkison WO, Eberle AN. Interactions of alpha-melanotropin and agouti on B16 melanoma cells: evidence for inverse agonism of agouti. *J Recept Signal Transduct Res*. 1997;17(1-3):75–98.
 120. Vergoni AV, Bertolini A, Wikberg JE, Schioth HB. Selective melanocortin MC4 receptor blockage reduces immobilization stress-induced anorexia in rats. *Eur J Pharmacol*. 1999;369(1):11–15.
 121. Vergoni AV, Bertolini A, Guidetti G, Karefilakis V, Filaferrero M, Wikberg JE, et al. Chronic melanocortin 4 receptor blockage causes obesity without influencing sexual behavior in male rats. *J Endocrinol*. 2000;166(2):419–426.
 122. Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S. A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet*. 1998;20(2):111–112.
 123. Farooqi IS, Yeo GS, Keogh JM, Aminian S, Jebb SA, Butler G, et al. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clin Invest*. 2000;106(2):271–279.
 124. Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*. 2003;348(12):1085–1095.
 125. Vink T, Hinney A, van Elburg AA, van Goozen SH, Sandkuijl LA, Sinke RJ, et al. Association between an agouti-related protein gene polymorphism and anorexia nervosa. *Mol Psychiatry*. 2001;6(3):325–328.
 126. Adan RA, Vink T. Drug target discovery by pharmacogenetics: mutations in the melanocortin system and eating disorders. *Eur Neuropsychopharmacol*. 2001;11(6):483–490.
 127. Chagnon YC, Chen WJ, Perusse L, Chagnon M, Nadeau A, Wilkison WO, et al. Linkage and association studies between the melanocortin receptors 4 and 5 genes and obesity-related phenotypes in the Quebec Family Study. *Mol Med*. 1997;3(10):663–673.
 128. Farooqi IS, O'Rahilly S. Monogenic obesity in humans. *Annu Rev Med*. 2005;56:443–458.
 129. Jacobson P, Ukkola O, Rankinen T, Snyder EE, Leon AS, Rao DC, et al. Melanocortin 4 receptor sequence variations are seldom a cause of human obesity: the Swedish Obese Subjects, the HERITAGE Family Study, and a Memphis cohort. *J Clin Endocrinol Metab*. 2002;87(10):4442–4446.
 130. Miraglia Del Giudice E, Cirillo G, Nigro V, Santoro N, D'Urso L, Raimondo P, et al. Low frequency of melanocortin-4 receptor (MC4R) mutations in a Mediterranean population with early-onset obesity. *Int J Obes Relat Metab Disord*. 2002;26(5):647–651.
 131. Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest*. 2000;106(2):253–262.
 132. Herraiz C, Garcia-Borron JC, Jimenez-Cervantes C, Olivares C. MC1R signaling. Intracellular partners and pathophysiological implications. *Biochim Biophys Acta Mol Dis*. 2017;1863(10 Pt A):2448–2461.
 133. Swope VB, Abdel-Malek ZA. MC1R: Front and Center in the Bright Side of Dark Eumelanin and DNA Repair. *Int J Mol Sci*. 2018;19(9).
 134. Wakamatsu K, Graham A, Cook D, Thody AJ. Characterisation of ACTH peptides in human skin and their activation of the melanocortin-1 receptor. *Pigment Cell Res*. 1997;10(5):288–297.
 135. Graham A, Wakamatsu K, Hunt G, Ito S, Thody AJ. Agouti protein inhibits the production of eumelanin and pheomelanin in the presence and absence of alpha-melanocyte stimulating hormone. *Pigment Cell Res*. 1997;10(5):298–303.
 136. Mendiratta MS, Yang Y, Balazs AE, Willis AS, Eng CM, Karaviti LP, et al. Early onset obesity and adrenal insufficiency associated with a homozygous POMC mutation. *Int J Pediatr Endocrinol*. 2011;1(1):5.
 137. Rees JL. Genetics of hair and skin color. *Annu Rev Genet*. 2003;37:67–90.
 138. Challis BG, Pritchard LE, Creemers JW, Delplanque J, Keogh JM, Luan J, et al. A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. *Hum Mol Genet*. 2002;11(17):1997–2004.
 139. Farooqi IS, Drop S, Clements A, Keogh JM, Biernacka J, Lowenbein S, et al. Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes*. 2006;55(9):2549–2553.
 140. Lee YS, Challis BG, Thompson DA, Yeo GS, Keogh JM, Madonna ME, et al. A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. *Cell Metab*. 2006;3(2):135–140.
 141. Farooqi IS, Volders K, Stanhope R, Hueschkel R, White A, Lank E, et al. Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J Clin Endocrinol Metab*. 2007;92(9):3369–3373.
 142. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. 1997;16(3):303–306.
 143. Creemers JW, Choquet H, Stijnen P, Vatin V, Pigeyre M, Beckers S, et al. Heterozygous mutations causing partial prohormone convertase 1 deficiency contribute to human obesity. *Diabetes*. 2012;61(2):383–390.
 144. Drucker DJ. The role of gut hormones in glucose homeostasis. *J Clin Invest*. 2007;117(1):24–32.
 145. Pasel K, Schulz A, Timmermann K, Linnemann K, Hoeltzenbein M, Jaaskelainen J, et al. Functional characterization of the molecular defects causing nephrogenic diabetes insipidus in eight families. *J Clin Endocrinol Metab*. 2000;85(4):1703–1710.
 146. Bichet DG, Henty GN, Lonergan M, Arthus MF, Ligier S, Pausova Z, et al. X-linked nephrogenic diabetes insipidus: from the ship Hope-well to RFLP studies. *Am J Hum Genet*. 1992;51(5):1089–1092.
 147. Rosenthal W, Seibold A, Antaramian A, Lonergan M, Arthus MF, Henty GN, et al. Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature*. 1992;359(6392):233–235.
 148. Lolait SJ, O'Carroll AM, McBride OW, Konig M, Morel A, Brownstein MJ. Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. *Nature*. 1992;357(6376):336–339.
 149. Knoers N, van den Ouweland A, Dreesen J, Verdijk M, Monnens LA, van Oost BA. Nephrogenic diabetes insipidus: identification of the genetic defect. *Pediatr Nephrol*. 1993;7(5):685–688.
 150. Anesi L, de Gemmis P, Galla D, Hladnik U. Two new large deletions of the AVPR2 gene causing nephrogenic diabetes insipidus and a review of previously published deletions. *Nephrol Dial Transplant*. 2012;27(10):3705–3712.

151. Wesche D, Deen PM, Knoers NV. Congenital nephrogenic diabetes insipidus: the current state of affairs. *Pediatr Nephrol*. 2012;27(12):2183–2204.
152. Ala Y, Morin D, Mouillac B, Sabatier N, Vargas R, Cotte N, et al. Functional studies of twelve mutant V2 vasopressin receptors related to nephrogenic diabetes insipidus: molecular basis of a mild clinical phenotype. *J Am Soc Nephrol*. 1998;9(10):1861–1872.
153. van Lieburg AF, Verdijk MA, Knoers VV, van Essen AJ, Proesmans W, Mallmann R, et al. Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet*. 1994;55(4):648–652.
154. Feldman BJ, Rosenthal SM, Vargas GA, Fenwick RG, Huang EA, Matsuda-Abedini M, et al. Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*. 2005;352(18):1884–1890.
155. Hague J, Casey R, Bruty J, Legerton T, Abbs S, Oddy S, et al. Adult female with symptomatic AVPR2-related nephrogenic syndrome of inappropriate antidiuresis (NSIAD). *Endocrinol Diabetes Metabol Case Rep*. 2018;2018:17–0139.
156. Cheung CC, Cadnapaphornchai MA, Ranadive SA, Gitelman SE, Rosenthal SM. Persistent elevation of urine aquaporin-2 during water loading in a child with nephrogenic syndrome of inappropriate antidiuresis (NSIAD) caused by a R137L mutation in the V2 vasopressin receptor. *Int J Pediatr Endocrinol*. 2012;2012(1):3.
157. Ben-Menahem D, Hyde R, Pixley M, Berger P, Boime I. Synthesis of multi-subunit domain gonadotropin complexes: a model for alpha/beta heterodimer formation. *Biochemistry*. 1999;38(46):15070–15077.
158. Lustbader JW, Lobel L, Wu H, Elliott MM. Structural and molecular studies of human chorionic gonadotropin and its receptor. *Recent Prog Horm Res*. 1998;53:395–424.
159. Themmen AP, Martens JW, Brunner HG. Activating and inactivating mutations in LH receptors. *Mol Cell Endocrinol*. 1998;145(1-2):137–142.
160. Tsai-Morris CH, Buczko E, Wang W, Xie XZ, Dufau ML. Structural organization of the rat luteinizing hormone (LH) receptor gene. *J Biol Chem*. 1991;266(17):11355–11359.
161. Minegishi T, Nakamura K, Takakura Y, Miyamoto K, Hasegawa Y, Ibuki Y, et al. Cloning and sequencing of human LH/hCG receptor cDNA [published erratum appears in *Biochem Biophys Res Commun* 1994 Jun 15;201(2):1057]. *Biochem Biophys Res Commun*. 1990;172(3):1049–1054.
162. Schwartz M, Imperato-McGinley J, Peterson RE, Cooper G, Morris PL, MacGillivray M, et al. Male pseudohermaphroditism secondary to an abnormality in Leydig cell differentiation. *J Clin Endocrinol Metab*. 1981;53(1):123–127.
163. Toledo SP, Arnhold IJ, Luthold W, Russo EM, Saldanha PH. Leydig cell hypoplasia determining familial hypergonadotropic hypogonadism. *Prog Clin Biol Res*. 1985;200:311–314.
164. el-Awady MK, Temtamy SA, Salam MA, Gad YZ. Familial Leydig cell hypoplasia as a cause of male pseudohermaphroditism. *Hum Hered*. 1987;37(1):36–40.
165. Martínez-Mora J, Saez JM, Toran N, Isnard R, Perez-Iribarne MM, Egozcue J, et al. Male pseudohermaphroditism due to Leydig cell agenesis and absence of testicular LH receptors [see comments]. *Clin Endocrinol (Oxf)*. 1991;34(6):485–491.
166. Toledo SP. Leydig cell hypoplasia leading to two different phenotypes: male pseudohermaphroditism and primary hypogonadism not associated with this [letter; comment]. *Clin Endocrinol (Oxf)*. 1992;36(5):521–522.
167. Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, et al. Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet*. 1995;9(2):160–164.
168. Latronico AC, Anastasi J, Arnhold IJ, Rapaport R, Mendonca BB, Bloise W, et al. Brief report: testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. *N Engl J Med*. 1996;334(8):507–512.
169. Chan WY. Molecular genetic, biochemical, and clinical implications of gonadotropin receptor mutations. *Mol Genet Metab*. 1998;63(2):75–84.
170. Latronico AC, Chai Y, Arnhold IJ, Liu X, Mendonca BB, Segaloff DL. A homozygous microdeletion in helix 7 of the luteinizing hormone receptor associated with familial testicular and ovarian resistance is due to both decreased cell surface expression and impaired effector activation by the cell surface receptor. *Mol Endocrinol*. 1998;12(3):442–450.
171. Martens JW, Verhoef-Post M, Abelin N, Ezabella M, Toledo SP, Brunner HG, et al. A homozygous mutation in the luteinizing hormone receptor causes partial Leydig cell hypoplasia: correlation between receptor activity and phenotype. *Mol Endocrinol*. 1998;12(6):775–784.
172. Laue LL, Wu SM, Kudo M, Bourdony CJ, Cutler Jr GB, Hsueh AJ, et al. Compound heterozygous mutations of the luteinizing hormone receptor gene in Leydig cell hypoplasia. *Mol Endocrinol*. 1996;10(8):987–997.
173. Misrahi M, Meduri G, Pissard S, Bouvattier C, Beau I, Loosfelt H, et al. Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *J Clin Endocrinol Metab*. 1997;82(7):2159–2165.
174. Gromoll J, Eiholzer U, Nieschlag E, Simoni M. Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. *J Clin Endocrinol Metab*. 2000;85(6):2281–2286.
175. Arnhold IJ, Lofrano-Porto A, Latronico AC. Inactivating mutations of luteinizing hormone beta-subunit or luteinizing hormone receptor cause oligo-amenorrhea and infertility in women. *Horm Res*. 2009;71(2):75–82.
176. Toledo SP, Brunner HG, Kraaij R, Post M, Dahia PL, Hayashida CY, et al. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab*. 1996;81(11):3850–3854.
177. Latronico AC, Arnhold IJ. Inactivating mutations of the human luteinizing hormone receptor in both sexes. *Semin Reprod Med*. 2012;30(5):382–386.
178. Yuan P, He Z, Zheng L, Wang W, Li Y, Zhao H, et al. Genetic evidence of ‘genuine’ empty follicle syndrome: a novel effective mutation in the LHCGR gene and review of the literature. *Hum Reprod*. 2017;32(4):944–953.
179. Schedewie HK, Reiter EO, Beitins IZ, Seyed S, Wooten VD, Jimenez JF, et al. Testicular leydig cell hyperplasia as a cause of familial sexual precocity. *J Clin Endocrinol Metab*. 1981;52(2):271–278.
180. Rosenthal SM, Grumbach MM, Kaplan SL. Gonadotropin-independent familial sexual precocity with premature Leydig and germinal cell maturation (familial testotoxicosis): effects of a potent luteinizing hormone-releasing factor agonist and medroxyprogesterone acetate therapy in four cases. *J Clin Endocrinol Metab*. 1983;57(3):571–579.
181. Gondos B, Egli CA, Rosenthal SM, Grumbach MM. Testicular changes in gonadotropin-independent familial male sexual precocity. Familial testotoxicosis. *Arch Pathol Lab Med*. 1985;109(11):990–995.
182. Egli CA, Rosenthal SM, Grumbach MM, Montalvo JM, Gondos B. Pituitary gonadotropin-independent male-limited autosomal dominant sexual precocity in nine generations: familial testotoxicosis. *J Pediatr*. 1985;106(1):33–40.
183. Kremer H, Mariman E, Otten BJ, Moll Jr GW, Stoelinga GB, Wit JM, et al. Cosegregation of missense mutations of the luteinizing hormone receptor gene with familial male-limited precocious puberty. *Hum Mol Genet*. 1993;2(11):1779–1783.
184. Rosenthal IM, Refetoff S, Rich B, Barnes RB, Sunthornthevarakul T, Parma J, et al. Response to challenge with gonadotropin-releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor—a clinical research center study. *J Clin Endocrinol Metab*. 1996;81(10):3802–3806.
185. Oerter KE, Uriarte MM, Rose SR, Barnes KM, Cutler Jr GB. Gonadotropin secretory dynamics during puberty in normal girls and boys. *J Clin Endocrinol Metab*. 1990;71(5):1251–1258.
186. Latronico AC, Abell AN, Arnhold IJ, Liu X, Lins TS, Brito VN, et al. A unique constitutively activating mutation in third transmembrane helix of luteinizing hormone receptor causes sporadic male gonadotropin-independent precocious puberty. *J Clin Endocrinol Metab*. 1998;83(7):2435–2440.

187. Nagasaki K, Katsumata N, Ogawa Y, Kikuchi T, Uchiyama M. Novel C617Y mutation in the 7th transmembrane segment of luteinizing hormone/choriogonadotropin receptor in a Japanese boy with peripheral precocious puberty. *Endocrine J*. 2011;57(12):1055–1060.
188. Goji K, Teraoka Y, Hosokawa Y, Okuno M, Ozaki K, Yoshida M, et al. Gonadotropin-independent precocious puberty associated with a somatic activating mutation of the LH receptor gene: detection of a mutation present in only a small fraction of cells from testicular tissue using wild-type blocking polymerase chain reaction and laser-capture microdissection. *Endocrine*. 2009;35(3):397–401.
189. Sangkhathat S, Kanngum S, Jaruratanasirikul S, Tubtawee T, Chaiyapan W, Patrapinyokul S, et al. Peripheral precocious puberty in a male caused by Leydig cell adenoma harboring a somatic mutation of the LHR gene: report of a case. *J Med Assoc Thai*. 2010;93(9):1093–1097.
190. Tapanainen JS, Vaskivuo T, Aittomaki K, Huhtaniemi IT. Inactivating FSH receptor mutations and gonadal dysfunction. *Mol Cell Endocrinol*. 1998;145(1-2):129–135.
191. Minegishi T, Nakamura K, Takakura Y, Ibuki Y, Igarashi M, Minegishi T. Cloning and sequencing of human FSH receptor cDNA [published erratum appears in *Biochem Biophys Res Commun* 1994 Jun 15;201(2):1057]. *Biochem Biophys Res Commun*. 1991;175(3):1125–1130.
192. Heckert LL, Daley JJ, Griswold MD. Structural organization of the follicle-stimulating hormone receptor gene. *Mol Endocrinol*. 1992;6(1):70–80.
193. Jones GS, Acosta AA, Garcia JE, Bernardus RE, Rosenwaks Z. The effect of follicle-stimulating hormone without additional luteinizing hormone on follicular stimulation and oocyte development in normal ovulatory women. *Fertil Steril*. 1985;43(5):696–702.
194. Durham CR, Zhu H, Masters BS, Simpson ER, Mendelson CR. Regulation of aromatase activity of rat granulosa cells: induction of synthesis of NADPH-cytochrome P-450 reductase by FSH and dibutyryl cyclic AMP. *Mol Cell Endocrinol*. 1985;40(2-3):211–219.
195. Knobil E. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res*. 1980;36:53–88.
196. Aittomaki K, Herva R, Stenman UH, Juntunen K, Ylostalo P, Hovatta O, et al. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J Clin Endocrinol Metab*. 1996;81(10):3722–3726.
197. Aittomaki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, et al. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell*. 1995;82(6):959–968.
198. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet*. 1997;15(2):205–206.
199. Jiang M, Aittomaki K, Nilsson C, Pakarinen P, Iitia A, Torresani T, et al. The frequency of an inactivating point mutation (566C->T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry. *J Clin Endocrinol Metab*. 1998;83(12):4338–4343.
200. Touraine P, Beau I, Gougeon A, Meduri G, Desroches A, Pichard C, et al. New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. *Mol Endocrinol*. 1999;13(11):1844–1854.
201. Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab*. 1996;81(4):1367–1370.
202. Doherty E, Pakarinen P, Tiitinen A, Kiilavuori A, Huhtaniemi I, Forrest S, et al. A Novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure. *J Clin Endocrinol Metab*. 2002;87(3):1151–1155.
203. Kotlar TJ, Young RH, Albanese C, Crowley Jr WF, Scully RE, Jameson JL. A mutation in the follicle-stimulating hormone receptor occurs frequently in human ovarian sex cord tumors. *J Clin Endocrinol Metab*. 1997;82(4):1020–1026.
204. Allen LA, Achermann JC, Pakarinen P, Kotlar TJ, Huhtaniemi IT, Jameson JL, et al. A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics. *Hum Reprod*. 2003;18(2):251–256.
205. Meduri G, Touraine P, Beau I, Lahuna O, Desroches A, Vacher-Lavenu MC, et al. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. *J Clin Endocrinol Metab*. 2003;88(8):3491–3498.
206. He WB, Du J, Yang XW, Li W, Tang WL, Dai C, et al. Novel inactivating mutations in the FSH receptor cause premature ovarian insufficiency with resistant ovary syndrome. *Reprod Biomed Online*. 2019;38(3):397–406.
207. Beau I, Touraine P, Meduri G, Gougeon A, Desroches A, Matuchansky C, et al. A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *J Clin Invest*. 1998;102(7):1352–1359.
208. Laue L, Chan WY, Hsueh AJ, Kudo M, Hsu SY, Wu SM, et al. Genetic heterogeneity of constitutively activating mutations of the human luteinizing hormone receptor in familial male-limited precocious puberty. *Proc Natl Acad Sci U S A*. 1995;92(6):1906–1910.
209. Tonacchera M, Van Sande J, Cetani F, Swillens S, Schwartz C, Winiszewski P, et al. Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. *J Clin Endocrinol Metab*. 1996;81(2):547–554.
210. Tonacchera M, Van Sande J, Parma J, Duprez L, Cetani F, Costagliola S, et al. TSH receptor and disease. *Clin Endocrinol (Oxf)*. 1996;44(6):621–633.
211. Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med*. 2003;349(8):760–766.
212. Vasseur C, Rodien P, Beau I, Desroches A, Gerard C, de Poncheville L, et al. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N Engl J Med*. 2003;349(8):753–759.
213. Montanelli L, Delbaere A, Di Carlo C, Nappi C, Smits G, Vassart G, et al. A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab*. 2004;89(4):1255–1258.
214. De Leener A, Montanelli L, Van Durme J, Chae H, Smits G, Vassart G, et al. Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J Clin Endocrinol Metab*. 2006;91(2):555–562.
215. Libert F, Lefort A, Gerard C, Parmentier M, Perret J, Ludgate M, et al. Cloning, sequencing and expression of the human thyrotropin (TSH) receptor: evidence for binding of autoantibodies. *Biochem Biophys Res Commun*. 1989;165(3):1250–1255.
216. Nagayama Y, Kaufman KD, Seto P, Rapoport B. Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. *Biochem Biophys Res Commun*. 1989;165(3):1184–1190.
217. Mirrahi M, Loosfelt H, Atger M, Sar S, Guiochon-Mantel A, Milgrom E. Cloning, sequencing and expression of human TSH receptor. *Biochem Biophys Res Commun*. 1990;166(1):394–403.
218. Gross B, Mirrahi M, Sar S, Milgrom E. Composite structure of the human thyrotropin receptor gene. *Biochem Biophys Res Commun*. 1991;177(2):679–687.
219. Dumont JE, Lamy F, Roger P, Maenhaut C. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. *Physiol Rev*. 1992;72(3):667–697.
220. Allgeier A, Offermanns S, Van Sande J, Spicher K, Schultz G, Dumont JE. The human thyrotropin receptor activates G-proteins Gs and Gq/11. *J Biol Chem*. 1994;269(19):13733–13735.
221. Russo D, Chazenbalk GD, Nagayama Y, Wadsworth HL, Seto P, Rapoport B. A new structural model for the thyrotropin (TSH) receptor, as determined by covalent cross-linking of TSH to the recombinant receptor in intact cells: evidence for a single polypeptide chain. *Mol Endocrinol*. 1991;5(11):1607–1612.

222. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies [published erratum appears in *Endocr Rev* 1999 Feb; 20(1):100]. *Endocr Rev*. 1998;19(6):673–716.
223. Chazenbalk GD, Tanaka K, McLachlan SM, Rapoport B. On the functional importance of thyrotropin receptor intramolecular cleavage. *Endocrinology*. 1999;140(10):4516–4520.
224. Tanaka K, Chazenbalk GD, McLachlan SM, Rapoport B. Thyrotropin receptor cleavage at site 1 involves two discontinuous segments at each end of the unique 50-amino acid insertion. *J Biol Chem*. 1999;274(4):2093–2096.
225. Kajita Y, Rickards CR, Buckland PR, Howells RD, Rees Smith B. Analysis of thyrotropin receptors by photoaffinity labelling. Orientation of receptor subunits in the cell membrane. *Biochem J*. 1985;227(2):413–420.
226. Loosfelt H, Pichon C, Jolivet A, Misrahi M, Caillou B, Jamous M, et al. Two-subunit structure of the human thyrotropin receptor. *Proc Natl Acad Sci U S A*. 1992;89(9):3765–3769.
227. Misrahi M, Ghinea N, Sar S, Saunier B, Jolivet A, Loosfelt H, et al. Processing of the precursors of the human thyroid-stimulating hormone receptor in various eukaryotic cells (human thyrocytes, transfected L cells and baculovirus-infected insect cells). *Eur J Biochem*. 1994;222(2):711–719.
228. Tanaka K, Chazenbalk GD, McLachlan SM, Rapoport B. Subunit structure of thyrotropin receptors expressed on the cell surface. *J Biol Chem*. 1999;274(48):33979–33984.
229. Nagayama, Y., Wadsworth, H.L., Chazenbalk, G.D., Russo, D., Seto, P., Rapoport, B. Thyrotropin-luteinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. *Proc Natl Acad Sci U S A*, 88 (3), 902–905.
230. Nagayama Y, Rapoport B. Role of the carboxyl-terminal half of the extracellular domain of the human thyrotropin receptor in signal transduction. *Endocrinology*. 1992;131(2):548–552.
231. Kopp P, Muirhead S, Jourdain N, Gu WX, Jameson JL, Rodd C. Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281→isoleucine) in the extracellular domain of the thyrotropin receptor. *J Clin Invest*. 1997;100(6):1634–1639.
232. Duprez L, Parma J, Costagliola S, Hermans J, Van Sande J, Dumont JE, et al. Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. *FEBS Lett*. 1997;409(3):469–474.
233. Gruters A, Schoneberg T, Biebermann H, Krude H, Krohn HP, Dralle H, et al. Severe congenital hyperthyroidism caused by a germ-line neo mutation in the extracellular portion of the thyrotropin receptor. *J Clin Endocrinol Metab*. 1998;83(5):1431–1436.
234. Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas [see comments]. *Nature*. 1993;365(6447):649–651.
235. Porcellini A, Ciullo I, Laviola L, Amabile G, Fenzi G, Avvedimento VE. Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas. Rapid identification by fine needle aspiration biopsy. *J Clin Endocrinol Metab*. 1994;79(2):657–661.
236. Paschke R, Tonacchera M, Van Sande J, Parma J, Vassart G. Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. *J Clin Endocrinol Metab*. 1994;79(6):1785–1789.
237. Russo D, Arturi F, Wicker R, Chazenbalk GD, Schlumberger M, DuVillard JA, et al. Genetic alterations in thyroid hyperfunctioning adenomas. *J Clin Endocrinol Metab*. 1995;80(4):1347–1351.
238. Russo D, Arturi F, Suarez HG, Schlumberger M, Du Villard JA, Crocetti U, et al. Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. *J Clin Endocrinol Metab*. 1996;81(4):1548–1551.
239. Parma J, Duprez L, Van Sande J, Hermans J, Rocmans P, Van Vliet G, et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs alpha genes as a cause of toxic thyroid adenomas. *J Clin Endocrinol Metab*. 1997;82(8):2695–2701.
240. Fuhrer D, Holzapfel HP, Wonerow P, Scherbaum WA, Paschke R. Somatic mutations in the thyrotropin receptor gene and not in the Gs alpha protein gene in 31 toxic thyroid nodules. *J Clin Endocrinol Metab*. 1997;82(11):3885–3891.
241. Krohn K, Fuhrer D, Holzapfel HP, Paschke R. Clonal origin of toxic thyroid nodules with constitutively activating thyrotropin receptor mutations. *J Clin Endocrinol Metab*. 1998;83(1):130–134.
242. Parma J, Van Sande J, Swillens S, Tonacchera M, Dumont J, Vassart G. Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3',5'-monophosphate and inositol phosphate-Ca²⁺ cascades. *Mol Endocrinol*. 1995;9(6):725–733.
243. Paschke R, Ludgate M. The thyrotropin receptor in thyroid diseases. *N Engl J Med*. 1997;337(23):1675–1681.
244. Takeshita A, Nagayama Y, Yokoyama N, Ishikawa N, Ito K, Yamashita T, et al. Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid nodules in Japan. *J Clin Endocrinol Metab*. 1995;80(9):2607–2611.
245. Nogueira CR, Kopp P, Arseven OK, Santos CL, Jameson JL, Medeiros-Neto G. Thyrotropin receptor mutations in hyperfunctioning thyroid adenomas from Brazil. *Thyroid*. 1999;9(11):1063–1068.
246. Holzapfel HP, Fuhrer D, Wonerow P, Weinland G, Scherbaum WA, Paschke R. Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goiters. *J Clin Endocrinol Metab*. 1997;82(12):4229–4233.
247. Russo D, Arturi F, Schlumberger M, Caillou B, Monier R, Filetti S, et al. Activating mutations of the TSH receptor in differentiated thyroid carcinomas. *Oncogene*. 1995;11(9):1907–1911.
248. Spambalg D, Sharifi N, Elisei R, Gross JL, Medeiros-Neto G, Fagin JA. Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. *J Clin Endocrinol Metab*. 1996;81(11):3898–3901.
249. Russo D, Tumino S, Arturi F, Vigneri P, Grasso G, Pontecorvi A, et al. Detection of an activating mutation of the thyrotropin receptor in a case of an autonomously hyperfunctioning thyroid insular carcinoma [see comments]. *J Clin Endocrinol Metab*. 1997;82(3):735–738.
250. O'Sullivan C, Barton CM, Staddon SL, Brown CL, Lemoine NR. Activating point mutations of the gsp oncogene in human thyroid adenomas. *Mol Carcinog*. 1991;4(5):345–34.
251. Suarez HG, du Villard JA, Caillou B, Schlumberger M, Parmentier C, Monier R. gsp mutations in human thyroid tumours. *Oncogene*. 1991;6(4):677–679.
252. Schaarschmidt J, Paschke S, Ozerden M, Jaschke H, Huth S, Eszlinger M, et al. Late manifestation of subclinical hyperthyroidism after goitrogenesis in an index patient with a N670S TSH receptor germline mutation masquerading as TSH receptor antibody negative Graves' disease. *Horm Metab Res*. 2012;44(13):962–965.
253. Duprez L, Parma J, Van Sande J, Allgeier A, Leclere J, Schwartz C, et al. Germline mutations in the thyrotropin receptor gene cause non- autoimmune autosomal dominant hyperthyroidism. *Nat Genet*. 1994;7(3):396–401.
254. Fuhrer D, Wonerow P, Willgerodt H, Paschke R. Identification of a new thyrotropin receptor germline mutation (Leu629Phe) in a family with neonatal onset of autosomal dominant nonautoimmune hyperthyroidism. *J Clin Endocrinol Metab*. 1997;82(12):4234–4238.
255. Schwab KO, Sohlmann P, Gerlich M, Broecker M, Petrykowski W, Holzapfel HP, et al. Mutations of the TSH receptor as cause of congenital hyperthyroidism. *Exp Clin Endocrinol Diabetes*. 1996;104(Suppl 4):124–128.
256. Kopp P, van Sande J, Parma J, Duprez L, Gerber H, Joss E, et al. Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene [see comments]. *N Engl J Med*. 1995;332(3):150–154.
257. de Roux N, Polak M, Couet J, Leger J, Czernichow P, Milgrom E, et al. A neonatal mutation of the thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism [see comments]. *J Clin Endocrinol Metab*. 1996;81(6):2023–2026.
258. Holzapfel HP, Wonerow P, von Petrykowski W, Henschen M, Scherbaum WA, Paschke R. Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. *J Clin Endocrinol Metab*. 1997;82(11):3879–3884.

259. Schwab KO, Gerlich M, Broecker M, Sohlmann P, Derwahl M, Lohse MJ. Constitutively active germline mutation of the thyrotropin receptor gene as a cause of congenital hyperthyroidism. *J Pediatr*. 1997;131(6):899–904.
260. Esapa CT, Duprez L, Ludgate M, Mustafa MS, Kendall-Taylor P, Vassart G, et al. A novel thyrotropin receptor mutation in an infant with severe thyrotoxicosis. *Thyroid*. 1999;9(10):1005–1010.
261. Biebertmann H, Winkler F, Handke D, Gruters A, Krude H, Kleinau G. Molecular description of non-autoimmune hyperthyroidism at a neonate caused by a new thyrotropin receptor germline mutation. *Thyroid Res*. 2011;4(Suppl 1):S8.
262. Costagliola S, Sunthornthepvarakul T, Migeotte I, Van Sande J, Kajava AM, Refetoff S, et al. Structure-function relationships of two loss-of-function mutations of the thyrotropin receptor gene. *Thyroid*. 1999;9(10):995–1000.
263. de Roux N, Misrahi M, Brauner R, Houang M, Carel JC, Granier M, et al. Four families with loss of function mutations of the thyrotropin receptor. *J Clin Endocrinol Metab*. 1996;81(12):4229–4235.
264. Biebertmann H, Winkler F, Handke D, Teichmann A, Gerling B, Cameron F, et al. New pathogenic thyrotropin receptor mutations decipher differentiated activity switching at a conserved helix 6 motif of family A GPCR. *J Clin Endocrinol Metab*. 2011;97(2):E228–E232.
265. Tonacchera M, Agretti P, Pinchera A, Rosellini V, Perri A, Collecchi P, et al. Congenital hypothyroidism with impaired thyroid response to thyrotropin (TSH) and absent circulating thyroglobulin: evidence for a new inactivating mutation of the TSH receptor gene. *J Clin Endocrinol Metab*. 2000;85(3):1001–1008.
266. Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S. Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene [see comments]. *N Engl J Med*. 1995;332(3):155–160.
267. Clifton-Bligh RJ, Gregory JW, Ludgate M, John R, Persani L, Asteria C, et al. Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. *J Clin Endocrinol Metab*. 1997;82(4):1094–1100.
268. Biebertmann H, Schoneberg T, Krude H, Schultz G, Gudermann T, Gruters A. Mutations of the human thyrotropin receptor gene causing thyroid hypoplasia and persistent congenital hypothyroidism. *J Clin Endocrinol Metab*. 1997;82(10):3471–3480.
269. Abramowicz MJ, Duprez L, Parma J, Vassart G, Heinrichs C. Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clin Invest*. 1997;99(12):3018–3024.
270. Gagne N, Parma J, Deal C, Vassart G, Van Vliet G. Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities? *J Clin Endocrinol Metab*. 1998;83(5):1771–1775.
271. Alves EA, Cruz CM, Pimentel CP, Ribeiro RC, Santos AK, Caldato MC, et al. High frequency of D727E polymorphisms in exon 10 of the TSHR gene in Brazilian patients with congenital hypothyroidism. *J Pediatr Endocrinol Metab*. 2010;23(12):1321–1328.
272. Cangul H, Aycan Z, Saglam H, Forman JR, Cetinkaya S, Tarim O, et al. TSHR is the main causative locus in autosomal recessively inherited thyroid dysgenesis. *J Pediatr Endocrinol Metab*. 2012;25(5-6):419–426.
273. Chang WC, Liao CY, Chen WC, Fan YC, Chiu SJ, Kuo HC, et al. R450H TSH receptor mutation in congenital hypothyroidism in Taiwanese children. *Clin Chim Acta*. 2012;413(11-12):1004–1007.
274. Narumi S, Muroya K, Abe Y, Yasui M, Asakura Y, Adachi M, et al. TSHR mutations as a cause of congenital hypothyroidism in Japan: a population-based genetic epidemiology study. *J Clin Endocrinol Metab*. 2009;94(4):1317–1323.
275. Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T, et al. Increased expression of the Na⁺/I⁻ symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. *J Clin Endocrinol Metab*. 1997;82(10):3331–3336.
276. Narumi S, Nagasaki K, Ishii T, Muroya K, Asakura Y, Adachi M, et al. Nonclassic TSH resistance: TSHR mutation carriers with discrepantly high thyroidal iodine uptake. *J Clin Endocrinol Metab*. 2011;96(8):E1340–E1345.
277. Mimouni M, Mimouni-Bloch A, Schachter J, Shohat M. Familial hypothyroidism with autosomal dominant inheritance. *Arch Dis Child*. 1996;75(3):245–246.
278. Xie J, Pannain S, Pohlenz J, Weiss RE, Moltz K, Morlot M, et al. Resistance to thyrotropin (TSH) in three families is not associated with mutations in the TSH receptor or TSH [see comments]. *J Clin Endocrinol Metab*. 1997;82(12):3933–3940.
279. Grossmann M, Weintraub BD, Szkudlinski MW. Novel insights into the molecular mechanisms of human thyrotropin action: structural, physiological, and therapeutic implications for the glycoprotein hormone family. *Endocr Rev*. 1997;18(4):476–501.
280. Harada A, Hershman JM, Reed AW, Braunstein GD, Dignam WJ, Harzko C, et al. Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. *J Clin Endocrinol Metab*. 1979;48(5):793–797.
281. Hershman JM. Human chorionic gonadotropin and the thyroid: hyperemesis gravidarum and trophoblastic tumors. *Thyroid*. 1999;9(7):653–657.
282. Karp PJ, Hershman JM, Richmond S, Goldstein DP, Selenkow HA. Thyrotoxicosis from molar thyrotropin. *Arch Intern Med*. 1973;132(3):432–436.
283. Kenimer JG, Hershman JM, Higgins HP. The thyrotropin in hydatidiform moles is human chorionic gonadotropin. *J Clin Endocrinol Metab*. 1975;40(3):482–491.
284. Higgins HP, Hershman JM, Kenimer JG, Patillo RA, Bayley TA, Walfish P. The thyrotoxicosis of hydatidiform mole. *Ann Intern Med*. 1975;83(3):307–311.
285. Nagataki S, Mizuno M, Sakamoto S, Irie M, Shizume K. Thyroid function in molar pregnancy. *J Clin Endocrinol Metab*. 1977;44(2):254–263.
286. Anderson NR, Lokich JJ, McDermott Jr WV, Trey C, Falchuk KR. Gestational choriocarcinoma and thyrotoxicosis. *Cancer*. 1979;44(1):304–306.
287. Walkington L, Webster J, Hancock BW, Everard J, Coleman RE. Hyperthyroidism and human chorionic gonadotrophin production in gestational trophoblastic disease. *Br J Cancer*. 2011;104(11):1665–1669.
288. Lockwood CM, Grenache DG, Gronowski AM. Serum human chorionic gonadotropin concentrations greater than 400,000 IU/L are invariably associated with suppressed serum thyrotropin concentrations. *Thyroid*. 2009;19(8):863–868.
289. Rodien P, Bremont C, Sanson ML, Parma J, Van Sande J, Costagliola S, et al. Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. *N Engl J Med*. 1998;339(25):1823–1826.
290. Fan NC, Jeung EB, Peng C, Olofsson JJ, Krisinger J, Leung PC. The human gonadotropin-releasing hormone (GnRH) receptor gene: cloning, genomic organization and chromosomal assignment. *Mol Cell Endocrinol*. 1994;103(1-2):R1–R6.
291. Kottler ML, Lorenzo F, Bergametti F, Commercon P, Souchier C, Counis R. Subregional mapping of the human gonadotropin-releasing hormone receptor (GnRH-R) gene to 4q between the markers D4S392 and D4S409. *Hum Genet*. 1995;96(4):477–480.
292. Kakar SS, Musgrove LC, Devor DC, Sellers JC, Neill JD. Cloning, sequencing, and expression of human gonadotropin releasing hormone (GnRH) receptor. *Biochem Biophys Res Commun*. 1992;189(1):289–295.
293. Chi L, Zhou W, Prikhozhan A, Flanagan C, Davidson JS, Golembo M, et al. Cloning and characterization of the human GnRH receptor. *Mol Cell Endocrinol*. 1993;91(1-2):R1–R6.
294. Stojilkovic SS, Reinhart J, Catt KJ. Gonadotropin-releasing hormone receptors: structure and signal transduction pathways. *Endocr Rev*. 1994;15(4):462–499.
295. Hokin LE. Receptors and phosphoinositide-generated second messengers. *Annu Rev Biochem*. 1985;54:205–235.
296. Berridge MJ. Inositol trisphosphate and diacylglycerol: two interacting second messengers. *Annu Rev Biochem*. 1987;56:159–193.
297. de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med*. 1997;337(22):1597–1602.
298. Seminara SB, Hayes FJ, Crowley Jr WF. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome):

- pathophysiological and genetic considerations. *Endocr Rev.* 1998;19(5):521–539.
299. Pralong FP, Gomez F, Castillo E, Cotecchia S, Abuin L, Aubert ML, et al. Complete hypogonadotropic hypogonadism associated with a novel inactivating mutation of the gonadotropin-releasing hormone receptor. *J Clin Endocrinol Metab.* 1999;84(10):3811–3816.
 300. Francou B, Paul C, Amazit L, Cartes A, Bouvattier C, Albarel F, et al. Prevalence of KISS1 Receptor mutations in a series of 603 patients with normosmic congenital hypogonadotropic hypogonadism and characterization of novel mutations: a single-centre study. *Hum Reprod.* 2016;31(6):1363–1374.
 301. de Roux N, Young J, Brailly-Tabard S, Misrahi M, Milgrom E, Schaison G. The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred. *J Clin Endocrinol Metab.* 1999;84(2):567–572.
 302. Seminara SB, Beranova M, Oliveira LM, Martin KA, Crowley Jr WF, Hall JE. Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *J Clin Endocrinol Metab.* 2000;85(2):556–562.
 303. Chanson P, De Roux N, Young J, Bidart JM, Jacquet P, Misrahi M, et al. Absence of activating mutations in the GnRH receptor gene in human pituitary gonadotroph adenomas. *Eur J Endocrinol.* 1998;139(2):157–160.
 304. Gershengorn MC. Mechanism of signal transduction by TRH. *Ann N Y Acad Sci.* 1989;553:191–196.
 305. Collu R, Tang J, Castagne J, Lagace G, Masson N, Huot C, et al. A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab.* 1997;82(5):1561–1565.
 306. Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, et al. A family with complete resistance to thyrotropin-releasing hormone. *N Engl J Med.* 2009;360(7):731–734.
 307. Rabeler R, Mittag J, Geffers L, Ruther U, Leitges M, Parlow AF, et al. Generation of thyrotropin-releasing hormone receptor 1-deficient mice as an animal model of central hypothyroidism. *Mol Endocrinol.* 2004;18(6):1450–1460.
 308. Tomita T, Masuzaki H, Iwakura H, Fujikura J, Noguchi M, Tanaka T, et al. Expression of the gene for a membrane-bound fatty acid receptor in the pancreas and islet cell tumours in humans: evidence for GPR40 expression in pancreatic beta cells and implications for insulin secretion. *Diabetologia.* 2006;49(5):962–968.
 309. Liou AP, Lu X, Sei Y, Zhao X, Pechhold S, Carrero RJ, et al. The G-protein-coupled receptor GPR40 directly mediates long-chain fatty acid-induced secretion of cholecystokinin. *Gastroenterology.* 2010;140(3):903–912.
 310. Edfalk S, Steneberg P, Edlund H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes.* 2008;57(9):2280–2287.
 311. Ichimura A, Hirasawa A, Hara T, Tsujimoto G. Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. *Prostaglandins Other Lipid Mediat.* 2009;89(3-4):82–88.
 312. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, et al. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature.* 2003;422(6928):173–176.
 313. Fujiwara K, Maekawa F, Yada T. Oleic acid interacts with GPR40 to induce Ca²⁺ signaling in rat islet beta-cells: mediation by PLC and L-type Ca²⁺ channel and link to insulin release. *Am J Physiol Endocrinol Metab.* 2005;289(4):E670–E677.
 314. Shapiro H, Shachar S, Sekler I, Hershfield M, Walker MD. Role of GPR40 in fatty acid action on the beta cell line INS-1E. *Biochem Biophys Res Commun.* 2005;335(1):97–104.
 315. Itoh Y, Hinuma S. GPR40, a free fatty acid receptor on pancreatic beta cells, regulates insulin secretion. *Hepatol Res.* 2005;33(2):171–173.
 316. Vettor R, Granzotto M, De Stefani D, Trevisan E, Rossato M, Farina MG, et al. Loss-of-function mutation of the GPR40 gene associates with abnormal stimulated insulin secretion by acting on intracellular calcium mobilization. *J Clin Endocrinol Metab.* 2008;93(9):3541–3550.
 317. Hamid YH, Vissing H, Holst B, Urhammer SA, Pyke C, Hansen SK, et al. Studies of relationships between variation of the human G protein-coupled receptor 40 Gene and Type 2 diabetes and insulin release. *Diabet Med.* 2005;22(1):74–80.
 318. Burant CF, Viswanathan P, Marcinek J, Cao C, Vakilynejad M, Xie B, et al. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet.* 2012;379(9824):1403–1411.
 319. Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H. The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab.* 2005;1(4):245–258.
 320. Ogawa T, Hirose H, Miyashita K, Saito I, Saruta T. GPR40 gene Arg211His polymorphism may contribute to the variation of insulin secretory capacity in Japanese men. *Metabolism.* 2005;54(3):296–299.
 321. Seminara SB. Metastin and its G protein-coupled receptor, GPR54: critical pathway modulating GnRH secretion. *Front Neuroendocrinol.* 2005;26(3-4):131–138.
 322. Dungan Lemko HM, Elias CF. Kiss of the mutant mouse: how genetically altered mice advanced our understanding of kisspeptin's role in reproductive physiology. *Endocrinology.* 2012;153(11):5119–5129.
 323. Bo-Abbas Y, Acierno Jr JS, Shagoury JK, Crowley Jr WF, Seminara SB. Autosomal recessive idiopathic hypogonadotropic hypogonadism: genetic analysis excludes mutations in the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes. *J Clin Endocrinol Metab.* 2003;88(6):2730–2737.
 324. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the Kiss1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A.* 2003;100(19):10972–10976.
 325. Seminara, S.B., Messenger, S., Chatzidaki, E.E., Thresher, R.R., Acierno, J.S., Jr., Shagoury JK, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med.* 2003;349(17):1614–1627.
 326. Wacker JL, Feller DB, Tang XB, Defino MC, Namkung Y, Lyssand JS, et al. Disease-causing mutation in GPR54 reveals the importance of the second intracellular loop for class A G-protein-coupled receptor function. *J Biol Chem.* 2008;283(45):31068–31078.
 327. Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, et al. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2005;90(3):1849–1855.
 328. Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N. Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab.* 2007;92(3):1137–1144.
 329. Kotani M, Dethoux M, Vandenberghe A, Communi D, Vanderwinden JM, Le Poul E, et al. The metastasis suppressor gene KISS-1 encodes kisspeptin, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem.* 2001;276(37):34631–34636.
 330. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, et al. Metastasis suppressor gene KISS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature.* 2001;411(6837):613–617.
 331. Dhillon WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab.* 2005;90(12):6609–6615.
 332. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, et al. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med.* 2008;358(7):709–715.
 333. Krstevska-Konstantinova M, Jovanovska J, Tasic VB, Montenegro LR, Beneduzzi D, Silveira LF, et al. Mutational analysis of KISS1 and KISS1R in idiopathic central precocious puberty. *J Pediatr Endocrinol Metab.* 2014;27(1-2):199–201.
 334. Oh YJ, Rhie YJ, Nam HK, Kim HR, Lee KH. Genetic variations of the KISS1R gene in Korean girls with central precocious puberty. *J Korean Med Sci.* 2017;32(1):108–114.
 335. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell.* 1998;92(4):573–585.
 336. Sutcliffe JG, de Lecea L. The hypocretins: excitatory neuromodulatory peptides for multiple homeostatic systems, including sleep and feeding. *J Neurosci Res.* 2000;62(2):161–168.
 337. Spinazzi R, Andreis PG, Rossi GP, Nussdorfer GG. Orexins in the regulation of the hypothalamic-pituitary-adrenal axis. *Pharmacol Rev.* 2006;58(1):46–57.

338. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A*. 1998;95(1):322–327.
339. Ammoun S, Holmqvist T, Shariatmadari R, Oonk HB, Detheux M, Parmentier M, et al. Distinct recognition of OX1 and OX2 receptors by orexin peptides. *J Pharmacol Exp Ther*. 2003;305(2):507–514.
340. Smart D, Jerman JC, Brough SJ, Rushton SL, Murdock PR, Jewitt F, et al. Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. *Br J Pharmacol*. 1999;128(1):1–3.
341. Kane JK, Tanaka H, Parker SL, Yanagisawa M, Li MD. Sensitivity of orexin-A binding to phospholipase C inhibitors, neuropeptide Y, and secretin. *Biochem Biophys Res Commun*. 2000;272(3):959–965.
342. Holmqvist T, Johansson L, Ostman M, Ammoun S, Akerman KE, Kukkonen JP. OX1 orexin receptors couple to adenylyl cyclase regulation via multiple mechanisms. *J Biol Chem*. 2005;280(8):6570–6579.
343. Sakurai T. Orexins and orexin receptors: implication in feeding behavior. *Regul Pept*. 1999;85(1):25–30.
344. Xu YL, Jackson VR, Civelli O. Orphan G protein-coupled receptors and obesity. *Eur J Pharmacol*. 2004;500(1-3):243–253.
345. Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*. 2000;355(9197):39–40.
346. Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med*. 2000;6(9):991–997.
347. Nishino S, Ripley B, Overeem S, Nevsimalova S, Lammers GJ, Vankova J, et al. Low cerebrospinal fluid hypocretin (Orexin) and altered energy homeostasis in human narcolepsy. *Ann Neurol*. 2001;50(3):381–388.
348. Sutcliffe JG, de Lecea L. The hypocretins: setting the arousal threshold. *Nat Rev Neurosci*. 2002;3(5):339–349.
349. Nishino S, Okuro M, Kotorii N, Aneqawa E, Ishimaru Y, Matsumura M, et al. Hypocretin/orexin and narcolepsy: new basic and clinical insights. *Acta Physiol (Oxf)*. 2010;198(3):209–222.
350. Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*. 1999;98(3):365–376.
351. Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, et al. International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol Rev*. 2005;57(4):541–546.
352. Zhang JV, Ren PG, Aysian-Kretschmer O, Luo CW, Rauch R, Klein C, et al. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*. 2005;310(5750):996–999.
353. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656–660.
354. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology*. 2000;141(11):4255–4261.
355. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714–1719.
356. Tschöp M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, et al. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest*. 2001;24(6):RC19–21.
357. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*. 2001;86(12):5992.
358. Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, et al. Elevated plasma ghrelin levels in Prader Willi syndrome. *Nat Med*. 2002;8(7):643–644.
359. Wang HJ, Geller F, Dempfle A, Schauble N, Friedel S, Lichtner P, et al. Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J Clin Endocrinol Metab*. 2004;89(1):157–162.
360. Wang W, Tao YX. Ghrelin receptor mutations and human obesity. *Prog Mol Biol Transl Sci*. 2016;140:131–150.
361. Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, Morisset S, et al. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest*. 2006;116(3):760–768.
362. Pantel J, Legendre M, Nivot S, Morisset S, Vie-Luton MP, le Bouc Y, et al. Recessive isolated growth hormone deficiency and mutations in the ghrelin receptor. *J Clin Endocrinol Metab*. 2009;94(11):4334–4341.
363. Inoue H, Kangawa N, Kinouchi A, Sakamoto Y, Kimura C, Horikawa R, et al. Identification and functional analysis of novel human growth hormone secretagogue receptor (GHSR) gene mutations in Japanese subjects with short stature. *J Clin Endocrinol Metab*. 2012;96(2):E373–E378.
364. Pugliese-Pires PN, Fortin JP, Arthur T, Latronico AC, Mendonca BB, Villares SM, et al. Novel inactivating mutations in the GH secretagogue receptor gene in patients with constitutional delay of growth and puberty. *Eur J Endocrinol*. 2011;165(2):233–241.
365. Chambers J, Ames RS, Bergsma D, Muir A, Fitzgerald LR, Hervieu G, et al. Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1. *Nature*. 1999;400(6741):261–265.
366. Saito Y, Nothacker HP, Wang Z, Lin SH, Leslie F, Civelli O. Molecular characterization of the melanin-concentrating-hormone receptor. *Nature*. 1999;400(6741):265–269.
367. Wang S, Behan J, O'Neill K, Weig B, Fried S, Laz T, et al. Identification and pharmacological characterization of a novel human melanin-concentrating hormone receptor, mch-r2. *J Biol Chem*. 2001;276(37):34664–34670.
368. Hawes BE, Kil E, Green B, O'Neill K, Fried S, Graziano MP. The melanin-concentrating hormone receptor couples to multiple G proteins to activate diverse intracellular signaling pathways. *Endocrinology*. 2000;141(12):4524–4532.
369. Hill J, Duckworth M, Murdock P, Rennie G, Sabido-David C, Ames RS, et al. Molecular cloning and functional characterization of MCH2, a novel human MCH receptor. *J Biol Chem*. 2001;276(23):20125–20129.
370. Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Lagu B, et al. Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. *Nat Med*. 2002;8(8):825–830.
371. Shearman LP, Camacho RE, Sloan Stribling D, Zhou D, Bednarek MA, Hreniuk DL, et al. Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. *Eur J Pharmacol*. 2003;475(1-3):37–47.
372. Gehlert DR, Rasmussen K, Shaw J, Li X, Ardayfio P, Craft L, et al. Preclinical evaluation of melanin-concentrating hormone receptor 1 antagonism for the treatment of obesity and depression. *J Pharmacol Exp Ther*. 2009;329(2):429–438.
373. Chung S, Parks GS, Lee C, Civelli O. Recent updates on the melanin-concentrating hormone (MCH) and its receptor system: lessons from MCH1R antagonists. *J Mol Neurosci*. 2010;43(1):115–121.
374. Sherwood A, Holland PC, Adamantidis A, Johnson AW. Deletion of Melanin Concentrating Hormone Receptor-1 disrupts overeating in the presence of food cues. *Physiol Behav*. 2015;152(Pt B):402–407.
375. Goldstein C, Schroeder JC, Fortin JP, Goss JM, Schaus SE, Beinborn M, et al. Two naturally occurring mutations in the type 1 melanin-concentrating hormone receptor abolish agonist-induced signaling. *J Pharmacol Exp Ther*. 2010;335(3):799–806.
376. Wernter AK, Reichwald K, Buch T, Geller F, Platzer C, Huse K, et al. Mutation analysis of the MCH1R gene in human obesity. *Eur J Endocrinol*. 2005;152(6):851–862.
377. Gibson WT, Pissios P, Trombly DJ, Luan J, Keogh J, Wareham NJ, et al. Melanin-concentrating hormone receptor mutations and human obesity: functional analysis. *Obes Res*. 2004;12(5):743–749.
378. Maheshwari HG, Silverman BL, Dupuis J, Baumann G. Phenotype and genetic analysis of a syndrome caused by an inactivating mutation in the growth hormone-releasing hormone receptor: Dwarfism of Sindh. *J Clin Endocrinol Metab*. 1998;83(11):4065–4074.

379. Salvatori R, Hayashida CY, Aguiar-Oliveira MH, Phillips 3rd JA, Souza AH, Gondo RG, et al. Familial dwarfism due to a novel mutation of the growth hormone-releasing hormone receptor gene. *J Clin Endocrinol Metab*. 84(3): 917–923.
380. Vallar L, Spada A, Giannattasio G. Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. *Nature*. 1987;330(6148):566–568.
381. Alatzoglou KS, Turton JP, Kelberman D, Clayton PE, Mehta A, Buchanan C, et al. Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. *J Clin Endocrinol Metab*. 94(9): 3191–3199.
382. Martar M, Salvatori R. Diseases associated with growth hormone-releasing hormone receptor (GHRHR) mutations. *Prog Mol Biol Transl Sci*. 2009;88:57–84.
383. Shohreh R, Sherfat-Kazemzadeh R, Jee YH, Blitz A, Salvatori R. A novel frame shift mutation in the GHRH receptor gene in familial isolated GH deficiency: early occurrence of anterior pituitary hypoplasia. *J Clin Endocrinol Metab*. 2012;96(10): 2982–2986.
384. Wang Q, Diao Y, Xu Z, Li X, Luo XP, Xu H, et al. Identification of a novel splicing mutation in the growth hormone (GH)-releasing hormone receptor gene in a Chinese family with pituitary dwarfism. *Mol Cell Endocrinol*. 2009;313(1-2):50–56.
385. Godfrey P, Rahal JO, Beamer WG, Copeland NG, Jenkins NA, Mayo KE. GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. *Nat Genet*. 1993;4(3):227–232.
386. Lin SC, Lin CR, Gukovsky I, Lusic AJ, Sawchenko PE, Rosenfeld MG. Molecular basis of the little mouse phenotype and implications for cell type-specific growth. *Nature*. 1993;364(6434):208–213.
387. Gaylinn BD, Dealmeida VI, Lyons Jr CE, Wu KC, Mayo KE, Thorne MO. The mutant growth hormone-releasing hormone (GHRH) receptor of the little mouse does not bind GHRH. *Endocrinology*. 1999;140(11):5066–5074.
388. Wajnrajch MP, Gertner JM, Harbison MD, Chua Jr SC, Leibel RL. Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet*. 1996;12(1):88–90.
389. Baumann G, Maheshwari H. The Dwarfs of Sindh: severe growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. *Acta Paediatrica*. 1997; 423:33–38.
390. Netchine I, Talon P, Dastot F, Vitaux F, Goossens M, Amselem S. Extensive phenotypic analysis of a family with growth hormone (GH) deficiency caused by a mutation in the GH-releasing hormone receptor gene. *J Clin Endocrinol Metab*. 1998;83(2): 432–436.
391. Salvatori R, Fan X, Phillips 3rd JA, Prince M, Levine MA. Isolated growth hormone (GH) deficiency due to compound heterozygosity for two new mutations in the GH-releasing hormone receptor gene. *Clin Endocrinol (Oxf)*. 2001;54(5):681–687.
392. Salvatori R, Fan X, Mullis PE, Haile A, Levine MA. Decreased expression of the GHRH receptor gene due to a mutation in a Pit-1 binding site. *Mol Endocrinol*. 2002;16(3):450–458.
393. Aguiar-Oliveira MH, Souza AHO, Oliveira CRP, Campos VC, Oliveira-Neto LA, Salvatori R. Mechanisms in Endocrinology: The multiple facets of GHRH/GH/IGF-I axis: lessons from lifetime, untreated, isolated GH deficiency due to a GHRH receptor gene mutation. *Eur J Endocrinol*. 2017;177(2):R85–R97.
394. Aguiar-Oliveira MH, Davalos C, Campos VC, Oliveira Neto LA, Marinho CG, Oliveira CRP. Hypothalamic abnormalities: growth failure due to defects of the GHRH receptor. *Growth Horm IGF Res*. 2018;38:14–18.
395. Siklar Z, Berberoglu M, Legendre M, Amselem S, Evliyaoglu O, Hacıhamdioglu B, et al. Two siblings with isolated GH deficiency due to loss-of-function mutation in the GHRHR gene: successful treatment with growth hormone despite late admission and severe growth retardation. *J Clin Res Pediatr Endocrinol*. 2011;2(4):164–167.
396. Menezes Oliveira JL, Marques-Santos C, Barreto-Filho JA, Ximenes Filho R, de Oliveira Britto AV, Oliveira Souza AH, et al. Lack of evidence of premature atherosclerosis in untreated severe isolated growth hormone (GH) deficiency due to a GH-releasing hormone receptor mutation. *J Clin Endocrinol Metab*. 2006;91(6):2093–2099.
397. Oliveira JL, Aguiar-Oliveira MH, D'Oliveira Jr A, Pereira RM, Oliveira CR, Farias CT, et al. Congenital growth hormone (GH) deficiency and atherosclerosis: effects of GH replacement in GH-naive adults. *J Clin Endocrinol Metab*. 2007;92(12): 4664–4670.
398. Araujo VP, Aguiar-Oliveira MH, Oliveira JL, Rocha HM, Oliveira CR, Rodrigues TM, et al. Arrest of atherosclerosis progression after interruption of GH replacement in adults with congenital isolated GH deficiency. *Eur J Endocrinol*. 2012;166(6): 977–982.
399. Oliveira FT, Salvatori R, Marcondes J, Macena LB, Oliveira-Santos AA, Faro ACN, et al. Altered sleep patterns in patients with non-functional GHRH receptor. *Eur J Endocrinol*. 2017;177(1): 51–57.
400. Pereira RM, Aguiar-Oliveira MH, Sagazio A, Oliveira CR, Oliveira FT, Campos VC, et al. Heterozygosity for a mutation in the growth hormone-releasing hormone receptor gene does not influence adult stature, but affects body composition. *J Clin Endocrinol Metab*. 2007;92(6):2353–2357.
401. Camats N, Fernandez-Cancio M, Carrascosa A, Andaluz P, Albisu MA, Clemente M, et al. Contribution of human growth hormone-releasing hormone receptor (GHRHR) gene sequence variation to isolated severe growth hormone deficiency (ISGHD) and normal adult height. *Clin Endocrinol*. 2012;77(4):564–574.
402. Johansson A, Jonasson I, Gyllensten U. Extended haplotypes in the growth hormone releasing hormone receptor gene (GHRHR) are associated with normal variation in height. *PLoS One*. 2009;4(2). e4464.
403. Franca MM, Jorge AA, Alatzoglou KS, Carvalho LR, Mendonca BB, Audi L, et al. Absence of GH-releasing hormone (GHRH) mutations in selected patients with isolated GH deficiency. *J Clin Endocrinol Metab*. 2011;96(9):E1457–E1460.
404. Yamada Y, Hayami T, Nakamura K, Kaisaki PJ, Someya Y, Wang CZ, et al. Human gastric inhibitory polypeptide receptor: cloning of the gene (GIPR) and cDNA. *Genomics*. 1995;29(3): 773–776.
405. Gremlich S, Porret A, Hani EH, Cherif D, Vionnet N, Froguel P, et al. Cloning, functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. *Diabetes*. 1995;44(10):1202–1208.
406. Wheeler MB, Gelling RW, McIntosh CH, Georgiou J, Brown JC, Pederson RA. Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signaling properties. *Endocrinology*. 1995;136(10):4629–4639.
407. Tseng CC, Zhang XY. Role of regulator of G protein signaling in desensitization of the glucose-dependent insulinotropic peptide receptor. *Endocrinology*. 1998;139(11):4470–4475.
408. Beck B. Gastric inhibitory polypeptide: a gut hormone with anabolic functions. *J Mol Endocrinol*. 1989;2(3):169–174.
409. Fehmann HC, Goke R, Goke B. Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev*. 1995; 16(3):390–410.
410. Sauber J, Grothe J, Behm M, Scherag A, Gallert H, Illig T, et al. Association of variants in gastric inhibitory polypeptide receptor gene with impaired glucose homeostasis in obese children and adolescents from Berlin. *Eur J Endocrinol*. 2010;163(2):259–264.
411. Almind K, Ambye L, Urhammer SA, Hansen T, Echwald SM, Holst JJ, et al. Discovery of amino acid variants in the human glucose-dependent insulinotropic polypeptide (GIP) receptor: the impact on the pancreatic beta cell responses and functional expression studies in Chinese hamster fibroblast cells. *Diabetologia*. 1998;41(10):1194–1198.
412. N'Diaye N, Tremblay J, Hamet P, De Herder WW, Lacroix A. Adrenocortical overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. *J Clin Endocrinol Metab*. 1998;83(8):2781–2785.
413. Lebrethon MC, Avallat O, Reznik Y, Archambeaud F, Combes J, Usdin TB, et al. Food-dependent Cushing's syndrome: characterization and functional role of gastric inhibitory polypeptide receptor in the adrenals of three patients. *J Clin Endocrinol Metab*. 1998;83(12):4514–4519.

414. de Herder WW, Hofland LJ, Usdin TB, de Jong FH, Uitterlinden P, van Koetsveld P, et al. Food-dependent Cushing's syndrome resulting from abundant expression of gastric inhibitory polypeptide receptors in adrenal adenoma cells. *J Clin Endocrinol Metab.* 1996;81(9):3168–3172.
415. Mannstadt M, Juppner H, Gardella TJ. Receptors for PTH and PTHrP: their biological importance and functional properties. *Am J Physiol.* 1999;277(5 Pt 2):F665–F675.
416. Usdin TB, Gruber C, Bonner TI. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. *J Biol Chem.* 1995;270(26):15455–15458.
417. Behar V, Pines M, Nakamoto C, Greenberg Z, Bisello A, Stueckle SM, et al. The human PTH2 receptor: binding and signal transduction properties of the stably expressed recombinant receptor. *Endocrinology.* 1996;137(7):2748–2757.
418. Turner PR, Mefford S, Bambino T, Nissenson RA. Transmembrane residues together with the amino terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide. *J Biol Chem.* 1998;273(7):3830–3837.
419. Bhattacharya P, Yan YL, Postlethwait J, Rubin DA. Evolution of the vertebrate pth2 (tip39) gene family and the regulation of PTH type 2 receptor (pth2r) and its endogenous ligand pth2 by hedgehog signaling in zebrafish development. *J Endocrinol.* 2011;211(2):187–200.
420. Dobolyi A, Palkovits M, Usdin TB. The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. *Prog Neurobiol.* 2010;90(1):29–59.
421. Usdin TB. The PTH2 receptor and TIP39: a new peptide-receptor system. *Trends Pharmacol Sci.* 2000;21(4):128–130.
422. Abou-Samra AB, Jueppner H, Westerberg D, Potts Jr JT, Segre GV. Parathyroid hormone causes translocation of protein kinase-C from cytosol to membranes in rat osteosarcoma cells. *Endocrinology.* 1989;124(3):1107–1113.
423. Tamura T, Sakamoto H, Filburn CR. Parathyroid hormone 1-34, but not 3-34 or 7-34, transiently translocates protein kinase C in cultured renal (OK) cells. *Biochem Biophys Res Commun.* 1989;159(3):1352–1358.
424. Dunlay R, Hruska K. PTH receptor coupling to phospholipase C is an alternate pathway of signal transduction in bone and kidney. *Am J Physiol.* 1990;258(2 Pt 2):F223–F231.
425. Partridge NC, Bloch SR, Pearman AT. Signal transduction pathways mediating parathyroid hormone regulation of osteoblastic gene expression. *J Cell Biochem.* 1994;55(3):321–327.
426. Offermanns S, Iida-Klein A, Segre GV, Simon MI. G alpha q family members couple parathyroid hormone (PTH)/PTH-related peptide and calcitonin receptors to phospholipase C in COS-7 cells. *Mol Endocrinol.* 1996;10(5):566–574.
427. Schwindinger WF, Fredericks J, Watkins L, Robinson H, Bathon JM, Pines M, et al. Coupling of the PTH/PTHrP receptor to multiple G-proteins. Direct demonstration of receptor activation of Gs, Gq/11, and Gi(1) by [alpha-32P]GTP-gamma-azidoanilide photoaffinity labeling. *Endocrine.* 1998;8(2):201–209.
428. Cheloha RW, Gellman SH, Vilaradaga JP, Gardella TJ. PTH receptor-1 signalling-mechanistic insights and therapeutic prospects. *Nat Rev Endocrinol.* 2015;11(12):712–724.
429. Iida-Klein A, Guo J, Takemura M, Drake MT, Potts Jr JT, Abou-Samra A, et al. Mutations in the second cytoplasmic loop of the rat parathyroid hormone (PTH)/PTH-related protein receptor result in selective loss of PTH-stimulated phospholipase C activity. *J Biol Chem.* 1997;272(11):6882–6889.
430. Huang Z, Chen Y, Pratt S, Chen TH, Bambino T, Nissenson RA, et al. The N-terminal region of the third intracellular loop of the parathyroid hormone (PTH)/PTH-related peptide receptor is critical for coupling to cAMP and inositol phosphate/Ca2+ signal transduction pathways. *J Biol Chem.* 1996;271(52):33382–33389.
431. Ferrandon S, Feinstein TN, Castro M, Wang B, Bouley R, Potts JT, et al. Sustained cyclic AMP production by parathyroid hormone receptor endocytosis. *Nat Chem Biol.* 2009;5(10):734–742.
432. Wysolmerski JJ, Cormier S, Philbrick WM, Dann P, Zhang JP, Roume J, et al. Absence of functional type 1 parathyroid hormone (PTH)/PTH-related protein receptors in humans is associated with abnormal breast development and tooth impaction. *J Clin Endocrinol Metab.* 2001;86(4):1788–1794.
433. Karaplis AC, He B, Nguyen MT, Young ID, Semeraro D, Ozawa H, et al. Inactivating mutation in the human parathyroid hormone receptor type 1 gene in Blomstrand chondrodysplasia [see comments]. *Endocrinology.* 1998;139(12):5255–5258.
434. Zhang P, Jobert AS, Couvineau A, Silve C. A homozygous inactivating mutation in the parathyroid hormone/parathyroid hormone-related peptide receptor causing Blomstrand chondrodysplasia. *J Clin Endocrinol Metab.* 1998;83(9):3365–3368.
435. Karperien M, van der Harten HJ, van Schooten R, Farih-Sips H, den Hollander NS, Kneppers SL, et al. A frame-shift mutation in the type I parathyroid hormone (PTH)/PTH-related peptide receptor causing Blomstrand lethal osteochondrodysplasia. *J Clin Endocrinol Metab.* 1999;84(10):3713–3720.
436. Jobert AS, Zhang P, Couvineau A, Bonaventure J, Roume J, Le Merrer M, et al. Absence of functional receptors for parathyroid hormone and parathyroid hormone-related peptide in Blomstrand chondrodysplasia. *J Clin Invest.* 1998;102(1):34–40.
437. Hopyan S, Gokgoz N, Poon R, Gensure RC, Yu C, Cole WG, et al. A mutant PTH/PTHrP type I receptor in enchondromatosis. *Nat Genet.* 2002;30(3):306–310.
438. Alderton GK. Genetics: IDH mosaicism in enchondromatosis syndromes. *Nat Rev Cancer.* 2011;12(1):6.
439. Pansuriya TC, van Eijk R, d'Adamo P, van Ruler MA, Kuijjer ML, Oosting J, et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. *Nat Genet.* 2011;43(12):1256–1261.
440. Eiken M, Prag J, Petersen KE, Kaufmann HJ. A new familial skeletal dysplasia with severely retarded ossification and abnormal modeling of bones especially of the epiphyses, the hands, and feet. *Eur J Pediatr.* 1984;141(4):231–235.
441. Duchatelet S, Ostergaard E, Cortes D, Lemainque A, Julier C. Recessive mutations in PTHR1 cause contrasting skeletal dysplasias in Eiken and Blomstrand syndromes. *Hum Mol Genet.* 2005;14(1):1–5.
442. Moirangthem A, Narayanan DL, Jacob P, Nishimura G, Mortier G, Girisha KM. Report of second case and clinical and molecular characterization of Eiken syndrome. *Clin Genet.* 2018;94(5):457–460.
443. Decker E, Stellzig-Eisenhauer A, Fiebig BS, Rau C, Kress W, Saar K, et al. PTHR1 loss-of-function mutations in familial, nonsyndromic primary failure of tooth eruption. *Am J Hum Genet.* 2008;83(6):781–786.
444. Schipani E, Kruse K, Juppner H. A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science.* 1995;268(5207):98–100.
445. Schipani E, Langman CB, Parfitt AM, Jensen GS, Kikuchi S, Kooh SW, et al. Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia [see comments]. *N Engl J Med.* 1996;335(10):708–714.
446. Schipani E, Langman C, Hunzelman J, Le Merrer M, Loke KY, Dillon MJ, et al. A novel parathyroid hormone (PTH)/PTH-related peptide receptor mutation in Jansen's metaphyseal chondrodysplasia. *J Clin Endocrinol Metab.* 1999;84(9):3052–3057.
447. Holz GG, Chepurny OG. Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Curr Med Chem.* 2003;10(22):2471–2483.
448. Holz GG, Leech CA, Habener JF. Insulinotropic toxins as molecular probes for analysis of glucagon-like-peptide-1 receptor-mediated signal transduction in pancreatic beta-cells. *Biochimie.* 2000;82(9-10):915–926.
449. Janicic N, Soliman E, Pausova Z, Seldin MF, Riviere M, Szpirer J, et al. Mapping of the calcium-sensing receptor gene (CASR) to human chromosome 3q13.3-21 by fluorescence in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16. *Mamm Genome.* 1995;6(11):798–801.
450. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature.* 1993;366(6455):575–580.

451. Mancilla EE, De Luca F, Baron J. Activating mutations of the Ca²⁺-sensing receptor. *Mol Genet Metab.* 1998;64(3):198–204.
452. Brown EM, Pollak M, Chou YH, Seidman CE, Seidman JG, Hebert SC. The cloning of extracellular Ca(2+)-sensing receptors from parathyroid and kidney: molecular mechanisms of extracellular Ca(2+)-sensing. *J Nutr.* 1995;125(7 Suppl):1965S–1970S.
453. Brown EM, Hebert SC. A cloned Ca(2+)-sensing receptor: a mediator of direct effects of extracellular Ca²⁺ on renal function? *J Am Soc Nephrol.* 1995;6(6):1530–1540.
454. Cholist IN, Steinberg SF, Tropper PJ, Fox HE, Segre GV, Bilezikian JP. The influence of hypermagnesemia on serum calcium and parathyroid hormone levels in human subjects. *N Engl J Med.* 1984;310(19):1221–1225.
455. Hebert SC, Brown EM, Harris HW. Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol.* 1997;200(Pt 2):295–302.
456. Chattopadhyay N. Biochemistry, physiology and pathophysiology of the extracellular calcium-sensing receptor. *Int J Biochem Cell Biol.* 2000;32(8):789–804.
457. Hannan FM, Babinsky VN, Thakker RV. Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Mol Endocrinol.* 2016;57(3):R127–R142.
458. Thakker RV. Disorders of the calcium-sensing receptor. *Biochim Biophys Acta.* 1998;1448(2):166–170.
459. Pearce S, Steinmann B. Casting new light on the clinical spectrum of neonatal severe hyperparathyroidism. *Clin Endocrinol (Oxf).* 1999;50(6):691–693.
460. Chou YH, Pollak MR, Brandi ML, Toss G, Arnqvist H, Atkinson AB, et al. Mutations in the human Ca(2+)-sensing-receptor gene that cause familial hypocalciuric hypercalcemia. *Am J Hum Genet.* 1995;56(5):1075–1079.
461. Heath 3rd H, Odelberg S, Jackson CE, Teh BT, Hayward N, Larsson C, et al. Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. *J Clin Endocrinol Metab.* 1996;81(4):1312–1317.
462. Marx SJ, Attie MF, Spiegel AM, Levine MA, Lasker RD, Fox M. An association between neonatal severe primary hyperparathyroidism and familial hypocalciuric hypercalcemia in three kindreds. *N Engl J Med.* 1982;306(5):257–264.
463. Marx SJ, Fraser D, Rapoport A. Familial hypocalciuric hypercalcemia. Mild expression of the gene in heterozygotes and severe expression in homozygotes. *Am J Med.* 1985;78(1):15–22.
464. Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, et al. Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism [see comments]. *Cell.* 1993;75(7):1297–1303.
465. Janicic N, Pausova Z, Cole DE, Hendy GN. Insertion of an Alu sequence in the Ca(2+)-sensing receptor gene in familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Am J Hum Genet.* 1995;56(4):880–886.
466. Pearce SH, Trump D, Wooding C, Besser GM, Chew SL, Grant DB, et al. Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism. *J Clin Invest.* 1995;96(6):2683–2692.
467. Lietman SA, Tenenbaum-Rakover Y, Jap TS, Yi-Chi W, De-Ming Y, Ding C, et al. A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab.* 2009;94(11):4372–4379.
468. Marx SJ, Attie MF, Levine MA, Spiegel AM, Downs Jr RW, Lasker RD. The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine (Baltimore).* 1981;60(6):397–412.
469. Marx SJ, Spiegel AM, Levine MA, Rizzoli RE, Lasker RD, Santora AC, et al. Familial hypocalciuric hypercalcemia: the relation to primary parathyroid hyperplasia. *N Engl J Med.* 1982;307(7):416–426.
470. Nesbit MA, Hannan FM, Howles SA, Babinsky VN, Head RA, Cranston T, et al. Mutations affecting G-protein subunit alpha11 in hypercalcemia and hypocalcemia. *N Engl J Med.* 2013;368(26):2476–2486.
471. Nesbit MA, Hannan FM, Howles SA, Reed AA, Cranston T, Thakker CE, et al. Mutations in AP2S1 cause familial hypocalciuric hypercalcemia type 3. *Nat Genet.* 2013;45(1):93–97.
472. Thakker RV. Diseases associated with the extracellular calcium-sensing receptor. *Cell Calcium.* 2004;35(3):275–282.
473. Heath 3rd H, Jackson CE, Otterud B, Leppert MF. Genetic linkage analysis in familial benign (hypocalciuric) hypercalcemia: evidence for locus heterogeneity. *Am J Hum Genet.* 1993;53(1):193–200.
474. Lloyd SE, Pannett AA, Dixon PH, Whyte MP, Thakker RV. Localization of familial benign hypercalcemia, Oklahoma variant (FBHOk), to chromosome 19q13. *Am J Hum Genet.* 1999;64(1):189–195.
475. McMurtry CT, Schranck FW, Walkenhorst DA, Murphy WA, Kocher DB, Teitelbaum SL, et al. Significant developmental elevation in serum parathyroid hormone levels in a large kindred with familial benign (hypocalciuric) hypercalcemia. *Am J Med.* 1992;93(3):247–258.
476. Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, et al. Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. *Nat Genet.* 1994;8(3):303–307.
477. Finegold DN, Armitage MM, Galiani M, Matise TC, Pandian MR, Perry YM, et al. Preliminary localization of a gene for autosomal dominant hypoparathyroidism to chromosome 3q13. *Pediatr Res.* 1994;36(3):414–417.
478. Baron J, Winer KK, Yanovski JA, Cunningham AW, Laue L, Zimmerman D, et al. Mutations in the Ca(2+)-sensing receptor gene cause autosomal dominant and sporadic hypoparathyroidism. *Hum Mol Genet.* 1996;5(5):601–606.
479. Pearce SH, Williamson C, Kifor O, Bai M, Coulthard MG, Davies M, et al. A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor [see comments]. *N Engl J Med.* 1996;335(15):1115–1122.
480. De Luca F, Ray K, Mancilla EE, Fan GF, Winer KK, Gore P, et al. Sporadic hypoparathyroidism caused by de novo gain-of-function mutations of the Ca(2+)-sensing receptor. *J Clin Endocrinol Metab.* 1997;82(8):2710–2715.
481. Pearce S. Extracellular "calciostat" in health and disease [comment]. *Lancet.* 1999;353(9147):83–84.
482. Mannstadt M, Harris M, Bravenboer B, Chitturi S, Dreijerink KM, Lambright DG, et al. Germline mutations affecting Galpha11 in hypoparathyroidism. *N Engl J Med.* 2013;368(26):2532–2534.
483. Li D, Opas EE, Tuluc F, Metzger DL, Hou C, Hakonarson H, et al. Autosomal dominant hypoparathyroidism caused by germline mutation in GNA11: phenotypic and molecular characterization. *J Clin Endocrinol Metab.* 2014;99(9):E1774–E1783.
484. Vargas-Poussou R, Huang C, Hulin P, Houillier P, Jeunemaitre X, Paillard M, et al. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol.* 2002;13(9):2259–2266.
485. Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, et al. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet.* 2002;360(9334):692–694.
486. Hebert SC. Bartter syndrome. *Curr Opin Nephrol Hypertens.* 2003;12(5):527–532.
487. Cole DE, Peltekova VD, Rubin LA, Hawker GA, Vieth R, Liew CC, et al. A986S polymorphism of the calcium-sensing receptor and circulating calcium concentrations [see comments]. *Lancet.* 1999;353(9147):112–115.
488. Cole DE, Vieth R, Trang HM, Wong BY, Hendy GN, Rubin LA. Association between total serum calcium and the A986S polymorphism of the calcium-sensing receptor gene. *Mol Genet Metab.* 2001;72(2):168–174.
489. Scillitani A, Guarnieri V, De Geronimo S, Muscarella LA, Battista C, D'Aguma L, et al. Blood ionized calcium is associated with clustered polymorphisms in the carboxyl-terminal tail of the calcium-sensing receptor. *J Clin Endocrinol Metab.* 2004;89(11):5634–5638.
490. Miedlich S, Lamesch P, Mueller A, Paschke R. Frequency of the calcium-sensing receptor variant A986S in patients with primary hyperparathyroidism. *Eur J Endocrinol.* 2001;145(4):421–427.
491. Donath J, Speer G, Poor G, Gergely Jr P, Tabak A, Lakatos P. Vitamin D receptor, oestrogen receptor-alpha and calcium-sensing receptor

- genotypes, bone mineral density and biochemical markers in Paget's disease of bone. *Rheumatology (Oxf)*. 2004;43(6):692–695.
492. Scillitani A, Guarnieri V, Battista C, De Geronimo S, Muscarella LA, Chiodini I, et al. Primary hyperparathyroidism and the presence of kidney stones are associated with different haplotypes of the calcium-sensing receptor. *J Clin Endocrinol Metab*. 2007;92(1):277–283.
 493. Vezzoli G, Tanini A, Ferrucci L, Soldati L, Bianchin C, Franceschelli F, et al. Influence of calcium-sensing receptor gene on urinary calcium excretion in stone-forming patients. *J Am Soc Nephrol*. 2002;13(10):2517–2523.
 494. Kifor O, Moore Jr FD, Delaney M, Garber J, Hendy GN, Butters R, et al. A syndrome of hypocalcemic hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *J Clin Endocrinol Metab*. 2003;88(1):60–72.
 495. Li Y, Song YH, Rais N, Connor E, Schatz D, Muir A, et al. Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J Clin Invest*. 1996;97(4):910–914.
 496. Mayer A, Ploix C, Orgiazzi J, Desbos A, Moreira A, Vidal H, et al. Calcium-sensing receptor autoantibodies are relevant markers of acquired hypoparathyroidism. *J Clin Endocrinol Metab*. 2004;89(9):4484–4488.
 497. Milligan G, Kostenis E. Heterotrimeric G-proteins: a short history. *Br J Pharmacol*. 2006;147(Suppl 1):S46–S55.
 498. Haldeman-Englert CR, Hurst ACE, Levine MA. *Disorders of GNAS inactivation*. In: Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J.H., Stephens, K., et al., eds. *GeneReviews* ((R)). University of Washington, Seattle (WA), Seattle: GeneReviews is a registered trademark of the University of Washington; 1993.
 499. Linglart A, Levine MA, Juppner H. Pseudohypoparathyroidism. *Endocrinol Metab Clin North Am*. 2018;47(4):865–888.
 500. Hayward BE, Kamiya M, Strain L, Moran V, Campbell R, Hayashizaki Y, et al. The human GNAS1 gene is imprinted and encodes distinct paternally and biallelically expressed G proteins. *Proc Natl Acad Sci U S A*. 1998;95(17):10038–10043.
 501. Hayward BE, Moran V, Strain L, Bonthron DT. Bidirectional imprinting of a single gene: GNAS1 encodes maternally, paternally, and biallelically derived proteins. *Proc Natl Acad Sci U S A*. 1998;95(26):15475–15480.
 502. Juppner H, Schipani E, Bastepe M, Cole DE, Lawson ML, Mannstadt M, et al. The gene responsible for pseudohypoparathyroidism type 1b is paternally imprinted and maps in four unrelated kindreds to chromosome 20q13.3. *Proc Natl Acad Sci U S A*. 1998;95(20):11798–11803.
 503. Nakamoto JM, Sandstrom AT, Brickman AS, Christenson RA, Van Dop C. Pseudohypoparathyroidism type 1a from maternal but not paternal transmission of a Gsalpha gene mutation. *Am J Med Genet*. 1998;77(4):261–267.
 504. Wilson LC, Oude Luttikhuis ME, Clayton PT, Fraser WD, Trembath RC. Parental origin of Gs alpha gene mutations in Albright's hereditary osteodystrophy. *J Med Genet*. 1994;31(11):835–839.
 505. Weinstein LS, Yu S, Warner DR, Liu J. Endocrine manifestations of stimulatory G protein alpha-subunit mutations and the role of genomic imprinting. *Endocr Rev*. 2001;22(5):675–705.
 506. Lebrun M, Richard N, Abeguile G, David A, Coeslier DA, Journel H, et al. Progressive osseous heteroplasia: a model for the imprinting effects of GNAS inactivating mutations in humans. *J Clin Endocrinol Metab*. 2010;95(6):3028–3038.
 507. Lau K, Willig RP, Hiort O, Hoeger PH. Linear skin atrophy preceding calcinosis cutis in pseudo-pseudohypoparathyroidism. *Clin Exp Dermatol*. 2012;37(6):646–648.
 508. Martin J, Tucker M, Browning JC. Infantile osteoma cutis as a presentation of a GNAS mutation. *Pediatr Dermatol*. 2012;29(4):483–484.
 509. Ward S, Sugo E, Verge CF, Wargon O. Three cases of osteoma cutis occurring in infancy. A brief overview of osteoma cutis and its association with pseudo-pseudohypoparathyroidism. *Australas J Dermatol*. 2011;52(2):127–131.
 510. Silve C, Santora A, Breslau N, Moses A, Spiegel A. Selective resistance to parathyroid hormone in cultured skin fibroblasts from patients with pseudohypoparathyroidism type 1b. *J Clin Endocrinol Metab*. 1986;62(4):640–644.
 511. Fukumoto S, Suzawa M, Kikuchi T, Matsumoto T, Kato S, Fujita T. Cloning and characterization of kidney-specific promoter of human PTH/PTHrP receptor gene: absence of mutation in patients with pseudohypoparathyroidism type 1b. *Mol Cell Endocrinol*. 1998;141(1–2, 41–47).
 512. Bettoun JD, Minagawa M, Kwan MY, Lee HS, Yasuda T, Hendy GN, et al. Cloning and characterization of the promoter regions of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene: analysis of deoxyribonucleic acid from normal subjects and patients with pseudohypoparathyroidism type 1b. *J Clin Endocrinol Metab*. 1997;82(4):1031–1040.
 513. Liu J, Litman D, Rosenberg MJ, Yu S, Biesecker LG, Weinstein LS. A GNAS1 imprinting defect in pseudohypoparathyroidism type 1b. *J Clin Invest*. 2000;106(9):1167–1174.
 514. Izzi B, Van Geet C, Freson K. Recent advances in GNAS epigenetic research of pseudohypoparathyroidism. *Curr Mol Med*. 2012;12(5):566–573.
 515. Mantovani G. Clinical review: Pseudohypoparathyroidism: diagnosis and treatment. *J Clin Endocrinol Metab*. 2011;96(10):3020–3030.
 516. Mantovani G, Elli FM, Spada A. GNAS epigenetic defects and pseudohypoparathyroidism: time for a new classification? *Horm Metab Res*. 2012;44(10):716–723.
 517. Chillambhi S, Turan S, Hwang DY, Chen HC, Juppner H, Bastepe M. Deletion of the noncoding GNAS antisense transcript causes pseudohypoparathyroidism type 1b and biparental defects of GNAS methylation in cis. *J Clin Endocrinol Metab*. 2010;95(8):3993–4002.
 518. Fernandez-Rebollo E, Perez DN, Lecumberri B, Turan S, Anda E, Perez-Nanclares G, et al. Exclusion of the GNAS locus in PHP-1b patients with broad GNAS methylation changes: evidence for an autosomal recessive form of PHP-1b? *J Bone Miner Res*. 2011;26(8):1854–1863.
 519. Bastepe M, Altug-Teber O, Agarwal C, Oberfield SE, Bonin M, Juppner H. Paternal uniparental isodisomy of the entire chromosome 20 as a molecular cause of pseudohypoparathyroidism type 1b (PHP-1b). *Bone*. 2011;48(3):659–662.
 520. Spiegel AM, Weinstein LS. Inherited diseases involving G proteins and G protein-coupled receptors. *Annu Rev Med*. 2004;55:27–39.
 521. Williamson EA, Ince PG, Harrison D, Kendall-Taylor P, Harris PE. G-protein mutations in human pituitary adrenocorticotrophic hormone-secreting adenomas. *Eur J Clin Invest*. 1995;25(2):128–131.
 522. Okamoto S, Hisaoka M, Ushijima M, Nakahara S, Toyoshima S, Hashimoto H. Activating Gs(alpha) mutation in intramuscular myxomas with and without fibrous dysplasia of bone. *Virchows Arch*. 2000;437(2):133–137.
 523. Shenker A, Weinstein LS, Sweet DE, Spiegel AM. An activating Gs alpha mutation is present in fibrous dysplasia of bone in the McCune-Albright syndrome. *J Clin Endocrinol Metab*. 1994;79(3):750–755.
 524. Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med*. 1991;325(24):1688–1695.
 525. Collins MT, Singer FR, Eugster E. McCune-Albright syndrome and the extraskeletal manifestations of fibrous dysplasia. *Orphanet J Rare Dis*. 2012;7(Suppl 1):S4.
 526. Dumitrescu CE, Collins MT. McCune-Albright syndrome. *Orphanet J Rare Dis*. 2008;3:12.
 527. Lietman SA, Schwindinger WF, Levine MA. Genetic and molecular aspects of McCune-Albright syndrome. *Pediatr Endocrinol Rev*. 2007;4(Suppl 4):380–385.
 528. Boyce AM, Florenzano P, de Castro LF, Collins MT. Fibrous dysplasia/McCune-Albright Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., eds. *GeneReviews* ((R)). Seattle (WA); 1993.
 529. Robinson C, Collins MT, Boyce AM. Fibrous dysplasia/McCune-Albright syndrome: clinical and translational perspectives. *Curr Osteoporos Rep*. 2016;14(5):178–186.
 530. Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, White KE, et al. FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. *J Clin Invest*. 2003;112(5):683–692.

531. Yamamoto T, Imanishi Y, Kinoshita E, Nakagomi Y, Shimizu N, Miyauchi A, et al. The role of fibroblast growth factor 23 for hypophosphatemia and abnormal regulation of vitamin D metabolism in patients with McCune-Albright syndrome. *J Bone Miner Metab.* 2005;23(3):231–237.
532. Shenker A, Weinstein LS, Moran A, Pescovitz OH, Charest NJ, Boney CM, et al. Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein GS. *J Pediatr.* 1993;123(4):509–518.
533. Boyce AM, Glover M, Kelly MH, Brillante BA, Butman JA, Fitzgibbon EJ, et al. Optic neuropathy in McCune-Albright syndrome: effects of early diagnosis and treatment of growth hormone excess. *J Clin Endocrinol Metab.* 2013;98(1):E126–E134.
534. Lee JS, FitzGibbon E, Butman JA, Dufresne CR, Kushner H, Wientroub S, et al. Normal vision despite narrowing of the optic canal in fibrous dysplasia. *N Engl J Med.* 2002;347(21):1670–1676.
535. Cohen S, Bigazzi PE, Yoshida T. Commentary. Similarities of T cell function in cell-mediated immunity and antibody production. *Cell Immunol.* 1974;12(1):150–159.
536. Leonard WJ, Lin J-X. Cytokine receptor signaling pathways. *J Allergy Clin Immunol.* 2000;105(5):877–888.
537. Davies DR, Wlodawer A. Cytokines and their receptor complexes. *Faseb J.* 1995;9(1):50–56.
538. de Vos AM, Ultsch M, Kossiakoff AA. Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science.* 1992;255(5042):306–312.
539. Greenlund AC, Morales MO, Viviano BL, Yan H, Krolewski J, Schreiber RD. Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. *Immunity.* 1995;2(6):677–687.
540. Darnell Jr JE, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science.* 1994;264(5164):1415–1421.
541. Horvath CM, Darnell JE. The state of the STATs: recent developments in the study of signal transduction to the nucleus. *Curr Opin Cell Biol.* 1997;9(2):233–239.
542. Darnell Jr JE. STATs and gene regulation. *Science.* 1997;277(5332):1630–1635.
543. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. *Annu Rev Immunol.* 1998;16:293–322.
544. Witthuhn BA, Silvennoinen O, Miura O, Lai KS, Cwik C, Liu ET, et al. Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature.* 1994;370(6485):153–157.
545. Johnston JA, Kawamura M, Kirken RA, Chen YQ, Blake TB, Shibuya K, et al. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature.* 1994;370(6485):151–153.
546. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, et al. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature.* 1987;330(6148):537–543.
547. Kelly PA, Ali S, Rozakis M, Goujon L, Nagano M, Pellegrini I, et al. The growth hormone/prolactin receptor family. *Recent Prog Horm Res.* 1993;48:123–164.
548. Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev.* 1994;15(3):369–390.
549. Campbell GS. Growth-hormone signal transduction. *J Pediatr.* 1997;131(1 Pt 2):S42–S44.
550. Rosenbloom AL. Growth hormone insensitivity: physiologic and genetic basis, phenotype, and treatment. *J Pediatr.* 1999;135(3):280–289.
551. Argetsinger LS, Campbell GS, Yang X, Witthuhn BA, Silvennoinen O, Ihle JN, et al. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell.* 1993;74(2):237–244.
552. Meyer DJ, Campbell GS, Cochran BH, Argetsinger LS, Larner AC, Finbloom DS, et al. Growth hormone induces a DNA binding factor related to the interferon- γ -stimulated 91-kDa transcription factor. *J Biol Chem.* 1994;269(7):4701–4704.
553. Wood TJ, Sliva D, Lobie PE, Pircher TJ, Gouilleux F, Wakao H, et al. Mediation of growth hormone-dependent transcriptional activation by mammary gland factor/Stat 5. *J Biol Chem.* 1995;270(16):9448–9453.
554. Smit LS, Meyer DJ, Billestrup N, Norstedt G, Schwartz J, Carter-Su C. The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in the activation of Stats 1, 3, and 5 by GH. *Mol Endocrinol.* 1996;10(5):519–533.
555. Hansen LH, Wang X, Kopchick JJ, Bouchelouche P, Nielsen JH, Galsgaard ED, et al. Identification of tyrosine residues in the intracellular domain of the growth hormone receptor required for transcriptional signaling and Stat5 activation. *J Biol Chem.* 1996;271(21):12669–12673.
556. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. *Endocr Rev.* 1997;18(6):801–831.
557. Vanderkuur J, Allevato G, Billestrup N, Norstedt G, Carter-Su C. Growth hormone-promoted tyrosyl phosphorylation of SHC proteins and SHC association with Grb2. *J Biol Chem.* 1995;270(13):7587–7593.
558. Argetsinger LS, Hsu GW, Myers Jr MG, Billestrup N, White MF, Carter-Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. *J Biol Chem.* 1995;270(24):14685–14692.
559. Argetsinger LS, Norstedt G, Billestrup N, White MF, Carter-Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor utilize insulin receptor substrate-2 in intracellular signaling. *J Biol Chem.* 1996;271(46):29415–29421.
560. Savage MO, Hwa V, David A, Rosenfeld RG, Metherell LA. Genetic defects in the growth hormone-IGF-I axis causing growth hormone insensitivity and impaired linear growth. *Front Endocrinol (Lausanne).* 2011;2(95).
561. Laron Z, Pertzalan A, Mannheimer S. Genetic pituitary dwarfism with high serum concentration of growth hormone—a new inborn error of metabolism? *Isr J Med Sci.* 1966;2(2):152–155.
562. Laron Z, Pertzalan A, Karp M. Pituitary dwarfism with high serum levels of growth hormone. *Isr J Med Sci.* 1968;4(4):883–894.
563. Laron Z. The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone. *Birth Defects Orig Artic Ser.* 1974;10(4):231–238.
564. Rosenbloom AL, Guevara-Aguirre J, Rosenfeld RG, Francke U. Growth hormone receptor deficiency in Ecuador. *J Clin Endocrinol Metab.* 1999;84(12):4436–4443.
565. Wojcik J, Berg MA, Esposito N, Geffner ME, Sakati N, Reiter EO, et al. Four contiguous amino acid substitutions, identified in patients with Laron syndrome, differently affect the binding affinity and intracellular trafficking of the growth hormone receptor. *J Clin Endocrinol Metab.* 1998;83(12):4481–4489.
566. Walker JL, Crock PA, Behncken SN, Rowlinson SW, Nicholson LM, Boulton TJ, et al. A novel mutation affecting the interdomain link region of the growth hormone receptor in a Vietnamese girl, and response to long-term treatment with recombinant human insulin-like growth factor-I and luteinizing hormone-releasing hormone analogue. *J Clin Endocrinol Metab.* 1998;83(7):2554–2561.
567. Aisenberg J, Auyeung V, Pedro HF, Sugalski R, Chartoff A, Rothenberg R, et al. Atypical GH insensitivity syndrome and severe insulin-like growth factor-I deficiency resulting from compound heterozygous mutations of the GH receptor, including a novel frameshift mutation affecting the intracellular domain. *Horm Res Paediatr.* 2010;74(6):406–411.
568. Akinci A, Rosenfeld RG, Hwa V. A novel exonic GHR splicing mutation (c.784G > C) in a patient with classical growth hormone insensitivity syndrome. *Horm Res Paediatr.* 2013;79(1):32–38.
569. Arman A, Ozon A, Isguven PS, Coker A, Peker I, Yordam N. Novel splice site mutation in the growth hormone receptor gene in Turkish patients with Laron-type dwarfism. *J Pediatr Endocrinol Metab.* 2008;21(1):47–58.
570. David A, Miraki-Moud F, Shaw NJ, Savage MO, Clark AJ, Metherell LA. Identification and characterisation of a novel GHR defect disrupting the polypyridine tract and resulting in GH insensitivity. *Eur J Endocrinol.* 2010;162(1):37–42.
571. David A, Hwa V, Metherell LA, Netchine I, Camacho-Hubner C, Clark AJ, et al. Evidence for a continuum of genetic, phenotypic,

- and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev.* 2011;32(4):472–497.
572. Fang P, Riedl S, Amselem S, Pratt KL, Little BM, Haeusler G, et al. Primary growth hormone (GH) insensitivity and insulin-like growth factor deficiency caused by novel compound heterozygous mutations of the GH receptor gene: genetic and functional studies of simple and compound heterozygous states. *J Clin Endocrinol Metab.* 2007;92(6):2223–2231.
 573. Kang JH, Kim OS, Kim JH, Lee SK, Park YJ, Baik HW. A novel mutation of exon 7 in growth hormone receptor mRNA in a patient with growth hormone insensitivity syndrome and neurofibromatosis type I. *Int J Mol Med.* 2012;30(3):713–717.
 574. Woods K. Genetic defects of the growth-hormone-IGF axis associated with growth hormone insensitivity. *Endocr Dev.* 2007;11:6–15.
 575. Derr MA, Aisenberg J, Fang P, Tenenbaum-Rakover Y, Rosenfeld RG, Hwa V. The growth hormone receptor (GHR) c.899dupC mutation functions as a dominant negative: insights into the pathophysiology of intracellular GHR defects. *J Clin Endocrinol Metab.* 2011;96(11):E1896–E1904.
 576. Goddard AD, Covello R, Luoh SM, Clackson T, Attie KM, Gesundheit N, et al. Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group [see comments]. *N Engl J Med.* 1995;333(17):1093–1098.
 577. Goddard AD, Dowd P, Chernausk S, Geffner M, Gertner J, Hintz R, et al. Partial growth-hormone insensitivity: the role of growth-hormone receptor mutations in idiopathic short stature. *J Pediatr.* 1997;131(1 Pt 2):S51–S55.
 578. Ayling RM, Ross R, Townner P, Von Laue S, Finidori J, Moutoussamy S, et al. A dominant-negative mutation of the growth hormone receptor causes familial short stature [letter]. *Nat Genet.* 1997;16(1):13–14.
 579. Iida K, Takahashi Y, Kaji H, Nose O, Okimura Y, Abe H, et al. Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. *J Clin Endocrinol Metab.* 1998;83(2):531–537.
 580. Gorbenko, del Blanco D, de Graaff LC, Visser TJ, Hokken-Koelega AC. Growth hormone insensitivity syndrome caused by a heterozygous GHR mutation: phenotypic variability owing to moderation by nonsense-mediated decay. *Clin Endocrinol.* 2012;76(5):706–712.
 581. Freeth JS, Silva CM, Whatmore AJ, Clayton PE. Activation of the signal transducers and activators of transcription signaling pathway by growth hormone (GH) in skin fibroblasts from normal and GH binding protein-positive Laron Syndrome children. *Endocrinology.* 1998;139(1):20–28.
 582. Hwa V, Little B, Adiyaman P, Kofoed EM, Pratt KL, Ocal G, et al. Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab.* 2005;90(7):4260–4266.
 583. Hwa V, Nadeau K, Wit JM, Rosenfeld RG. STAT5b deficiency: lessons from STAT5b gene mutations. *Best Pract Res Clin Endocrinol Metab.* 2011;25(1):61–75.
 584. Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, et al. Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med.* 2003;349(12):1139–1147.
 585. Walenkamp MJ, Vidarsdottir S, Pereira AM, Karperien M, van Doorn J, van Duyvenvoorde HA, et al. Growth hormone secretion and immunological function of a male patient with a homozygous STAT5b mutation. *Eur J Endocrinol.* 2007;156(2):155–165.
 586. Wit JM, van Duyvenvoorde HA, Scheltinga SA, de Bruin S, Hafkenscheid L, Kant SG, et al. Genetic analysis of short children with apparent growth hormone insensitivity. *Horm Res Paediatr.* 2012;77(5):320–333.
 587. Hwa V. STAT5B deficiency: impacts on human growth and immunity. *Growth Horm IGF Res.* 2016;28:16–20.
 588. Laron Z. Insulin-like growth factor-I treatment of children with Laron syndrome (primary growth hormone insensitivity). *Pediatr Endocrinol Rev.* 2008;5(3):766–771.
 589. Chen D, Garg A. Monogenic disorders of obesity and body fat distribution. *J Lipid Res.* 1999;40(10):1735–1746.
 590. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction [see comments]. *Nature.* 1998;392(6674):398–401.
 591. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med.* 2007;356(3):237–247.
 592. Kimber W, Peelman F, Prieur X, Wangensteen T, O'Rahilly S, Tavernier J, et al. Functional characterization of naturally occurring pathogenic mutations in the human leptin receptor. *Endocrinology.* 2008;149(12):6043–6052.
 593. Andiran N, Celik N, Andiran F. Homozygosity for two missense mutations in the leptin receptor gene (P316:W646C) in a Turkmenian girl with severe early-onset obesity. *J Pediatr Endocrinol Metab.* 2012;24(11-12):1043–1045.
 594. Mazen I, El-Gammal M, Abdel-Hamid M, Farooqi IS, Amr K. Homozygosity for a novel missense mutation in the leptin receptor gene (P316T) in two Egyptian cousins with severe early onset obesity. *Mol Genet Metab.* 2011;102(4):461–464.
 595. Hubbard SR. Structural analysis of receptor tyrosine kinases. *Prog Biophys Mol Biol.* 1999;71(3-4):343–358.
 596. Spivak-Kroizman T, Lemmon MA, Dilic I, Ladbury JE, Pinchasi D, Huang J, et al. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell.* 1994;79(6):1015–1024.
 597. Heldin CH. Dimerization of cell surface receptors in signal transduction. *Cell.* 1995;80(2):213–223.
 598. Pawson T. Protein modules and signalling networks. *Nature.* 1995;373(6515):573–580.
 599. Patel TB. Single transmembrane spanning heterotrimeric G protein-coupled receptors and their signaling cascades. *Pharmacol Rev.* 2004;56(3):371–385.
 600. Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, et al. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell.* 1985;40(4):747–758.
 601. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, et al. Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *Embo J.* 1986;5(10):2503–2512.
 602. Urso B, Cope DL, Kalloo-Hosein HE, Hayward AC, Whitehead JP, O'Rahilly S, et al. Differences in signaling properties of the cytoplasmic domains of the insulin receptor and insulin-like growth factor receptor in 3T3-L1 adipocytes. *J Biol Chem.* 1999;274(43):30864–30873.
 603. Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, et al. Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. *Nature.* 1998;394(6691):95–99.
 604. Treadway JL, Morrison BD, Soos MA, Siddle K, Olefsky J, Ullrich A, et al. Transdominant inhibition of tyrosine kinase activity in mutant insulin/insulin-like growth factor I hybrid receptors. *Proc Natl Acad Sci U S A.* 1991;88(1):214–218.
 605. Frattali AL, Treadway JL, Pessin JE. Transmembrane signaling by the human insulin receptor kinase. Relationship between intramolecular beta subunit trans- and cis-autophosphorylation and substrate kinase activation. *J Biol Chem.* 1992;267(27):19521–19528.
 606. Lee CH, Li W, Nishimura R, Zhou M, Batzer AG, Myers Jr MG, et al. Nck associates with the SH2 domain-docking protein IRS-1 in insulin-stimulated cells. *Proc Natl Acad Sci U S A.* 1993;90(24):11713–11717.
 607. Skolnik EY, Batzer A, Li N, Lee CH, Lowenstein E, Mohammadi M, et al. The function of GRB2 in linking the insulin receptor to Ras signaling pathways. *Science.* 1993;260(5116):1953–1955.
 608. Myers Jr MG, Grammer TC, Wang LM, Sun XJ, Pierce JH, Blenis J, et al. Insulin receptor substrate-1 mediates phosphatidylinositol 3'-kinase and p70S6k signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation. *J Biol Chem.* 1994;269(46):28783–28789.
 609. Myers Jr MG, Wang LM, Sun XJ, Zhang Y, Yenush L, Schlessinger J, et al. Role of IRS-1-GRB-2 complexes in insulin signaling. *Mol Cell Biol.* 1994;14(6):3577–3587.
 610. White MF. The insulin signalling system and the IRS proteins. *Diabetologia.* 1997;40(Suppl 2):S2–17.
 611. Gustafson TA, He W, Craparo A, Schaub CD, O'Neill TJ. Phosphotyrosine-dependent interaction of SHC and insulin receptor

- substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. *Mol Cell Biol.* 1995;15(5):2500–2508.
612. Craparo A, O'Neill TJ, Gustafson TA. Non-SH2 domains within insulin receptor substrate-1 and SHC mediate their phosphotyrosine-dependent interaction with the NPEY motif of the insulin-like growth factor I receptor. *J Biol Chem.* 1995;270(26):15639–15643.
 613. White MF, Yenush L. The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol.* 1998;228:179–208.
 614. Denton RM, Tavare JM. Does mitogen-activated-protein kinase have a role in insulin action? The cases for and against. *Eur J Biochem.* 1995;227(3):597–611.
 615. Hara K, Yonezawa K, Sakaue H, Ando A, Kotani K, Kitamura T, et al. 1-Phosphatidylinositol 3-kinase activity is required for insulin-stimulated glucose transport but not for RAS activation in CHO cells. *Proc Natl Acad Sci U S A.* 1994;91(16):7415–7419.
 616. Clarke JF, Young PW, Yonezawa K, Kasuga M, Holman GD. Inhibition of the translocation of GLUT1 and GLUT4 in 3T3-L1 cells by the phosphatidylinositol 3-kinase inhibitor, wortmannin. *Biochem J.* 1994;300(Pt 3):631–635.
 617. Okada T, Kawano Y, Sakakibara T, Hazeki O, Uli M. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. *J Biol Chem.* 1994;269(5):3568–3573.
 618. Lammers R, Gray A, Schlessinger J, Ullrich A. Differential signaling potential of insulin- and IGF-1-receptor cytoplasmic domains. *Embo J.* 1989;8(5):1369–1375.
 619. Kallou-Hosein HE, Whitehead JP, Soos M, Tavare JM, Siddle K, O'Rahilly S. Differential signaling to glycogen synthesis by the intracellular domain of the insulin versus the insulin-like growth factor-1 receptor. Evidence from studies of TrkC-chimeras [published erratum appears in *J Biol Chem*, 272(47), 29984]. *J Biol Chem.* 1997;272(39):24325–24332.
 620. Seino S, Seino M, Bell GI. Human insulin-receptor gene. *Diabetes.* 1990;39(2):129–133.
 621. Frattali AL, Treadway JL, Pessin JE. Insulin/IGF-1 hybrid receptors: implications for the dominant-negative phenotype in syndromes of insulin resistance. *J Cell Biochem.* 1992;48(1):43–50.
 622. Treadway JL, Frattali AL, Pessin JE. Intramolecular subunit interactions between insulin and insulin-like growth factor 1 alpha beta half-receptors induced by ligand and Mn/MgATP binding. *Biochemistry.* 1992;31(47):11801–11805.
 623. Donohue W, Uchida I. Leprechaunism: a euphemism for a rare familial disorder. *J Pediatr.* 1954;45:505–519.
 624. Whitehead JP, Soos MA, Jackson R, Tasic V, Kocova M, O'Rahilly S. Multiple molecular mechanisms of insulin receptor dysfunction in a patient with Donohue syndrome. *Diabetes.* 1998;47(8):1362–1364.
 625. Mantzoros CS, Flier JS. Insulin resistance: the clinical spectrum. *Adv Endocrinol Metab.* 1995;6:193–232.
 626. Longo N, Wang Y, Pasquali M. Progressive decline in insulin levels in Rabson-Mendenhall syndrome. *J Clin Endocrinol Metab.* 1999;84(8):2623–2629.
 627. Mendenhall E. Tumor of the pineal gland with high insulin resistance. *J Indiana State Med Assoc.* 1950;43:32–36.
 628. Hunter SJ, Garvey WT. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. *Am J Med.* 1998;105(4):331–345.
 629. Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM, et al. The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *N Engl J Med.* 1976;294(14):739–745.
 630. Moller DE, Flier JS. Insulin resistance—mechanisms, syndromes, and implications [see comments]. *N Engl J Med.* 1991;325(13):938–948.
 631. Tritos NA, Mantzoros CS. Clinical review 97: Syndromes of severe insulin resistance. *J Clin Endocrinol Metab.* 1998;83(9):3025–3030.
 632. Taylor SI, Grunberger G, Marcus-Samuels B, Underhill LH, Dons RF, Ryan J, et al. Hypoglycemia associated with antibodies to the insulin receptor. *N Engl J Med.* 1982;307(23):1422–1426.
 633. Whitehead JP, Humphreys P, Krook A, Jackson R, Hayward A, Lewis H, et al. Molecular scanning of the insulin receptor substrate 1 gene in subjects with severe insulin resistance: detection and functional analysis of a naturally occurring mutation in a YMXM motif. *Diabetes.* 1998;47(5):837–839.
 634. Taylor S, Wertheimer E, Hone J, et al. Mutations in the insulin receptor gene in patients with genetic syndromes of extreme insulin resistance. In: Draznin B, LeRoith D, eds. *Molecular Biology of Diabetes*. Philadelphia: Humana Press; 1994:1–23.
 635. Kadowaki T, Bevins CL, Cama A, Ojamaa K, Marcus-Samuels B, Kadowaki H, et al. Two mutant alleles of the insulin receptor gene in a patient with extreme insulin resistance. *Science.* 1988;240(4853):787–790.
 636. Krook A, Brueton L, O'Rahilly S. Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism [see comments]. *Lancet.* 1993;42(8866):277–278.
 637. Wertheimer E, Lu SP, Backeljauw PF, Davenport ML, Taylor SI. Homozygous deletion of the human insulin receptor gene results in leprechaunism. *Nat Genet.* 1993;5(1):71–73.
 638. Psiachou H, Mitton S, Alagband-Zadeh J, Hone J, Taylor SI, Sinclair L. Leprechaunism and homozygous nonsense mutation in the insulin receptor gene [letter; comment]. *Lancet.* 1993;342(8876):924.
 639. Takahashi Y, Kadowaki H, Momomura K, Fukushima Y, Orban T, Okai T, et al. A homozygous kinase-defective mutation in the insulin receptor gene in a patient with leprechaunism. *Diabetologia.* 1997;40(4):412–420.
 640. Kawashima Y, Nishimura R, Utsunomiya A, Kagawa R, Funata H, Fujimoto M, et al. Leprechaunism (Donohue syndrome): a case bearing novel compound heterozygous mutations in the insulin receptor gene. *Endocr J.* 2013;60(1):107–112.
 641. Kim D, Cho SY, Yeau SH, Park SW, Sohn YB, Kwon MJ, et al. Two novel insulin receptor gene mutations in a patient with Rabson-Mendenhall syndrome: the first Korean case confirmed by biochemical, and molecular evidence. *J Korean Med Sci.* 2012;27(5):565–568.
 642. Nobile S, Semple RK, Carnielli VP. A novel mutation of the insulin receptor gene in a preterm infant with Donohue syndrome and heart failure. *J Pediatr Endocrinol Metab.* 2012;25(3-4):363–366.
 643. Thiel CT, Knebel B, Knerr I, Sticht H, Muller-Wieland D, Zenker M, et al. Two novel mutations in the insulin binding subunit of the insulin receptor gene without insulin binding impairment in a patient with Rabson-Mendenhall syndrome. *Mol Genet Metab.* 2008;94(3):356–362.
 644. Tuthill A, Semple RK, Day R, Soos MA, Sweeney E, Seymour PJ, et al. Functional characterization of a novel insulin receptor mutation contributing to Rabson-Mendenhall syndrome. *Clin Endocrinol (Oxf).* 2007;66(1):21–26.
 645. Unal S, Aycan Z, Halsall DJ, Kibar AE, Eker S, Ozyaydin E. Donohue syndrome in a neonate with homozygous deletion of exon 3 of the insulin receptor gene. *J Pediatr Endocrinol Metab.* 2009;22(7):669–674.
 646. Moller DE, Flier JS. Detection of an alteration in the insulin-receptor gene in a patient with insulin resistance, acanthosis nigricans, and the polycystic ovary syndrome (type A insulin resistance). *N Engl J Med.* 1988;319(23):1526–1529.
 647. Odawara M, Kadowaki T, Yamamoto R, Shibasaki Y, Tobe K, Accili D, et al. Human diabetes associated with a mutation in the tyrosine kinase domain of the insulin receptor. *Science.* 1989;245(4913):66–68.
 648. Taira M, Hashimoto N, Shimada F, Suzuki Y, Kanatsuka A, Nakamura F, et al. Human diabetes associated with a deletion of the tyrosine kinase domain of the insulin receptor. *Science.* 1989;245(4913):63–66.
 649. Cama A, de la Luz Sierra M, Ottini L, Kadowaki T, Gorden P, Imperato-McGinley J, et al. A mutation in the tyrosine kinase domain of the insulin receptor associated with insulin resistance in an obese woman. *J Clin Endocrinol Metab.* 1991;73(4):894–901.
 650. Moller DE, Cohen O, Yamaguchi Y, Assiz R, Grigorescu F, Eberle A, et al. Prevalence of mutations in the insulin receptor gene in subjects with features of the type A syndrome of insulin resistance. *Diabetes.* 1994;43(2):247–255.
 651. Yoshimasa Y, Seino S, Whittaker J, Kakehi T, Kosaki A, Kuzuya H, et al. Insulin-resistant diabetes due to a point mutation that prevents insulin proreceptor processing. *Science.* 1988;240(4853):784–787.

652. Accili D, Frapier C, Mosthaf L, McKeon C, Elbein SC, Permutt MA, et al. A mutation in the insulin receptor gene that impairs transport of the receptor to the plasma membrane and causes insulin-resistant diabetes. *Embo J*. 1989;8(9):2509–2517.
653. Kadowaki T, Kadowaki H, Rechler MM, Serrano-Rios M, Roth J, Gorden P, et al. Five mutant alleles of the insulin receptor gene in patients with genetic forms of insulin resistance. *J Clin Invest*. 1990;86(1):254–264.
654. Kusari J, Takata Y, Hatada E, Freidenberg G, Kolterman O, Olefsky JM. Insulin resistance and diabetes due to different mutations in the tyrosine kinase domain of both insulin receptor gene alleles. *J Biol Chem*. 1991;266(8):5260–5267.
655. O'Rahilly S, Moller DE. Mutant insulin receptors in syndromes of insulin resistance. *Clin Endocrinol (Oxf)*. 1992;36(2):121–132.
656. Jain S, Golde DW, Bailey R, Geffner ME. Insulin-like growth factor-I resistance. *Endocr Rev*. 1998;19(5):625–646.
657. Butler MG, Fogo AB, Fuchs DA, Collins FS, Dev VG, Phillips JAD. Two patients with ring chromosome 15 syndrome. *Am J Med Genet*. 1988;29(1):149–154.
658. de Lacerda L, Carvalho JA, Stannard B, Werner H, Boguszewski MC, Sandrini R, et al. In vitro and in vivo responses to short-term recombinant human insulin-like growth factor-1 (IGF-I) in a severely growth-retarded girl with ring chromosome 15 and deletion of a single allele for the type 1 IGF receptor gene. *Clin Endocrinol (Oxf)*. 1999;51(5):541–550.
659. Pasquali F, Zuffardi O, Severi F, Colombo A, Burgio GR. Tandem translocation 15-13. *Ann Genet*. 1973;16(1):47–50.
660. Kristoffersson U, Heim S, Mandahl N, Sundkvist L, Szelest J, Hagerstrand I. Monosomy and trisomy of 15q24—qter in a family with a translocation t(6;15)(p25;q24). *Clin Genet*. 1987;32(3):169–171.
661. Roback EW, Barakat AJ, Dev VG, Mbikay M, Chretien M, Butler MG. An infant with deletion of the distal long arm of chromosome 15 (q26.1—qter) and loss of insulin-like growth factor 1 receptor gene. *Am J Med Genet*. 1991;38(1):74–79.
662. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*. 1993;75(1):73–82.
663. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell*. 1993;75(1):59–72.
664. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med*. 2003;349(23):2211–2222.
665. Fang P, Cho YH, Derr MA, Rosenfeld RG, Hwa V, Cowell CT. Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *J Clin Endocrinol Metab*. 2012;97(2):E243–E247.
666. Labarta JI, Barrio E, Audi L, Fernandez-Cancio M, Andaluz P, de Arriba A, et al. Familial short stature and intrauterine growth retardation associated with a novel mutation in the IGF-I receptor (IGF1R) gene. *Clin Endocrinol*. 2013;78(2):255–262.
667. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruihof MF, et al. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab*. 2006;91(8):3062–3070.
668. Rechler MM. Leprechaunism and related syndromes with primary insulin resistance: heterogeneity of molecular defects. *Prog Clin Biol Res*. 1982;97:245–281.
669. Kaplowitz PB, D'Ercole AJ. Fibroblasts from a patient with leprechaunism are resistant to insulin, epidermal growth factor, and somatomedin C. *J Clin Endocrinol Metab*. 1982;55(4):741–748.
670. Backeljauw PF, Alves C, Eidson M, Cleveland W, Underwood LE, Davenport ML. Effect of intravenous insulin-like growth factor I in two patients with leprechaunism. *Pediatr Res*. 1994;36(6):749–754.
671. Longo N, Singh R, Griffin LD, Langley SD, Parks JS, Elsas LJ. Impaired growth in Rabson-Mendenhall syndrome: lack of effect of growth hormone and insulin-like growth factor-I. *J Clin Endocrinol Metab*. 1994;79(3):799–805.
672. Desbois-Mouthon C, Danan C, Amselem S, Blivet-Van Eggelpoel MJ, Sert-Langeron C, Goossens M, et al. Severe resistance to insulin and insulin-like growth factor-I in cells from a patient with leprechaunism as a result of two mutations in the tyrosine kinase domain of the insulin receptor. *Metabolism*. 1996;45(12):1493–1500.
673. Kuzuya H, Matsuura N, Sakamoto M, Makino H, Sakamoto Y, Kadowaki T, et al. Trial of insulinlike growth factor I therapy for patients with extreme insulin resistance syndromes. *Diabetes*. 1993;42(5):696–705.
674. Lin Y, van Duyvenvoorde HA, Liu H, Yang C, Warsito D, Yin C, et al. Characterization of an activating R1353H insulin-like growth factor 1 receptor variant in a male with extreme tall height. *Eur J Endocrinol*. 2018;179(2):85–95.
675. Burke D, Wilkes D, Blundell TL, Malcolm S. Fibroblast growth factor receptors: lessons from the genes. *Trends Biochem Sci*. 1998;23(2):59–62.
676. Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin Cancer Res*. 2012;18(7):1855–1862.
677. Mohammadi M, Dionne CA, Li W, Li N, Spivak T, Honegger AM, et al. Point mutation in FGF receptor eliminates phosphatidylinositol hydrolysis without affecting mitogenesis. *Nature*. 1992;358(6388):681–684.
678. Peters KG, Marie J, Wilson E, Ives HE, Escobedo J, Del Rosario M, et al. Point mutation of an FGF receptor abolishes phosphatidylinositol turnover and Ca²⁺ flux but not mitogenesis. *Nature*. 1992;358(6388):678–681.
679. Su WC, Kitagawa M, Xue N, Xie B, Garofalo S, Cho J, et al. Activation of Stat1 by mutant fibroblast growth-factor receptor in thanatophoric dysplasia type II dwarfism. *Nature*. 1997;386(6622):288–292.
680. Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*. 2003;33(4):463–465.
681. Oliveira LM, Seminara SB, Beranova M, Hayes FJ, Valkenburgh SB, Schipani E, et al. The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteristics. *J Clin Endocrinol Metab*. 2001;86(4):1532–1538.
682. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2002;87(1):152–160.
683. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*. 1991;353(6344):529–536.
684. Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*. 1991;67(2):423–435.
685. Gonzalez-Martinez D, Kim SH, Hu Y, Guimond S, Schofield J, Winyard P, et al. Anosmin-1 modulates fibroblast growth factor receptor 1 signaling in human gonadotropin-releasing hormone olfactory neuroblasts through a heparan sulfate-dependent mechanism. *J Neurosci*. 2004;24(46):10384–10392.
686. Bick DP, Schorderet DF, Price PA, Campbell L, Huff RW, Shapiro LJ, et al. Prenatal diagnosis and investigation of a fetus with chondrodysplasia punctata, ichthyosis, and Kallmann syndrome due to an Xp deletion. *Prenat Diagn*. 1992;12(1):19–29.
687. Albuissou J, Pecheux C, Carel JC, Lacombe D, Leheup B, Lapuzina P, et al. Kallmann syndrome: 14 novel mutations in KAL1 and FGFR1 (KAL2). *Hum Mutat*. 2005;25(1):98–99.
688. Pitteloud N, Meysing A, Quinton R, Aciermo Jr JS, Dwyer AA, Plummer L, et al. Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol*. 2006;254-255:60–69.
689. White KE, Cabral JM, Davis SI, Fishburn T, Evans WE, Ichikawa S, et al. Mutations that cause osteoglyphic dysplasia define novel roles for FGFR1 in bone elongation. *Am J Hum Genet*. 2005;76(2):361–367.
690. Bonaventure J, Rousseau F, Legeai-Mallet L, Le Merrer M, Munnich A, Maroteaux P. Common mutations in the gene

- encoding fibroblast growth factor receptor 3 account for achondroplasia, hypochondroplasia and thanatophoric dysplasia. *Acta Paediatr Suppl.* 1996;417:33–38.
691. Bonaventure J, Rousseau F, Legeai-Mallet L, Le Merrer M, Munnich A, Maroteaux P. Common mutations in the fibroblast growth factor receptor 3 (FGFR3) gene account for achondroplasia, hypochondroplasia, and thanatophoric dwarfism. *Am J Med Genet.* 1996;63(1):148–154.
692. Brodie SG, Kitoh H, Lipson M, Sifry-Platt M, Wilcox WR. Thanatophoric dysplasia type I with syndactyly. *Am J Med Genet.* 1998;80(3):260–262.
693. Brodie SG, Kitoh H, Lachman RS, Nolasco LM, Mekikian PB, Wilcox WR. Platypondylic lethal skeletal dysplasia, San Diego type, is caused by FGFR3 mutations. *Am J Med Genet.* 1999;84(5):476–480.
694. Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, et al. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell.* 1994;78(2):335–342.
695. Bellus GA, Hefferon TW, Ortiz de Luna RI, Hecht JT, Horton WA, Machado M, et al. Achondroplasia is defined by recurrent G380R mutations of FGFR3. *Am J Hum Genet.* 1995;56(2):368–373.
696. Ezquieta Zubizaray B, Iguacel AO, Varela Junquera JM, Jariago Fente CM, Gonzalez Gancedo P, Gracia Bouthelie R. Gly380Arg and Asn540Lys mutations of fibroblast growth factor receptor 3 in achondroplasia and hypochondroplasia in the Spanish population. *Med Clin (Barc).* 1999;112(8):290–293.
697. Bellus GA, McIntosh I, Smith EA, Aylsworth AS, Kaitila I, Horton WA, et al. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet.* 1995;10(3):357–359.
698. Prinos P, Costa T, Sommer A, Kilpatrick MW, Tsipouras P. A common FGFR3 gene mutation in hypochondroplasia. *Hum Mol Genet.* 1995;4(11):2097–2101.
699. Ramaswami U, Rumsby G, Hindmarsh PC, Brook CG. Genotype and phenotype in hypochondroplasia [see comments]. *J Pediatr.* 1998;133(1):99–102.
700. Fofanova OV, Takamura N, Kinoshita E, Meerson EM, Iljina VK, Nechvolodova OL, et al. A missense mutation of C1659 in the fibroblast growth factor receptor 3 gene in Russian patients with hypochondroplasia. *Endocr J.* 1998;45(6):791–795.
701. Bellus GA, Bamshad MJ, Przylepa KA, Dorst J, Lee RR, Hurko O, et al. Severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN): phenotypic analysis of a new skeletal dysplasia caused by a Lys650Met mutation in fibroblast growth factor receptor 3. *Am J Med Genet.* 1999;85(1):53–65.
702. Peters K, Ornitz D, Werner S, Williams L. Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Dev Biol.* 1993;155(2):423–430.
703. Werner S, Weinberg W, Liao X, Peters KG, Blessing M, Yuspa SH, et al. Targeted expression of a dominant-negative FGF receptor mutant in the epidermis of transgenic mice reveals a role of FGF in keratinocyte organization and differentiation. *Embo J.* 1993;12(7):2635–2643.
704. Naski MC, Colvin JS, Coffin JD, Ornitz DM. Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development.* 1998;125(24):4977–4988.
705. Legeai-Mallet L, Benoist-Lasselin C, Delezoide AL, Munnich A, Bonaventure J. Fibroblast growth factor receptor 3 mutations promote apoptosis but do not alter chondrocyte proliferation in thanatophoric dysplasia [published erratum appears in J Biol Chem 1998 Jul 24;273(30):19358]. *J Biol Chem.* 1998;273(21):13007–13014.
706. Wongmongkolrit T, Bush M, Roessmann U. Neuropathological findings in thanatophoric dysplasia. *Arch Pathol Lab Med.* 1983;107(3):132–135.
707. Ho KL, Chang CH, Yang SS, Chason JL. Neuropathologic findings in thanatophoric dysplasia. *Acta Neuropathol.* 1984;63(3):218–228.
708. Shigematsu H, Takashima S, Otani K, Ieshima A. Neuropathological and Golgi study on a case of thanatophoric dysplasia. *Brain Dev.* 1985;7(6):628–632.
709. Toydemir RM, Brassington AE, Bayrak-Toydemir P, Krakowiak PA, Jorde LB, Whitby FC, et al. A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet.* 2006;79(5):935–941.
710. Foldynova-Trantirkova S, Wilcox WR, Krejci P. Sixteen years and counting: the current understanding of fibroblast growth factor receptor 3 (FGFR3) signaling in skeletal dysplasias. *Hum Mutat.* 2012;33(1):29–41.
711. Laudet V. Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. *J Mol Endocrinol.* 1997;19(3):207–226.
712. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, et al. Dominant negative mutations in human PPAR-gamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature.* 1999;402(6764):880–883.
713. Baker ME. Steroid receptor phylogeny and vertebrate origins. *Mol Cell Endocrinol.* 1997;135(2):101–107.
714. Alzahrani AS, Zou M, Baitei EY, Parhar RS, Al-Kahtani N, Raef H, et al. Molecular characterization of a novel p.R118C mutation in the insulin receptor gene from patients with severe insulin resistance. *Clin Endocrinol.* 2011;76(4):540–547.
715. Miyamoto K, Yazawa T, Mizutani T, Imamichi Y, Kawabe SY, Kanno M, et al. Stem cell differentiation into steroidogenic cell lineages by NR5A family. *Mol Cell Endocrinol.* 2011;336(1–2):123–126.
716. Greschik H, Schule R. Germ cell nuclear factor: an orphan receptor with unexpected properties. *J Mol Med.* 1998;76(12):800–810.
717. Kumar R, Thompson EB. The structure of the nuclear hormone receptors. *Steroids.* 1999;64(5):310–319.
718. Ford J, McEwan IJ, Wright AP, Gustafsson JA. Involvement of the transcription factor IID protein complex in gene activation by the N-terminal transactivation domain of the glucocorticoid receptor in vitro. *Mol Endocrinol.* 1997;11(10):1467–1475.
719. Henriksson A, Almlöf T, Ford J, McEwan IJ, Gustafsson JA, Wright AP. Role of the Ada adaptor complex in gene activation by the glucocorticoid receptor. *Mol Cell Biol.* 1997;17(6):3065–3073.
720. Hollenberg SM, Evans RM. Multiple and cooperative transactivation domains of the human glucocorticoid receptor. *Cell.* 1988;55(5):899–906.
721. Bocquel MT, Kumar V, Stricker C, Chambon P, Gronemeyer H. The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. *Nucleic Acids Res.* 1989;17(7):2581–2595.
722. Tasset D, Tora L, Fromental C, Scheer E, Chambon P. Distinct classes of transcriptional activating domains function by different mechanisms. *Cell.* 1990;62(6):1177–1187.
723. Freedman LP, Luisi BF, Korszun ZR, Basavappa R, Sigler PB, Yamamoto KR. The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. *Nature.* 1988;334(6182):543–546.
724. Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Sigler PB. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA [see comments]. *Nature.* 1991;352(6335):497–505.
725. Lee MS, Kliewer SA, Provencal J, Wright PE, Evans RM. Structure of the retinoid X receptor alpha DNA binding domain: a helix required for homodimeric DNA binding. *Science.* 1993;260(5111):1117–1121.
726. Wilson TE, Paulsen RE, Padgett KA, Milbrandt J. Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science.* 1992;256(5053):107–110.
727. Laudet V, Adelmant G. Nuclear receptors. Lonesome orphans. *Curr Biol.* 1995;5(2):124–127.
728. Tenbaum S, Baniahmad A. Nuclear receptors: structure, function and involvement in disease. *Int J Biochem Cell Biol.* 1997;29(12):1325–1341.
729. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature.* 1997;389(6652):753–758.
730. Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha [see comments]. *Nature.* 1995;375(6530):377–382.
731. Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, et al. Crystal structure of the RAR-gamma

- ligand-binding domain bound to all- trans retinoic acid. *Nature*. 1995;378(6558):681–689.
732. Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ. A structural role for hormone in the thyroid hormone receptor. *Nature*. 1995;378(6558):690–697.
 733. Danielian PS, White R, Lees JA, Parker MG. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors [published erratum appears in *EMBO J* 1992 11(6):2366]. *Embo J*. 1992;11(3):1025–1033.
 734. Baretino D, Vivanco Ruiz MM, Stunnenberg HG. Characterization of the ligand-dependent transactivation domain of thyroid hormone receptor. *Embo J*. 1994;13(13):3039–3049.
 735. Durand B, Saunders M, Gaudon C, Roy B, Losson R, Chambon P. Activation function 2 (AF-2) of retinoic acid receptor and 9-cis retinoic acid receptor: presence of a conserved autonomous constitutive activating domain and influence of the nature of the response element on AF-2 activity. *Embo J*. 1994;13(22):5370–5382.
 736. Schwabe JW. Transcriptional control: how nuclear receptors get turned on. *Curr Biol*. 1996;6(4):372–374.
 737. Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, et al. A canonical structure for the ligand-binding domain of nuclear receptors [see comments] [published erratum appears in *Nat Struct Biol* 1996 Feb;3(2):206]. *Nat Struct Biol*. 1996;3(1):87–94.
 738. Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, et al. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*. 1997;90(3):569–580.
 739. Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, et al. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell*. 1997;89(3):373–380.
 740. Alland L, Muhle R, Hou Jr H, Potes J, Chin L, Schreiber-Agus N, et al. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression [see comments]. *Nature*. 1997;387(6628):49–55.
 741. Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, et al. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression [see comments]. *Nature*. 1997;387(6628):43–48.
 742. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature*. 1997;389(6649):349–352.
 743. Jenster G. Coactivators and corepressors as mediators of nuclear receptor function: an update. *Mol Cell Endocrinol*. 1998;143(1-2):1–7.
 744. Smith DF, Toft DO. Steroid receptors and their associated proteins. *Mol Endocrinol*. 1993;7(1):4–11.
 745. Lee SK, Choi HS, Song MR, Lee MO, Lee JW. Estrogen receptor, a common interaction partner for a subset of nuclear receptors. *Mol Endocrinol*. 1998;12(8):1184–1192.
 746. Barrett TJ, Spelsberg TC. Steroid receptors at the nexus of transcriptional regulation. *J Cell Biochem Suppl*. 1998;31:185–193.
 747. Zhang XK, Lehmann J, Hoffmann B, Dawson MI, Cameron J, Graupner G, et al. Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. *Nature*. 1992;358(6387):587–591.
 748. Hermann T, Hoffmann B, Zhang XK, Tran P, Pfahl M. Heterodimeric receptor complexes determine 3,5,3'-triiodothyronine and retinoid signaling specificities. *Mol Endocrinol*. 1992;6(7):1153–1162.
 749. Zhang XK, Hoffmann B, Tran PB, Graupner G, Pfahl M. Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature*. 1992;355(6359):441–446.
 750. Chen ZP, Shemshedin L, Durand B, Noy N, Chambon P, Gronemeyer H. Pure and functionally homogeneous recombinant retinoid X receptor. *J Biol Chem*. 1994;269(41):25770–25776.
 751. Watson CS, Gametchu B. Membrane-initiated steroid actions and the proteins that mediate them. *Proc Soc Exp Biol Med*. 1999;220(1):9–19.
 752. Chen HC, Farese RV. Steroid hormones: Interactions with membrane-bound receptors. *Curr Biol*. 1999;9(13):R478–R481.
 753. Baldi E, Luconi M, Bonaccorsi L, Forti G. Nongenomic effects of progesterone on spermatozoa: mechanisms of signal transduction and clinical implications. *Front Biosci*. 1998;3:D1051–D1059.
 754. Bergeron R, de Montigny C, Debonnel G. Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J Neurosci*. 1996;16(3):1193–1202.
 755. Ke L, Lukas RJ. Effects of steroid exposure on ligand binding and functional activities of diverse nicotinic acetylcholine receptor subtypes. *J Neurochem*. 1996;67(3):1100–1112.
 756. Maitra R, Reynolds JN. Subunit dependent modulation of GABAA receptor function by neuroactive steroids. *Brain Res*. 1999;819(1-2):75–82.
 757. Grazzini E, Guillon G, Mouillac B, Zingg HH. Inhibition of oxytocin receptor function by direct binding of progesterone [see comments]. *Nature*. 1998;392(6675):509–512.
 758. Pietras RJ, Szego CM. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature*. 1977;265(5589):69–72.
 759. Berthois Y, Pourreau-Schneider N, Gandilhon P, Mittre H, Tubiana N, Martin PM. Estradiol membrane binding sites on human breast cancer cell lines. Use of a fluorescent estradiol conjugate to demonstrate plasma membrane binding systems. *J Steroid Biochem*. 1986;25(6):963–972.
 760. Gametchu B, Watson CS, Pasko D. Size and steroid-binding characterization of membrane-associated glucocorticoid receptor in S-49 lymphoma cells. *Steroids*. 1991;56(8):402–410.
 761. Nenci I, Marchetti E, Marzola A, Fabris G. Affinity cytochemistry visualizes specific estrogen binding sites on the plasma membrane of breast cancer cells. *J Steroid Biochem*. 1981;14(11):1139–1146.
 762. Refetoff S. Resistance to thyroid hormone: an historical overview. *Thyroid*. 1994;4(3):345–349.
 763. Apriletti JW, Ribeiro RC, Wagner RL, Feng W, Webb P, Kushner PJ, et al. Molecular and structural biology of thyroid hormone receptors. *Clin Exp Pharmacol Physiol Suppl*. 1998;25:S2–11.
 764. Usala SJ, Bale AE, Gesundheit N, Weinberger C, Lash RW, Wondisford FE, et al. Tight linkage between the syndrome of generalized thyroid hormone resistance and the human c-erbA beta gene. *Mol Endocrinol*. 1988;2(12):1217–1220.
 765. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. *Endocr Rev*. 1993;14(3):348–399.
 766. Lafranchi SH, Snyder DB, Sesser DE, Skeels MR, Singh N, Brent GA, et al. Follow-up of newborns with elevated screening T4 concentrations. *J Pediatr*. 2003;143(3):296–301.
 767. Pappa T, Refetoff S. Human Genetics of Thyroid Hormone Receptor Beta: Resistance to Thyroid Hormone Beta (RTHbeta). *Methods Mol Biol*. 2018;1801:225–240.
 768. Usala SJ, Menke JB, Watson TL, Wondisford FE, Weintraub BD, Berard J, et al. A homozygous deletion in the c-erbA beta thyroid hormone receptor gene in a patient with generalized thyroid hormone resistance: isolation and characterization of the mutant receptor. *Mol Endocrinol*. 1991;5(3):327–335.
 769. Piedrafita FJ, Ortiz MA, Pfahl M. Thyroid hormone receptor-beta mutants associated with generalized resistance to thyroid hormone show defects in their ligand-sensitive repression function. *Mol Endocrinol*. 1995;9(11):1533–1548.
 770. Yoh SM, Chatterjee VK, Privalsky ML. Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T3 receptors and transcriptional corepressors. *Mol Endocrinol*. 1997;11(4):470–480.
 771. Liu Y, Takeshita A, Misiti S, Chin WW, Yen PM. Lack of coactivator interaction can be a mechanism for dominant negative activity by mutant thyroid hormone receptors. *Endocrinology*. 1998;139(10):4197–4204.
 772. Takeda K, Sakurai A, DeGroot LJ, Refetoff S. Recessive inheritance of thyroid hormone resistance caused by complete deletion of the protein-coding region of the thyroid hormone receptor-beta gene. *J Clin Endocrinol Metab*. 1992;74(1):49–55.
 773. Ono S, Schwartz ID, Mueller OT, Root AW, Usala SJ, Bercu BB. Homozygosity for a dominant negative thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. *J Clin Endocrinol Metab*. 1991;73(5):990–994.
 774. Sakurai A, Miyamoto T, Refetoff S, DeGroot LJ. Dominant negative transcriptional regulation by a mutant thyroid hormone receptor-beta in a family with generalized resistance to thyroid hormone. *Mol Endocrinol*. 1990;4(12):1988–1994.
 775. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, et al. A mutation in the thyroid

- hormone receptor alpha gene. *N Engl J Med.* 2011;366(3):243–249.
776. van Mullem A, van Heerebeek R, Chrysis D, Visser E, Medici M, Andrikoula M, et al. Clinical phenotype and mutant TRalpha1. *N Engl J Med.* 2012;366(15):1451–1453.
777. Moran C, Schoenmakers N, Agostini M, Schoenmakers E, Offiah A, Kydd A, et al. An adult female with resistance to thyroid hormone mediated by defective thyroid hormone receptor alpha. *J Clin Endocrinol Metab.* 2013;98(11):4254–4261.
778. Zavacki AM, Larsen PR. RTHalpha, a newly recognized phenotype of the resistance to thyroid hormone (RTH) syndrome in patients with THRA gene mutations. *J Clin Endocrinol Metab.* 2013;98(7):2684–2686.
779. Tylki-Szymanska A, Acuna-Hidalgo R, Krajewska-Walasek M, Lecka-Ambroziak A, Steehouwer M, Gilissen C, et al. Thyroid hormone resistance syndrome due to mutations in the thyroid hormone receptor alpha gene (THRA). *J Med Genet.* 2015;52(5):312–316.
780. Moran C, Agostini M, Visser WE, Schoenmakers E, Schoenmakers N, Offiah AC, et al. Resistance to thyroid hormone caused by a mutation in thyroid hormone receptor (TR)alpha1 and TRalpha2: clinical, biochemical, and genetic analyses of three related patients. *Lancet Diabetes Endocrinol.* 2014;2(8):619–626.
781. Espiard S, Savagner F, Flamant F, Vlaeminck-Guillem V, Guyot R, Munier M, et al. A Novel Mutation in THRA Gene Associated With an Atypical Phenotype of Resistance to Thyroid Hormone. *J Clin Endocrinol Metab.* 2015;100(8):2841–2848.
782. van Gucht AL, Meima ME, Zwaveling-Soonawala N, Visser WE, Fliers E, Wennink JM, et al. Resistance to Thyroid Hormone Alpha in an 18-Month-Old Girl: Clinical, Therapeutic, and Molecular Characteristics. *Thyroid.* 2016;26(3):338–346.
783. Demir K, van Gucht AL, Buyukinan M, Catli G, Ayhan Y, Bas VN, et al. Diverse Genotypes and Phenotypes of Three Novel Thyroid Hormone Receptor-alpha Mutations. *J Clin Endocrinol Metab.* 2016;101(8):2945–2954.
784. Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, et al. Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. *Science.* 1988;242(4886):1702–1705.
785. Whitfield GK, Selznick SH, Haussler CA, Hsieh JC, Galligan MA, Jurutka PW, et al. Vitamin D receptors from patients with resistance to 1,25-dihydroxyvitamin D3: point mutations confer reduced transactivation in response to ligand and impaired interaction with the retinoid X receptor heterodimeric partner. *Mol Endocrinol.* 1996;10(12):1617–1631.
786. Kristjansson K, Rut AR, Hewison M, O'Riordan JL, Hughes MR. Two mutations in the hormone binding domain of the vitamin D receptor cause tissue resistance to 1,25 dihydroxyvitamin D3. *J Clin Invest.* 1993;92(1):12–16.
787. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *J Bone Miner Res.* 1999;14(5):740–746.
788. Gennari L, Becherini L, Masi L, Gonnelli S, Cepollaro C, Martini S, et al. Vitamin D receptor genotypes and intestinal calcium absorption in postmenopausal women. *Calcif Tissue Int.* 1997;61(6):460–463.
789. Ferrari S, Rizzoli R, Chevalley T, Slosman D, Eisman JA, Bonjour JP. Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density [see comments]. *Lancet.* 1995;345(8947):423–424.
790. Feskanich D, Hunter DJ, Willett WC, Hankinson SE, Hollis BW, Hough HL, et al. Vitamin D receptor genotype and the risk of bone fractures in women. *Epidemiology.* 1998;9(5):535–539.
791. Gennari L, Becherini L, Mansani R, Masi L, Falchetti A, Morelli A, et al. FokI polymorphism at translation initiation site of the vitamin D receptor gene predicts bone mineral density and vertebral fractures in postmenopausal Italian women. *J Bone Miner Res.* 1999;14(8):1379–1386.
792. Gong G, Stern HS, Cheng SC, Fong N, Mordeson J, Deng HW, et al. The association of bone mineral density with vitamin D receptor gene polymorphisms. *Osteoporos Int.* 1999;9(1):55–64.
793. Eccleshall TR, Garnero P, Gross C, Delmas PD, Feldman D. Lack of correlation between start codon polymorphism of the vitamin D receptor gene and bone mineral density in premenopausal French women: the OFELY study. *J Bone Miner Res.* 1998;13(1):31–35.
794. Cheng WC, Tsai KS. The vitamin D receptor start codon polymorphism (Fok1) and bone mineral density in premenopausal women in Taiwan. *Osteoporos Int.* 1999;9(6):545–549.
795. Deng HW, Li J, Li JL, Johnson M, Gong G, Recker RR. Association of VDR and estrogen receptor genotypes with bone mass in postmenopausal Caucasian women: different conclusions with different analyses and the implications. *Osteoporos Int.* 1999;9(6):499–507.
796. Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY, Feldman D. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *J Bone Miner Res.* 1998;13(11):1691–1699.
797. Hansen TS, Abrahamsen B, Henriksen FL, Hermann AP, Jensen LB, Horder M, et al. Vitamin D receptor alleles do not predict bone mineral density or bone loss in Danish perimenopausal women. *Bone.* 1998;22(5):571–575.
798. Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP. Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res.* 1998;13(3):363–370.
799. Gennari L, Becherini L, Masi L, Mansani R, Gonnelli S, Cepollaro C, et al. Vitamin D and estrogen receptor allelic variants in Italian postmenopausal women: evidence of multiple gene contribution to bone mineral density. *J Clin Endocrinol Metab.* 1998;83(3):939–944.
800. Suarez F, Rossignol C, Garabedian M. Interactive effect of estradiol and vitamin D receptor gene polymorphisms as a possible determinant of growth in male and female infants. *J Clin Endocrinol Metab.* 1998;83(10):3563–3568.
801. Tao C, Yu T, Garnett S, Briody J, Knight J, Woodhead H, et al. Vitamin D receptor alleles predict growth and bone density in girls. *Arch Dis Child.* 1998;79(6):488–493. discussion 93–94.
802. Carling T, Ridefelt P, Hellman P, Juhlin C, Lundgren E, Akerstrom G, et al. Vitamin D receptor gene polymorphism and parathyroid calcium sensor protein (CAS/gp330) expression in primary hyperparathyroidism. *World J Surg.* 1998;22(7):700–706, discussion 6–7.
803. Hennig BJ, Parkhill JM, Chapple IL, Heasman PA, Taylor JJ. Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases. *J Periodontol.* 1999;70(9):1032–1038.
804. Jackman SV, Kibel AS, Ovuworie CA, Moore RG, Kavoussi LR, Jarrett TW. Familial calcium stone disease: TaqI polymorphism and the vitamin D receptor. *J Endourol.* 1999;13(4):313–316.
805. Niimi T, Tomita H, Sato S, Kawaguchi H, Akita K, Maeda H, et al. Vitamin D receptor gene polymorphism in patients with sarcoidosis. *Am J Respir Crit Care Med.* 1999;160(4):1107–1109.
806. Ruggiero M, Pacini S, Amato M, Aterini S, Chiarugi V. Association between vitamin D receptor gene polymorphism and nephrolithiasis. *Miner Electrolyte Metab.* 1999;25(3):185–190.
807. Ruggiero M, Pacini S, Aterini S, Fallai C, Ruggiero C, Pacini P. Vitamin D receptor gene polymorphism is associated with metastatic breast cancer. *Oncol Res.* 1998;10(1):43–46.
808. Park BS, Park JS, Lee DY, Youn JI, Kim IG. Vitamin D receptor polymorphism is associated with psoriasis. *J Invest Dermatol.* 1999;112(1):113–116.
809. Roy S, Frodsham A, Saha B, Hazra SK, Mascie-Taylor CG, Hill AV. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis.* 1999;179(1):187–191.
810. Hill AV. The immunogenetics of human infectious diseases. *Annu Rev Immunol.* 1998;16:593–617.
811. Roth DE, Soto G, Arenas F, Bautista CT, Ortiz J, Rodriguez R, et al. Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. *J Infect Dis.* 2004;190(5):920–927.
812. Saito M, Eiraku N, Usuku K, Nobuhara Y, Matsumoto W, Kodama D, et al. ApaI polymorphism of vitamin D receptor gene is associated with susceptibility to HTLV-1-associated myelopathy/tropical spastic paraparesis in HTLV-1 infected individuals. *J Neurol Sci.* 2005;232(1–2):29–35.
813. Fiorenza CG, Chou SH, Mantzoros CS. Lipodystrophy: pathophysiology and advances in treatment. *Nat Rev Endocrinol.* 2010;7(3):137–150.
814. Savage DB, Tan GD, Acerini CL, Jebb SA, Agostini M, Gurnell M, et al. Human metabolic syndrome resulting from dominant-

- negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. *Diabetes*. 2003;52(4):910–917.
815. Agarwal AK, Garg A. A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. *J Clin Endocrinol Metab*. 2002;87(1):408–411.
816. Francis GA, Li G, Casey R, Wang J, Cao H, Leff T, et al. Peroxisomal proliferator activated receptor-gamma deficiency in a Canadian kindred with familial partial lipodystrophy type 3 (FPLD3). *BMC Med Genet*. 2006;7:3.
817. Hegele RA, Cao H, Frankowski C, Mathews ST, Leff T. PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes*. 2002;51(12):3586–3590.
818. Jeninga EH, van Beekum O, van Dijk AD, Hamers N, Hendriks-Stegeman BI, Bonvin AM, et al. Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. *Mol Endocrinol*. 2007;21(5):1049–1065.
819. Ludtke A, Buettner J, Schmidt HH, Worman HJ. New PPARG mutation leads to lipodystrophy and loss of protein function that is partially restored by a synthetic ligand. *J Med Genet*. 2007;44(9):e88.
820. Ludtke A, Buettner J, Wu W, Muchir A, Schroeter A, Zinn-Justin S, et al. Peroxisome proliferator-activated receptor-gamma C190S mutation causes partial lipodystrophy. *J Clin Endocrinol Metab*. 2007;92(6):2248–2255.
821. Monajemi H, Zhang L, Li G, Jeninga EH, Cao H, Maas M, et al. Familial partial lipodystrophy phenotype resulting from a single-base mutation in deoxyribonucleic acid-binding domain of peroxisome proliferator-activated receptor-gamma. *J Clin Endocrinol Metab*. 2007;92(5):1606–1612.
822. Visser ME, Kropman E, Kranendonk ME, Koppen A, Hamers N, Stroes ES, et al. Characterisation of non-obese diabetic patients with marked insulin resistance identifies a novel familial partial lipodystrophy-associated PPARgamma mutation (Y151C). *Diabetologia*. 2011;54(7):1639–1644.
823. Hegele RA, Ur E, Ransom TP, Cao H. A frameshift mutation in peroxisome-proliferator-activated receptor-gamma in familial partial lipodystrophy subtype 3 (FPLD3; MIM 604367). *Clin Genet*. 2006;70(4):360–362.
824. Campeau PM, Astapova O, Martins R, Bergeron J, Couture P, Hegele RA, et al. Clinical and molecular characterization of a severe form of partial lipodystrophy expanding the phenotype of PPARgamma deficiency. *J Lipid Res*. 2012;53(9):1968–1978.
825. Ristow M, Muller-Wieland D, Pfeiffer A, Krone W, Kahn CR. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N Engl J Med*. 1998;339(14):953–959.
826. Sladek FM, Dallas-Yang Q, Nepomuceno L. MODY1 mutation Q268X in hepatocyte nuclear factor 4alpha allows for dimerization in solution but causes abnormal subcellular localization. *Diabetes*. 1998;47(6):985–990.
827. Lindner T, Gagnoli C, Furuta H, Cockburn BN, Petzold C, Rietzsch H, et al. Hepatic function in a family with a nonsense mutation (R154X) in the hepatocyte nuclear factor-4alpha/MODY1 gene. *J Clin Invest*. 1997;100(6):1400–1405.
828. Furuta H, Iwasaki N, Oda N, Hinokio Y, Horikawa Y, Yamagata K, et al. Organization and partial sequence of the hepatocyte nuclear factor-4 alpha/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. *Diabetes*. 1997;46(10):1652–1657.
829. Bulman MP, Dronsfield MJ, Frayling T, Appleton M, Bain SC, Ellard S, et al. A missense mutation in the hepatocyte nuclear factor 4 alpha gene in a UK pedigree with maturity-onset diabetes of the young. *Diabetologia*. 1997;40(7):859–862.
830. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, et al. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1) [see comments]. *Nature*. 1996;384(6608):458–460.
831. Conn JJ, Simm PJ, Oats JJ, Nankervis AJ, Jacobs SE, Ellard S, et al. Neonatal hyperinsulinaemic hypoglycaemia and monogenic diabetes due to a heterozygous mutation of the HNF4A gene. *Aust N Z J Obstet Gynaecol*. 2009;49(3):328–330.
832. Fajans SS, Bell GI. Macrosomia and neonatal hypoglycaemia in RW pedigree subjects with a mutation (Q268X) in the gene encoding hepatocyte nuclear factor 4alpha (HNF4A). *Diabetologia*. 2007;50(12):2600–2601.
833. Flanagan SE, Kapoor RR, Mali G, Cody D, Murphy N, Schwahn B, et al. Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations. *Eur J Endocrinol*. 2010;162(5):987–992.
834. Kapoor RR, Locke J, Colclough K, Wales J, Conn JJ, Hattersley AT, et al. Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes*. 2008;57(6):1659–1663.
835. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med*. 2007;4(4):e118.
836. Hamilton AJ, Bingham C, McDonald TJ, Cook PR, Caswell RC, Weedon MN, et al. The HNF4A R76W mutation causes atypical dominant Fanconi syndrome in addition to a beta cell phenotype. *J Med Genet*. 2014;51(3):165–169.
837. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab*. 2012;97(10):E2026–E2030.
838. Marable SS, Chung E, Adam M, Potter SS, Park JS. Hnf4a deletion in the mouse kidney phenocopies Fanconi renotubular syndrome. *JCI Insight*. 2018;3(14).
839. Malecki MT, Yang Y, Antonellis A, Curtis S, Warram JH, Krolewski AS. Identification of new mutations in the hepatocyte nuclear factor 4alpha gene among families with early onset Type 2 diabetes mellitus. *Diabet Med*. 1999;16(3):193–200.
840. Fajans SS, Bell GI. Phenotypic heterogeneity between different mutations of MODY subtypes and within MODY pedigrees. *Diabetologia*. 2006;49(5):1106–1118.
841. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*. 2001;345(13):971–980.
842. Malecki MT, Klupa T. Type 2 diabetes mellitus: from genes to disease. *Pharmacol Rep*. 2005;57(Suppl):20–32.
843. Shih DQ, Stoffel M. Molecular etiologies of MODY and other early-onset forms of diabetes. *Curr Diab Rep*. 2002;2(2):125–134.
844. Charmandari E. Primary generalized glucocorticoid resistance and hypersensitivity. *Horm Res Paediatr*. 2011;76(3):145–155.
845. Lamberts SW, Koper JW, Biemond P, den Holder FH, de Jong FH. Cortisol receptor resistance: the variability of its clinical presentation and response to treatment. *J Clin Endocrinol Metab*. 1992;74(2):313–321.
846. Werner S, Thoren M, Gustafsson JA, Bronnegard M. Glucocorticoid receptor abnormalities in fibroblasts from patients with idiopathic resistance to dexamethasone diagnosed when evaluated for adrenocortical disorders. *J Clin Endocrinol Metab*. 1992;75(4):1005–1009.
847. Charmandari E, Kino T, Ichijo T, Chrousos GP. Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. *J Clin Endocrinol Metab*. 2008;93(5):1563–1572.
848. Malchoff CD, Javier EC, Malchoff DM, Martin T, Rogol A, Brandon D, et al. Primary cortisol resistance presenting as isosexual precocity. *J Clin Endocrinol Metab*. 1990;70(2):503–507.
849. Chrousos GP, Detera-Wadleigh SD, Karl M. Syndromes of glucocorticoid resistance. *Ann Intern Med*. 1993;119(11):1113–1124.
850. Chrousos GP, Vingerhoeds A, Brandon D, Eil C, Pugeat M, DeVroede M, et al. Primary cortisol resistance in man. A glucocorticoid receptor-mediated disease. *J Clin Invest*. 1982;69(6):1261–1269.
851. McMahon SK, Pretorius CJ, Ungerer JP, Salmon NJ, Conwell LS, Pearen MA, et al. Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in Helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). *J Clin Endocrinol Metab*. 2010;95(1):297–302.
852. Nader N, Bachrach BE, Hurt DE, Gajula S, Pittman A, Lescher R, et al. A novel point mutation in helix 10 of the human glucocorticoid receptor causes generalized glucocorticoid resistance by disrupting the structure of the ligand-binding domain. *J Clin Endocrinol Metab*. 2010;95(5):2281–2285.
853. Malchoff DM, Brufsky A, Reardon G, McDermott P, Javier EC, Bergh CH, et al. A mutation of the glucocorticoid receptor in primary cortisol resistance. *J Clin Invest*. 1993;91(5):1918–1925.

854. Mendonca BB, Leite MV, de Castro M, Kino T, Elias LL, Bachega TA, et al. Female pseudohermaphroditism caused by a novel homozygous missense mutation of the GR gene. *J Clin Endocrinol Metab.* 2002;87(4):1805–1809.
855. Charmandari E, Kino T, Ichijo T, Jubiz W, Mejia L, Zachman K, et al. A novel point mutation in helix 11 of the ligand-binding domain of the human glucocorticoid receptor gene causing generalized glucocorticoid resistance. *J Clin Endocrinol Metab.* 2007;92(10):3986–3990.
856. Charmandari E, Kino T, Souvatzoglou E, Vottero A, Bhattacharyya N, Chrousos GP. Natural glucocorticoid receptor mutants causing generalized glucocorticoid resistance: molecular genotype, genetic transmission, and clinical phenotype. *J Clin Endocrinol Metab.* 2004;89(4):1939–1949.
857. Karl M, Lamberts SW, Koper JW, Katz DA, Huizenga NE, Kino T, et al. Cushing's disease preceded by generalized glucocorticoid resistance: clinical consequences of a novel, dominant-negative glucocorticoid receptor mutation. *Proc Assoc Am Phys.* 1996;108(4):296–307.
858. Kino T, Stauber RH, Resau JH, Pavlakis GN, Chrousos GP. Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. *J Clin Endocrinol Metab.* 2001;86(11):5600–5608.
859. Charmandari E, Ichijo T, Jubiz W, Baid S, Zachman K, Chrousos GP, et al. A novel point mutation in the amino terminal domain of the human glucocorticoid receptor (hGR) gene enhancing hGR-mediated gene expression. *J Clin Endocrinol Metab.* 2008;93(12):4963–4968.
860. McPhaul MJ, Marcelli M, Tilley WD, Griffin JE, Wilson JD. Androgen resistance caused by mutations in the androgen receptor gene. *Faseb J.* 1991;5(14):2910–2915.
861. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M. The androgen receptor gene mutations database: 2012 update. *Hum Mutat.* 2012;33(5):887–894.
862. Hughes IA, Werner R, Bunch T, Hiort O. Androgen insensitivity syndrome. *Semin Reprod Med.* 2010;30(5):432–442.
863. Jirasek JE. Androgen-insensitive male pseudohermaphroditism. *Birth Defects Orig Artic Ser.* 1971;7(6):179–184.
864. Lubahn DB, Brown TR, Simental JA, Higgs HN, Migeon CJ, Wilson EM, et al. Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity [published erratum appears in *Proc Natl Acad Sci U S A* 87(11):4411]. *Proc Natl Acad Sci U S A.* 1990;86(23):9534–9538.
865. Bevan CL, Hughes IA, Patterson MN. Wide variation in androgen receptor dysfunction in complete androgen insensitivity syndrome. *J Steroid Biochem Mol Biol.* 1997;61(1-2):19–26.
866. Tincello DG, Saunders PT, Hodgins MB, Simpson NB, Edwards CR, Hargreaves TB, et al. Correlation of clinical, endocrine and molecular abnormalities with in vivo responses to high-dose testosterone in patients with partial androgen insensitivity syndrome. *Clin Endocrinol (Oxf).* 1997;46(4):497–506.
867. MacLean HE, Warne GL, Zajac JD. Defects of androgen receptor function: from sex reversal to motor neurone disease. *Mol Cell Endocrinol.* 1995;112(2):133–141.
868. Peterziel H, Culig Z, Stober J, Hobisch A, Radmayr C, Bartsch G, et al. Mutant androgen receptors in prostatic tumors distinguish between amino-acid-sequence requirements for transactivation and ligand binding. *Int J Cancer.* 1995;63(4):544–550.
869. Brown TR, Lubahn DB, Wilson EM, French FS, Migeon CJ, Corden JL. Functional characterization of naturally occurring mutant androgen receptors from subjects with complete androgen insensitivity. *Mol Endocrinol.* 1990;4(12):1759–1772.
870. McPhaul MJ, Marcelli M, Zoppi S, Griffin JE, Wilson JD. Genetic basis of endocrine disease. 4. The spectrum of mutations in the androgen receptor gene that causes androgen resistance. *J Clin Endocrinol Metab.* 1993;76(1):17–23.
871. Marcelli M, Zoppi S, Grino PB, Griffin JE, Wilson JD, McPhaul MJ. A mutation in the DNA-binding domain of the androgen receptor gene causes complete testicular feminization in a patient with receptor-positive androgen resistance. *J Clin Invest.* 1991;87(3):1123–1126.
872. Zoppi S, Wilson CM, Harbison MD, Griffin JE, Wilson JD, McPhaul MJ, et al. Complete testicular feminization caused by an amino-terminal truncation of the androgen receptor with downstream initiation. *J Clin Invest.* 1993;91(3):1105–1112.
873. Marcelli M, Tilley WD, Wilson CM, Griffin JE, Wilson JD, McPhaul MJ. Definition of the human androgen receptor gene structure permits the identification of mutations that cause androgen resistance: premature termination of the receptor protein at amino acid residue 588 causes complete androgen resistance. *Mol Endocrinol.* 1990;4(8):1105–1116.
874. Trifiro M, Gottlieb B, Pinsky L, Kaufman M, Prior L, Belsham DD, et al. The 56/58 kDa androgen-binding protein in male genital skin fibroblasts with a deleted androgen receptor gene. *Mol Cell Endocrinol.* 1991;75(1):37–47.
875. Quigley CA, Friedman KJ, Johnson A, Lafreniere RG, Silverman LM, Lubahn DB, et al. Complete deletion of the androgen receptor gene: definition of the null phenotype of the androgen insensitivity syndrome and determination of carrier status. *J Clin Endocrinol Metab.* 1992;74(4):927–933.
876. Ris-Stalpers C, Kuiper GG, Faber PW, Schweikert HU, van Rooij HC, Zegers ND, et al. Aberrant splicing of androgen receptor mRNA results in synthesis of a nonfunctional receptor protein in a patient with androgen insensitivity. *Proc Natl Acad Sci U S A.* 1990;87(20):7866–7870.
877. Holterhus PM, Wiebel J, Sinnecker GH, Bruggenwirth HT, Sippell WG, Brinkmann AO, et al. Clinical and molecular spectrum of somatic mosaicism in androgen insensitivity syndrome. *Pediatr Res.* 1999;46(6):684–690.
878. Holterhus PM, Bruggenwirth HT, Hiort O, Kleinkauf-Houcken A, Kruse K, Sinnecker GH, et al. Mosaicism due to a somatic mutation of the androgen receptor gene determines phenotype in androgen insensitivity syndrome. *J Clin Endocrinol Metab.* 1997;82(11):3584–3589.
879. Gast A, Neuschmid-Kaspar F, Klocker H, Cato AC. A single amino acid exchange abolishes dimerization of the androgen receptor and causes Reifenstein syndrome. *Mol Cell Endocrinol.* 1995;111(1):93–98.
880. Kaspar F, Klocker H, Denninger A, Cato AC. A mutant androgen receptor from patients with Reifenstein syndrome: identification of the function of a conserved alanine residue in the D box of steroid receptors. *Mol Cell Biol.* 1993;13(12):7850–7858.
881. Klocker H, Kaspar F, Eberle J, Uberreiter S, Radmayr C, Bartsch G. Point mutation in the DNA binding domain of the androgen receptor in two families with Reifenstein syndrome. *Am J Hum Genet.* 1992;50(6):1318–1327.
882. Nakao R, Yanase T, Sakai Y, Haji M, Nawata H. A single amino acid substitution (gly743 → val) in the steroid-binding domain of the human androgen receptor leads to Reifenstein syndrome. *J Clin Endocrinol Metab.* 1993;77(1):103–107.
883. McPhaul MJ, Marcelli M, Zoppi S, Wilson CM, Griffin JE, Wilson JD. Mutations in the ligand-binding domain of the androgen receptor gene cluster in two regions of the gene. *J Clin Invest.* 1992;90(5):2097–2101.
884. Wooster R, Mangion J, Eeles R, Smith S, Dowsett M, Averill D, et al. A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. *Nat Genet.* 1992;2(2):132–134.
885. MacLean HE, Warne GL, Zajac JD. Spinal and bulbar muscular atrophy: androgen receptor dysfunction caused by a trinucleotide repeat expansion. *J Neurol Sci.* 1996;135(2):149–157.
886. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res.* 1994;22(15):3181–3186.
887. Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, DeMartino GN, et al. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum Mol Genet.* 1999;8(5):731–741.
888. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology.* 1997;138(3):863–870.

889. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* 1996;392(1):49–53.
890. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U S A.* 1996;93(12):5925–5930.
891. McInerney EM, Tsai MJ, O'Malley BW, Katzenellenbogen BS. Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *Proc Natl Acad Sci U S A.* 1996;93(19):10069–10073.
892. Pettersson K, Grandien K, Kuiper GG, Gustafsson JA. Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Mol Endocrinol.* 1997;11(10):1486–1496.
893. Toran-Allerand CD. Minireview: a plethora of estrogen receptors in the brain: where will it end? *Endocrinology.* 2004;145(3):1069–1074.
894. Li L, Haynes MP, Bender JR. Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc Natl Acad Sci U S A.* 2003;100(8):4807–4812.
895. Toran-Allerand CD, Guan X, MacLusky NJ, Horvath TL, Diano S, Singh M, et al. ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. *J Neurosci.* 2002;22(19):8391–8401.
896. Rao BR. Isolation and characterization of an estrogen binding protein which may integrate the plethora of estrogenic actions in non-reproductive organs. *J Steroid Biochem Mol Biol.* 1998;65(1-6):3–41.
897. Asaithambi A, Mukherjee S, Thakur MK. Expression of 112-kDa estrogen receptor in mouse brain cortex and its autoregulation with age. *Biochem Biophys Res Commun.* 1997;231(3):683–685.
898. Ramirez VD, Kipp JL, Joe I. Estradiol, in the CNS, targets several physiologically relevant membrane-associated proteins. *Brain Res Rev.* 2001;37(1-3):141–152.
899. Joe I, Ramirez VD. Binding of estrogen and progesterone-BSA conjugates to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the effects of the free steroids on GAPDH enzyme activity: physiological implications. *Steroids.* 2001;66(6):529–538.
900. Zheng J, Ramirez VD. Purification and identification of an estrogen binding protein from rat brain: oligomycin sensitivity-conferring protein (OSCP), a subunit of mitochondrial F₀F₁-ATP synthase/ATPase. *J Steroid Biochem Mol Biol.* 1999;68(1-2):65–75.
901. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man [see comments] [published erratum appears in *N Engl J Med*, 332(2):131]. *N Engl J Med.* 1994;331(16):1056–1061.
902. Lehrer S, Rabin J, Stone J, Berkowitz GS. Association of an estrogen receptor variant with increased height in women. *Horm Metab Res.* 1994;26(10):486–488.
903. Kuhnle U, Nielsen MD, Tietze HU, Schroeter CH, Schlamp D, Bosson D, et al. Pseudohypoadosteronism in eight families: different forms of inheritance are evidence for various genetic defects. *J Clin Endocrinol Metab.* 1990;70(3):638–641.
904. Chitayat D, Spirer Z, Ayalon D, Golander A. Pseudohypoadosteronism in a female infant and her family: diversity of clinical expression and mode of inheritance. *Acta Paediatr Scand.* 1985;74(4):619–622.
905. Hanukoglu A, Fried D, Gotlieb A. Inheritance of pseudohypoadosteronism. *Lancet.* 1978;1(8078):1359.
906. Limal JM, Rappaport R, Dechaux M, Morin C. Familial dominant pseudohypoadosteronism [letter]. *Lancet.* 1978;1(8054):51.
907. Bonnici F. [Autosomal recessive transmission of familial pseudohypoadosteronism (letter)]. *Arch Fr Pediatr.* 1977;34(9):915–916.
908. Rosler A. The natural history of salt-wasting disorders of adrenal and renal origin. *J Clin Endocrinol Metab.* 1984;59(4):689–700.
909. Shigetomi S, Ojima M, Ueno S, Tosaki H, Kohno H, Fukuchi S. Two adult familial cases of selective hypoadosteronism due to insufficiency of conversion of corticosterone to aldosterone. *Endocrinol Jpn.* 1986;33(6):787–794.
910. Keszler M, Sivasubramanian KN. Pseudohypoadosteronism. *Am J Dis Child.* 1983;137(8):738–740.
911. Abramson O, Zmora E, Mazor M, Shinwell ES. Pseudohypoadosteronism in a preterm infant: intrauterine presentation as hydramnios [see comments]. *J Pediatr.* 1992;120(1):129–132.
912. Kuhnle U, Keller U, Armanini D, Funder J, Krozowski Z. Immunofluorescence of mineralocorticoid receptors in peripheral lymphocytes: presence of receptor-like activity in patients with the autosomal dominant form of pseudohypoadosteronism, and its absence in the recessive form. *J Steroid Biochem Mol Biol.* 1994;51(5-6):267–273.
913. Arai K, Chrousos GP. Syndromes of glucocorticoid and mineralocorticoid resistance. *Steroids.* 1995;60(1):173–179.
914. Oberfield SE, Levine LS, Carey RM, Bejar R, New MI. Pseudohypoadosteronism: multiple target organ unresponsiveness to mineralocorticoid hormones. *J Clin Endocrinol Metab.* 1979;48(2):228–234.
915. Furgeson SB, Linas S. Mechanisms of type I and type II pseudohypoadosteronism. *J Am Soc Nephrol.* 2010;21(11):1842–1845.
916. Hanukoglu A. Type I pseudohypoadosteronism includes two clinically and genetically distinct entities with either renal or multiple target organ defects. *J Clin Endocrinol Metab.* 1991;73(5):936–944.
917. Schambelan M, Sebastian A, Rector Jr FC. Mineralocorticoid-resistant renal hyperkalemia without salt wasting (type II pseudohypoadosteronism): role of increased renal chloride reabsorption. *Kidney Int.* 1981;19(5):716–727.
918. Zennaro MC, Hubert EL, Fernandes-Rosa FL. Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol.* 2011;350(2):206–215.
919. Fernandes-Rosa FL, de Castro M, Latronico AC, Sippell WG, Riepe FG, Antonini SR. Recurrence of the R947X mutation in unrelated families with autosomal dominant pseudohypoadosteronism type 1: evidence for a mutational hot spot in the mineralocorticoid receptor gene. *J Clin Endocrinol Metab.* 2006;91(9):3671–3675.
920. Geller DS, Rodriguez-Soriano J, Vallo Boado A, Schifter S, Bayer M, Chang SS, et al. Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoadosteronism type I. *Nat Genet.* 1998;19(3):279–281.
921. Geller DS, Zhang J, Zennaro MC, Vallo-Boado A, Rodriguez-Soriano J, Furu L, et al. Autosomal dominant pseudohypoadosteronism type 1: mechanisms, evidence for neonatal lethality, and phenotypic expression in adults. *J Am Soc Nephrol.* 2006;17(5):1429–1436.
922. Nystrom AM, Bondeson ML, Skanke N, Martensson J, Stromberg B, Gustafsson J, et al. A novel nonsense mutation of the mineralocorticoid receptor gene in a Swedish family with pseudohypoadosteronism type I (PHA1). *J Clin Endocrinol Metab.* 2004;89(1):227–231.
923. Riepe FG. Clinical and molecular features of type 1 pseudohypoadosteronism. *Horm Res.* 2009;72(1):1–9.
924. Riepe FG, Finkeldei J, de Sanctis L, Einaudi S, Testa A, Karges B, et al. Elucidating the underlying molecular pathogenesis of NR3C2 mutants causing autosomal dominant pseudohypoadosteronism type 1. *J Clin Endocrinol Metab.* 2006;91(11):4552–4561.
925. Riepe FG, Krone N, Morlot M, Ludwig M, Sippell WG, Partsch CJ. Identification of a novel mutation in the human mineralocorticoid receptor gene in a German family with autosomal-dominant pseudohypoadosteronism type 1: further evidence for marked interindividual clinical heterogeneity. *J Clin Endocrinol Metab.* 2003;88(4):1683–1686.
926. Riepe FG, Krone N, Morlot M, Peter M, Sippell WG, Partsch CJ. Autosomal-dominant pseudohypoadosteronism type 1 in a Turkish family is associated with a novel nonsense mutation in the human mineralocorticoid receptor gene. *J Clin Endocrinol Metab.* 2004;89(5):2150–2152.
927. Sartorato P, Lapeyraque AL, Armanini D, Kuhnle U, Khaldi Y, Salomon R, et al. Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type I pseudohypoadosteronism. *J Clin Endocrinol Metab.* 2003;88(6):2508–2517.
928. Tajima T, Kitagawa H, Yokoya S, Tachibana K, Adachi M, Nakae J, et al. A novel missense mutation of mineralocorticoid receptor gene in one Japanese family with a renal form of pseudohypoadosteronism type 1. *J Clin Endocrinol Metab.* 2000;85(12):4690–4694.

929. Viemann M, Peter M, Lopez-Siguero JP, Simic-Schleicher G, Sippell WG. Evidence for genetic heterogeneity of pseudohypoadosteronism type 1: identification of a novel mutation in the human mineralocorticoid receptor in one sporadic case and no mutations in two autosomal dominant kindreds. *J Clin Endocrinol Metab.* 2001;86(5):2056–2059.
930. Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science.* 2000;289(5476):119–123.
931. New MI, Stoner E, DiMartino-Nardi J. Apparent mineralocorticoid excess causing hypertension and hypokalemia in children. *Clin Exp Hypertens [A].* 1986;8(4-5):751–772.
932. Muller-Berghaus J, Homoki J, Michalk DV, Querfeld U. Diagnosis and treatment of a child with the syndrome of apparent mineralocorticoid excess type 1. *Acta Paediatr.* 1996;85(1):111–113.
933. Dave-Sharma S, Wilson RC, Harbison MD, Newfield R, Azar MR, Krozowski ZS, et al. Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab.* 1998;83(7):2244–2254.
934. Morineau G, Marc JM, Boudi A, Galons H, Gourmelen M, Corvol P, et al. Genetic, biochemical, and clinical studies of patients with A328V or R213C mutations in 11 β HSD2 causing apparent mineralocorticoid excess. *Hypertension.* 1999;34(3):435–441.
935. Ugrasbul F, Wiens T, Rubinstein P, New MI, Wilson RC. Prevalence of mild apparent mineralocorticoid excess in Mennonites. *J Clin Endocrinol Metab.* 1999;84(12):4735–4738.
936. Monder C, Shackleton CH, Bradlow HL, New MI, Stoner E, Iohan F, et al. The syndrome of apparent mineralocorticoid excess: its association with 11 β dehydrogenase and 5 β reductase deficiency and some consequences for corticosteroid metabolism. *J Clin Endocrinol Metab.* 1986;63(3):550–557.
937. Mune T, Rogerson FM, Nikkila H, Agarwal AK, White PC. Human hypertension caused by mutations in the kidney isozyme of 11 β hydroxysteroid dehydrogenase. *Nat Genet.* 1995;10(4):394–399.
938. Wilson RC, Harbison MD, Krozowski ZS, Funder JW, Shackleton CH, Hanauske-Abel HM, et al. Several homozygous mutations in the gene for 11 β hydroxysteroid dehydrogenase type 2 in patients with apparent mineralocorticoid excess. *J Clin Endocrinol Metab.* 1995;80(11):3145–3150.
939. McSherry E. Renal tubular acidosis in childhood. *Kidney Int.* 1981;20(6):799–809.
940. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley Jr WF. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. *J Clin Endocrinol Metab.* 1999;84(12):4501–4509.
941. Bassett JH, O'Halloran DJ, Williams GR, Beardwell CG, Shalet SM, Thakker RV. Novel DAX1 mutations in X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf).* 1999;50(1):69–75.
942. Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, et al. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. *Nature.* 1994;372(6507):635–641.
943. Lynch JP, Lala DS, Peluso JJ, Luo W, Parker KL, White BA. Steroidogenic factor 1, an orphan nuclear receptor, regulates the expression of the rat aromatase gene in gonadal tissues. *Mol Endocrinol.* 1993;7(6):776–786.
944. Ito M, Yu RN, Jameson JL. Steroidogenic factor-1 contains a carboxy-terminal transcriptional activation domain that interacts with steroid receptor coactivator-1. *Mol Endocrinol.* 1998;12(2):290–301.
945. Muscatelli F, Strom TM, Walker AP, Zhanaria E, Recan D, Meindl A, et al. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature.* 1994;372(6507):672–676.
946. Ito M, Yu R, Jameson JL. DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol.* 1997;17(3):1476–1483.
947. Zhang YH, Guo W, Wagner RL, Huang BL, McCabe L, Vilain E, et al. DAX1 mutations map to putative structural domains in a deduced three-dimensional model. *Am J Hum Genet.* 1998;62(4):855–864.
948. Hamaguchi K, Arikawa M, Yasunaga S, Kakuma T, Fukagawa K, Yanase T, et al. Novel mutation of the DAX1 gene in a patient with X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Am J Med Genet.* 1998;76(1):62–66.
949. Zhang YH, Huang BL, Niakan KK, McCabe LL, McCabe ER, Dipple KM. IL1RAPL1 is associated with mental retardation in patients with complex glycerol kinase deficiency who have deletions extending telomeric of DAX1. *Hum Mutat.* 2004;24(3):273.

4

Endocrinology Laboratory Testing

Jon Nakamoto

CHAPTER OUTLINE

INTRODUCTION	86
COLLABORATION AMONG CLINICIANS AND LABORATORIANS	86
LEARNING TO SPEAK (SOME OF) THE LANGUAGE OF THE LABORATORY	86
LABORATORY STATISTICS: THE BASICS OF EVIDENCE-BASED DIAGNOSIS	87
ANALYTICAL VALIDATION	89
Linearity/Reportable Range	89
Precision	89
Analytic Sensitivity	90
Analytic Specificity, Interferences, and Recovery	90
Accuracy/Method Comparison	90
Sample Types and Matrix Effects	90
Stability	91
Carryover	91
METHODOLOGY	91
Immunoassays	91
Mass Spectrometry	94
WHAT CAN GO WRONG? ERRORS, INTERFERENCE, AND THE LIKE	98
Potential Confounders in Immunoassays	99
STANDARDIZATION/HARMONIZATION: CLINICALLY RELEVANT AND CRUCIAL	100
REFERENCE INTERVALS	101
SUMMARY	104
References	104

INTRODUCTION

What makes one an effective user of the clinical laboratory?
Such a user:

- Identifies at least one sympathetic ally in the clinical laboratory to whom one can reach out for advice about laboratory testing. None of us can be expert in all the areas we need to know for the best possible care of patients, so including a laboratory expert as an ad hoc member of the care team should be a best practice for all clinicians.
- Is at least somewhat conversant with the language of the clinical laboratory to make it easier to communicate with laboratory experts, as well as to understand key articles published in the clinical laboratory literature.
- Understands that diagnostic testing, like so much of medicine, is “playing the odds,” and has enough awareness of basic probability theory and statistics to know: (1) when and when not to order laboratory tests and (2) how confident to be about a positive or negative result.
- Has insight into how laboratory tests are validated (for quality purposes).

- Knows enough about laboratory methodology to decide which test is most appropriate for the patient.
- Is aware of test limitations and possible sources of error (many of which can occur even before the sample is actually tested).
- Accepts the reality that different versions/kits/brands of the same test can give markedly different results, and adjusts diagnostic strategy accordingly.
- Recognizes the limitations and statistical fuzziness of all reference intervals, particularly those in pediatrics, and interprets results carefully with this inexactitude in mind.

COLLABORATION AMONG CLINICIANS AND LABORATORIANS

Even with a laboratory test as seemingly simple as thyroid-stimulating hormone (TSH), a collaborative relationship between an endocrinologist and a clinical laboratory expert can be mutually beneficial. An experienced clinician may be well aware of the significant circadian rhythm of this hormone but may or may not be aware of the substantial interindividual difference in how much daily variability is present¹, nor how much TSH results may vary among different assays. The laboratorian in turn may be intimately familiar with how often interfering antibodies may lead to falsely elevated results but may or may not realize how common it is to see teenaged congenital hypothyroidism patients with high TSH and high free thyroxine (T₄) because of last-minute double-dosing after months of chronic noncompliance. A clinical laboratory expert can explain how heterophilic antibody interference differs from TSH autoantibody interference (we will cover this later), and which diagnostic maneuvers make the most sense given the clinical scenario presented by the clinician. There is a time commitment involved in arranging regular clinician-laboratorian communications, but without a doubt patients benefit tremendously from what experts can teach each other.

LEARNING TO SPEAK (SOME OF) THE LANGUAGE OF THE LABORATORY

The language used in the clinical laboratory can be a barrier to learning. “Labspeak” is not quite a foreign language but represents a dialect with many terms unfamiliar to clinicians. Imagine the following conversation:

Clinician: “The results for test X on this patient seem really different between laboratory Y and your laboratory.”

Laboratorian: “I agree with you that these two results show more discordance than I’d expect from normal analytical and biological variation because the total allowable error is only 15% even though we’re down near the LOQ. The assay QC looks fine, so we should check for potential preanalytical issues and consider possible heterophilic antibody interference as well. And let’s check the platforms used, because the assays for this analyte are definitely not standardized or even harmonized to date.”

Although those working in the clinical laboratory should be savvy enough to avoid speaking like this to a clinician (especially

TABLE 4.1 Selected Laboratory Terminology

Term and/or Concept	Associated Acronyms	Pertinent Section
Aliquot		2
Analyte		2
Calibrator/calibration		7
Carryover		4
Chromatography	LC, HPLC, UPLC	5
Competitive immunoassay	RIA, EIA, CIA	5
Extraction		5
Harmonization		7
Heterophilic antibody	HAMA, HAAA	6
Immunometric assay	IRMA, ICMA, IFMA, ELISA	5
Interferences		6
Limit of quantitation (vs. limit of detection)	LOQ (vs. LOD)	4
Linearity		4
Mass spectrometry	MS, GC-MS, LC-MS/MS	5
Matrix		4
Method comparison		4
Platform		2
Positive (vs. negative) predictive value	PPV (vs. NPV)	3
Preanalytical		6
Precision		4
Receiver-operator characteristic curve	ROC curve	3
Recovery		4
Reportable range		4
Sensitivity & specificity (analytical)		4
Sensitivity & specificity (clinical)		4
Stability		3
Standardization		4
Validation (Analytical)		7

CIA, Competitive immunoassay; *ELISA*, enzyme-linked immunosorbent assay; *EIA*, enzyme immunoassay; *GC-MS*, gas chromatography-mass spectrometry; *ICMA*, immunochemiluminometric assay; *IFMA*, immunofluorescence assay; *IRMA*, immunoradiometric assay; *LC*, liquid chromatography; *HAAA*, human antianimal antibody; *HAMA*, human antimouse antibody; *HPLC*, high-performance liquid chromatography; *MS*, mass spectrometry; *RIA*, radioimmunoassay; *UPLC*, ultraperformance liquid chromatography.

using acronyms like LOQ), the earlier paragraph is perfectly plausible for a conversation between two laboratorians, and in fact might be the most concise way to convey key points in the investigation to follow. Knowing even just a few commonly used laboratory terms shown in Table 4.1 can help bridge the communication gap and will certainly help a clinician better understand key articles in a useful journal, such as *Clinical Chemistry*.

Many of these terms will be defined in sidebars in the appropriate section, but there are a few general ones that are worth mentioning right away:

- “Analyte” is a very common word in laboratory medicine, simple in concept, yet unfamiliar to most clinical ears. It is a generic term for “the thing being measured/analyzed.” Feeling comfortable with this word will make it much easier to talk with the laboratory and scour the pertinent laboratory literature.
- “Aliquots” are smaller portions of a sample, prepared from the original, or “mother” tube. You can use an aliquot to send out to another laboratory for corroboration, or use a “fresh aliquot” to repeat the test, if you think the original one may have had too many freeze-thaw cycles, or was potentially contaminated.

- “Platform” is a general, albeit somewhat ambiguous term, most often used to describe the manufacturer and model of automated testing instruments, for example, the Beckman Access versus the Roche Elecsys. Why is this of any importance to the clinician? Because platforms differ in their performance characteristics and vulnerability to interferences. We will see later on that comparing results for the same sample on two different platforms is sometimes the fastest way to investigate certain types of interferences. A clinician faced with an unexpected result must be aware enough to ask the laboratory if they can corroborate that result “on a different platform” when appropriate.

LABORATORY STATISTICS: THE BASICS OF EVIDENCE-BASED DIAGNOSIS

Biostatistics and epidemiology are often taught during the early preclinical years of training, when students are hungry to gain clinical experience, and are sometimes dismissive of what seems like more didactic study. Yet, both experienced clinicians and those involved in clinical research realize quickly how important it is to have at least a basic awareness of medical statistics to avoid making significant errors in diagnostic or treatment decisions.

If you call your laboratory asking about the sensitivity and specificity of a test, you will be asked whether you want “clinical sensitivity and specificity” (covered here) versus the completely different “analytical sensitivity and specificity” (discussed in the following methodology/validation section). The clinical laboratory will certainly have data on the latter, but likely only limited studies for the former, because establishing clinical sensitivity and specificity typically require significant clinical studies beyond the reach of the clinical laboratory.

- “Clinical sensitivity” is how often the test will be positive in a patient who has the disease being tested for. An excellent mnemonic (useful for examinations) is to think of the abbreviation for “positive in disease” as “PID” and consider how important it is to be “sensitive” when you have a patient with clinical PID (pelvic inflammatory disease).²
- “Clinical specificity” is how often the test will be negative in a patient who is “healthy” (at least, who does not have the disease being tested for). The mnemonic in this case is “negative in health,” or “NIH”—consider how important it is to be very “specific” when writing an NIH grant proposal.

Perhaps more relevant to clinical practice is to understand the concepts of “positive predictive value” (PPV) and “negative predictive value” (NPV)

- PPV is the probability of disease in a patient with a positive test result.
- NPV is the probability of “health” (nondisease) in a patient with a negative test result.

Both NPV and PPV are affected by the underlying prevalence of disease, or more precisely, by the probability that the patient in question has the disease (“pretest probability”). Fig. 4.1 summarizes the definitions of these terms, whereas Fig. 4.2 demonstrates the dramatic effect of increasing pretest probability on the PPV of a diagnostic test, which should be a testament to the importance of a good history and physical examination before deciding on laboratory test ordering. The best way to improve diagnostic test performance is to be as certain as possible about the diagnosis even before ordering the test!

In clinical practice, most endocrine laboratory tests give continuous rather than “yes/no” results and are therefore rarely used as strict positive/negative tests. A TSH of either 6.0 mU/L or 60 mU/L are both “positive,” but neither value will be used as a diagnostic cutoff for the diagnosis of primary hypothyroidism. Using a TSH of 6.0 mU/L would ensure that virtually

all patients with primary hypothyroidism are detected (maximum sensitivity) but at the expense of many false negatives (very poor specificity) and unnecessary referrals to the endocrine clinic. On the other hand, using a cutoff of 60 mU/L would minimize the number of false negatives (excellent specificity) but at the expense of missing many true cases of primary hypothyroidism (clinically unacceptable low sensitivity). The choice of a cutoff somewhere in between these two extremes should be determined by the clinical scenario (e.g., perhaps lower in a 14-month-old infant than in an obese but otherwise healthy 13-year-old teenager) rather than an arbitrary universal threshold.

TP = True positive (positive test in a patient with disease)
 FP = False positive (positive test in a patient without disease)
 TN = True negative (negative test in a patient without disease)
 FN = False negative (negative test in a patient with disease)

$$\text{Clinical sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Clinical specificity} = \frac{TN}{FP + TN}$$

$$\text{Positive predictive value (PPV)} = \frac{TP}{TP + FP}$$

$$\text{Negative predictive value (NPV)} = \frac{TN}{FN + TN}$$

Fig. 4.1 Definitions of basic diagnostic test statistics.

For tests that generate continuous results, the overall diagnostic effectiveness of a test can also be evaluated by plotting the true positive rate versus the false positive rate, producing a receiver-operating characteristics curve (ROC curve).

- An ROC curve plots, for various test results, the true positive rate (clinical sensitivity) of a test on the y-axis versus the false positive rate (1 – clinical specificity) on the x-axis (Fig. 4.3). The area under the curve (AUC) can be used to estimate the ability of the test to distinguish disease from nondisease, with an AUC of 0.50, indicating a test without diagnostic value, a test with an AUC of 0.90 or more generally considered as excellent, and one with an AUC of 0.70 considered to be a fair diagnostic test.

Two important caveats for evidence-based diagnosis:

- (1) Calculation of clinical sensitivity and specificity for a diagnostic test depends upon clear definition of who does have the disease in question and who does not. If there is no gold standard diagnostic test for comparison, or if the definition of the disease evolves and becomes less clear-cut over time (e.g., early definitions of severe/anatomically proven growth hormone deficiency [GHD] versus later, less well-defined cases of GHD), the definitions of sensitivity and specificity may be approximate at best.
- (2) Calculation of sensitivity, specificity, and true and false positive or negative rates will vary depending upon the nature of the population being studied. For example, a very good diagnostic test applied across the entire population of the United States will have a far higher false positive rate (as is seen with screening tests) than the same test applied to a carefully selected patient group who has been deemed

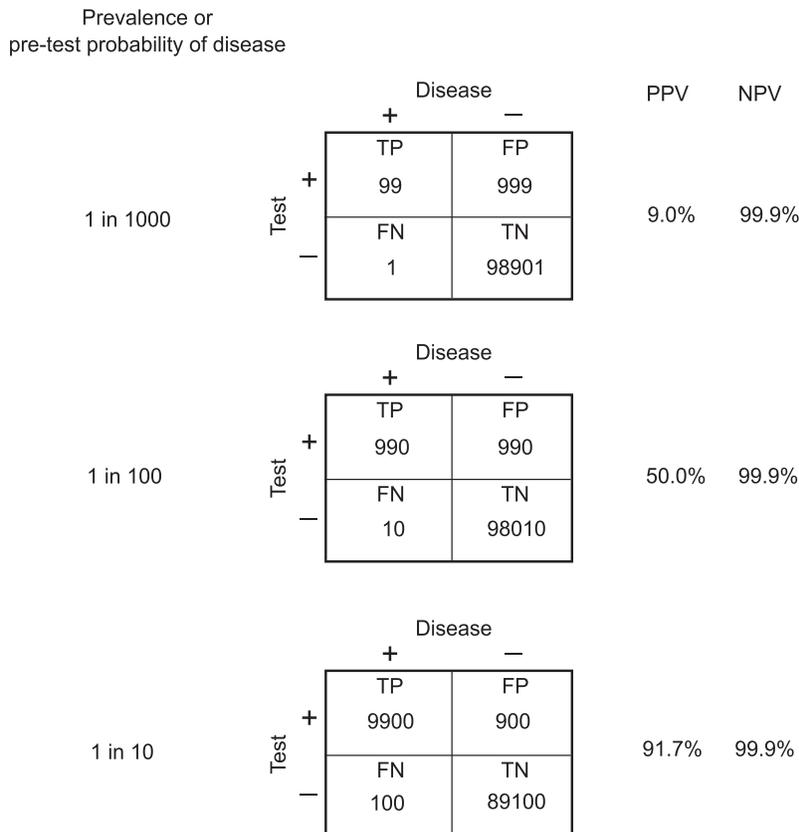


Fig. 4.2 Effect of disease prevalence/pretest probability on positive (PPV) and negative (NPV) predictive values, using a test with 99% clinical sensitivity and 99% clinical specificity. Numbers in boxes represent the distribution of 100,000 patients among true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN).

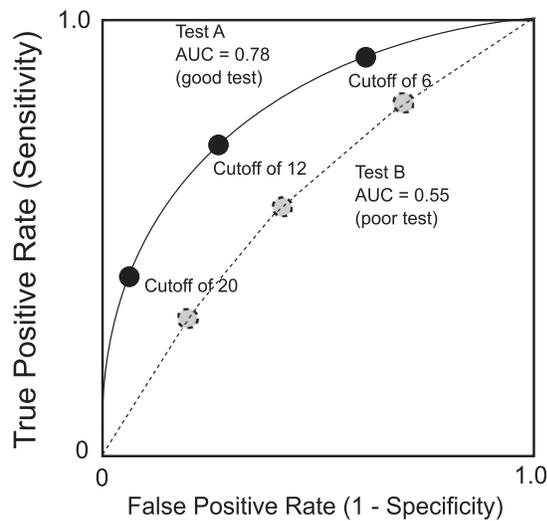


Fig. 4.3 Receiver-operating characteristic curves for two theoretical diagnostic tests.

likely to have the disease in question based on history and physical examination. This again emphasizes the need to increase pretest probability of disease as much as possible before ordering any diagnostic testing.

ANALYTICAL VALIDATION

Clinical Scenario 1

An investigator inadvertently runs two tubes containing nothing but water on a peptide classic radioimmunoassay (RIA) and is nonplussed when the results show a significant level of the peptide in these tubes.

Clinical Scenario 2

A laboratory declines to run a tumor marker immunoassay ordered on viscous cyst fluid because of a lack of analytic validation data but relents when the physician insists that the laboratory run the sample with a disclaimer "nonvalidated sample type; interpret with caution." Despite the disclaimer, the laboratory and the clinician are later both sued successfully for inappropriate diagnosis and unnecessary treatments based on what turns out to be a falsely positive result.

Both of these brief clinical vignettes illustrate why regulatory agencies and anyone concerned with quality laboratory testing place such emphasis on analytic method validation. A peptide RIA may give accurate results in serum, but totally inaccurate results in a protein-free fluid; perhaps the tracer (See Methodology: Immunoassays on page 91) in scenario 1 stuck to the sides of the plastic tube, leading to decreased tracer binding and an apparent detectable level of peptide where none was actually present. Scenario 2 represents a situation of misdiagnosis; the viscosity of the solution might have affected the interaction of the assay components, with substantial impact on the patient and legal consequences for all involved.

Analytical validation is meant to ensure that an assay method is accurate for its intended use. Components of an analytical validation include the following: (1) linearity/reportable range; (2) precision; (3) analytical sensitivity; (4) analytic specificity, interferences, and recovery; (5) accuracy/method comparison; (6) sample types and matrix effects; (7) stability; and (8) carryover. Determining reference intervals is an important part of many analytic validations and crosses over to clinical validation. Note that even though not all of these components are

always required from a regulatory point of view, all represent good-quality laboratory practice.

Linearity/Reportable Range

Also referred to as the analytic measurement range (AMR), this is the range of concentrations over which the assay is known to be reliable. Standards of known concentration (calibrators) are assayed and plotted against the signal generated in the assay. For the hypothetical study shown in Fig. 4.4, the upper limit of the AMR would likely be at the concentration represented by calibrator D, because the higher concentration represented by calibrator E does not result in a similar degree of increased signal. However, it may be possible to dilute the sample so that one can make a measurement within the AMR, thereby allowing for assay of concentrations above the upper limit of the AMR. The calibrator choice may alter the absolute value reported, particularly with peptides and proteins where the standard may represent only one of a mixture of differentially modified (e.g., glycosylated or cleaved) forms present in the circulation.

Precision

Also known as reproducibility or replicability, this defines whether the random error of the assay is small enough to make the assay clinically useful. A commonly used analogy is that of the target shooter: how close together are the bullet holes? Note that precision is distinct from accuracy (a different part of the validation study); precision addresses only if the shots are close together, not whether they are actually hitting the bulls-eye. Both intraassay (e.g., 20 measurements done on the same assay run) and interassay (1 measurement done daily for 20 days) precision are studied and the standard deviations (SDs) of the replicate measurements calculated. In general,

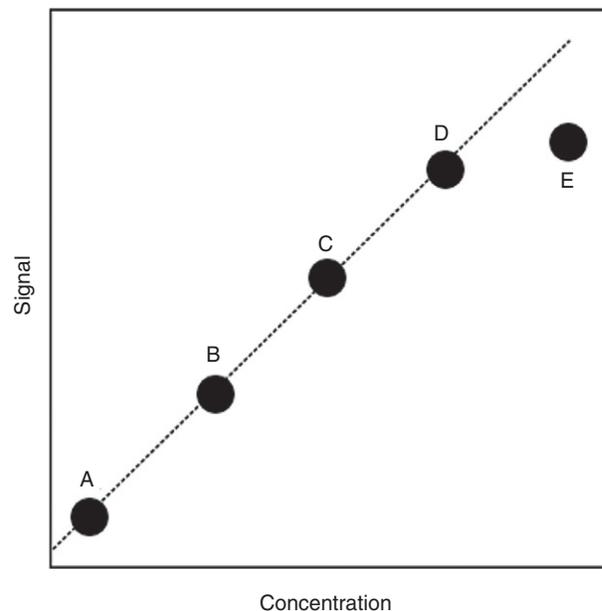


Fig. 4.4 Linearity study used to determine the analytic measurement range (AMR). The concentrations represented by points A and D represent the likely minimum and maximum concentrations of the AMR. Higher concentrations, such as that represented by point E, may still be measured if the sample can be diluted to bring the concentration to within the AMR, provided that previous studies have proven that the response remains linear when a dilution is performed.

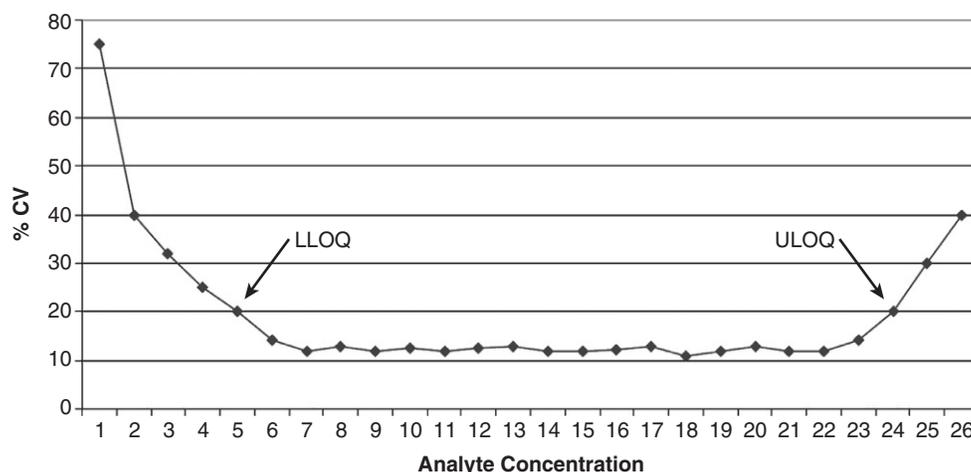


Fig. 4.5 Using a precision profile to determine the lower (LLOQ) and upper (ULOQ) limits of quantitation, based on acceptable interassay precision (commonly set at a coefficient of variation [CV] of 20% or less).

precision is presented as the coefficient of variation (CV), which is the SD divided by the mean, expressed as a percentage. For example, at a mean value of 100 ng/mL, an assay with an SD of 5 ng/mL would have a CV of 5%. Note that the concentration of the analyte factors into the assay's precision because CV values tend to be higher at either limit of the AMR (Fig. 4.5).

Analytic Sensitivity

This part of a validation study determines how low an analyte concentration can be measured with acceptable precision. It is distinct from clinical sensitivity (how often a result is positive in a patient with disease), which is a general part of a clinical validation study done well after the analytic validation has been completed. A frequent problem is the use of many different terms for analytic sensitivity, for example, minimal detectable concentration, limit of detection (LOD), limit of blank, or limit of absence, all of which describe the lowest possible concentration that can be confidently distinguished from zero. Although an assay developer might cite the LOD to make the assay look as sensitive as possible, the clinician should realize that values down near the LOD are hugely variable and not really quantitative. A more conservative and clinically useful analytic sensitivity limit is the limit of quantitation (LOQ), also known as *functional sensitivity* and typically defined as the lowest concentration that can be measured with a CV of less than 20%. Even this amount of variability is not trivial; if a testosterone assay has a CV of 20% at the LOQ of 30 ng/dL, one could obtain a result of either less than 30 ng/dL or 36 ng/dL on exactly the same sample, run on two different occasions.

Analytic Specificity, Interferences, and Recovery

Specificity in this context refers to the ability of the assay to measure a specific analyte without cross-reacting with other substances in the sample. Analytic specificity studies may involve the addition of known amounts of similar analytes to a sample; for example, a cortisol assay may be tested for cross-reactivity with cortisone, prednisone, prednisolone, dexamethasone, 17-hydroxyprogesterone, and other steroids. Closely related are interference studies to see if commonly encountered situations, such as hemolysis, hyperbilirubinemia, and lipemia affect the test results. Recovery studies are less often performed; here, a standard of known concentration is added into a sample, and the sample assayed to see what percentage of the added standard is detected (ideally 100%, but frequently less).

Accuracy/Method Comparison

Determining the accuracy of an assay is a multistep process, not all of which can be addressed in a typical analytic validation study. Accuracy may be part of the original test development decision-making process—for example, including an extraction and chromatography step to avoid otherwise problematic cross-reactions. Full determination of the clinical accuracy of a test may not be possible until the analytic validation is completed and the test released to investigators for clinical validation studies. Therefore so-called accuracy studies in an analytic validation are by necessity limited to just a small portion of the whole accuracy process. The interference and recovery studies mentioned earlier are pertinent to test accuracy, but the most common approach is to compare the new test method to another comparator method. Ideally, the method used for comparison will be some type of gold standard reference method, but often such a method is not available. As a proxy, the method under validation is compared with a well-accepted method, and the results shown (new method result on the y-axis, comparison method result on the x-axis) on a correlation plot (Fig. 4.6, upper panel). Also frequently used is a bias or difference plot (Fig. 4.6, lower panel), which better shows intermethod differences that may escape notice on a simple correlation plot.

Sample Types and Matrix Effects

Sample types are not automatically interchangeable. Ethylenediaminetetraacetic acid (EDTA) plasma from a lavender top tube may perform fine in an assay, whereas heparin plasma from a green top tube may not. One parathyroid hormone assay may give comparable results from both EDTA plasma and red top tube serum, whereas a different parathyroid hormone assay may not. These represent examples of a “matrix effect.”

- Matrix refers to the components of a sample other than the analyte being measured.

As noted in our vignettes, the matrix (e.g., protein-free, viscous, high-salt, containing high levels of paraproteins) may have a profound effect on the accuracy of a laboratory assay.

The matrix of serum is different than that of plasma, because of the absence of fibrinogen and anticoagulants, plus the presence of substances released by platelets. Differences in many analyte values may be more than is suggested by this apparently small difference; one study of metabolite profiles showed that 104 out of 122 metabolites had significantly higher concentrations in serum as compared with plasma.³ It is important to realize that an assay validated for one matrix cannot be automatically used for another.

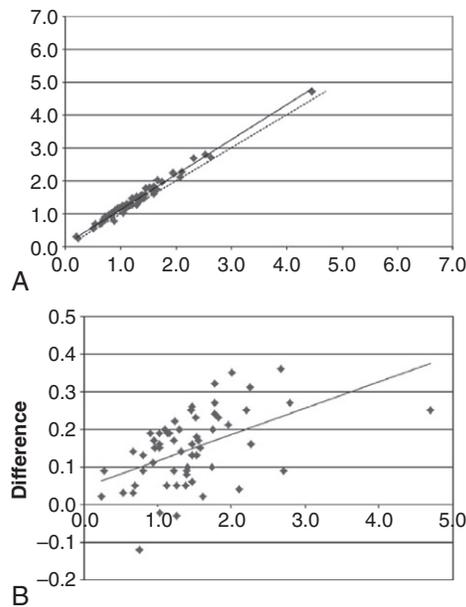


Fig. 4.6 Method comparison. A correlation plot (*upper panel*) versus difference/bias plot (*lower panel*). With a correlation plot (**A**), the values for a new assay (y-axis) are compared with values from an established assay (x-axis), showing a best fit (*solid line*) deviating slightly from the line of identity (*dotted line*). For the difference/bias plot (**B**), the difference between the values from the new versus the old assay is plotted on the y-axis, whereas the mean value of the results from both assays is plotted on the x-axis. The systematic upward bias of the new assay is seen more clearly on the difference/bias plot.

Stability

Typically, sample stability is studied at ambient (about 22°–26° C), refrigerated (about 2°–6° C), and regular frozen (around –18° to –20° C) temperatures. For assays used in clinical studies where specimens may be banked for prolonged periods, stability studies should also be performed at deeper frozen (e.g., –70° C) temperatures. Aliquots stored at these temperatures for varying periods of time are recovered and assayed to see if results are stable relative to baseline. Stability limits are an inherent property of the assay rather than the analyte: an osteocalcin assay designed to pick up only full-length molecules may have a short stability limit for samples at room temperature, whereas another assay that detects osteocalcin molecule fragments, as well as full-length protein, may have a much longer stability limit.

Carryover

Another important quality process is to ensure that there is no cross-contamination between samples, and that a sample with very high values will not carry over and falsely elevate results in the next sample to be assayed. Assays should be designed to minimize this problem, but there should also be awareness on the part of the laboratory staff to check for carryover whenever a sample with an extremely elevated value is encountered.

METHODOLOGY

Immunoassays

Immunoassay is an important methodology in the endocrine laboratory. Understanding the basic principles involved allows specialists to: (1) identify when a particular immunoassay is or is not appropriate for a particular clinical scenario,

(2) anticipate potential physiological and technical issues affecting the interpretation of laboratory results, and (3) understand how to work with the clinical laboratory to investigate unanticipated or clinically discordant test results.

Competitive Immunoassay versus Immunometric (Sandwich) Assay

There are two main immunoassay formats relevant to endocrinology laboratory testing, and understanding some of the basic differences between these formats helps both interpretation of results and troubleshooting of unexpected situations. The first class of assays is termed *competitive*, with the RIA as the archetypal example. A primary antibody against the analyte of interest is added to the patient's sample, together with a radiolabeled version of the analyte (tracer) that competes with the endogenous analyte for binding to the primary antibody (Fig. 4.7, upper panel). After a sufficiently long incubation time, the primary antibody is precipitated using a second anti-immunoglobulin (Ig)G antibody, polyethylene glycol, or (most commonly nowadays) by using primary antibody attached to a solid support, such as a bead, which allows a simple centrifugation step to collect the primary antibody. Any unbound tracer or analyte is washed away, and the amount of tracer in the precipitate is then quantified.

To convert this tracer signal into a concentration, a standard curve is prepared from samples where varying concentrations of a known amount of analyte have been added. For a competitive immunoassay, the amount of signal detected (amount of tracer bound to antibody) decreases as the analyte in the patient's sample increases. Importantly, anything that prevents the tracer from binding to the primary antibody would also decrease signal and increase apparent analyte concentration. For example, taking an immunoassay optimized for serum and using it for an extremely concentrated urine sample or in a body fluid where the protein concentration is extremely high can significantly alter the results. Because high-salt or high-protein concentration can inhibit tracer binding to antibody, tracer signal will be low and the apparent analyte concentration very high, even in the complete absence of any analyte.

The second class of immunoassays is termed *immunometric*, with the most important example being the noncompetitive, so-called *sandwich assay*. Typically, as shown in Fig. 4.7 (lower panel), an antibody attached to a solid support (plate, wall of a tube, or a bead) is used to capture the analyte of interest, followed by the addition of a second, labeled antibody that binds to a different site on the analyte, creating an antibody-analyte-antibody sandwich. After unbound detection antibody is washed away, and that which remains generates a radioactive (immunoradiometric [IRMA]), chemiluminescent (immunochemiluminometric [ICMA]), colorimetric (enzyme-linked immunosorbent assay [ELISA]), or fluorescent (immunofluorescence assay [IFMA]) signal, depending on the label chosen.

The immunometric assays offer certain inherent advantages over competitive immunoassays. First of all, the fact that two antibodies are involved, each binding to a separate epitope on the analyte, greatly increases analytic specificity, including the ability to select very specific isoforms of an analyte. For example, one antibody can be directed at the N-terminus of a peptide, whereas the other antibody can be directed at the C-terminus, ensuring that only full-length peptide is detected. A second advantage is that immunometric assays tend to be more analytically sensitive than their competitive assay counterparts.⁴ This arises from the fact that the sensitivity of a competitive immunoassay largely depends on the affinity of the antibody used in the assay—and developing very high-affinity antibodies is not an easy or predictable task. In contrast, the sensitivity of an immunometric assay can be improved by use of a

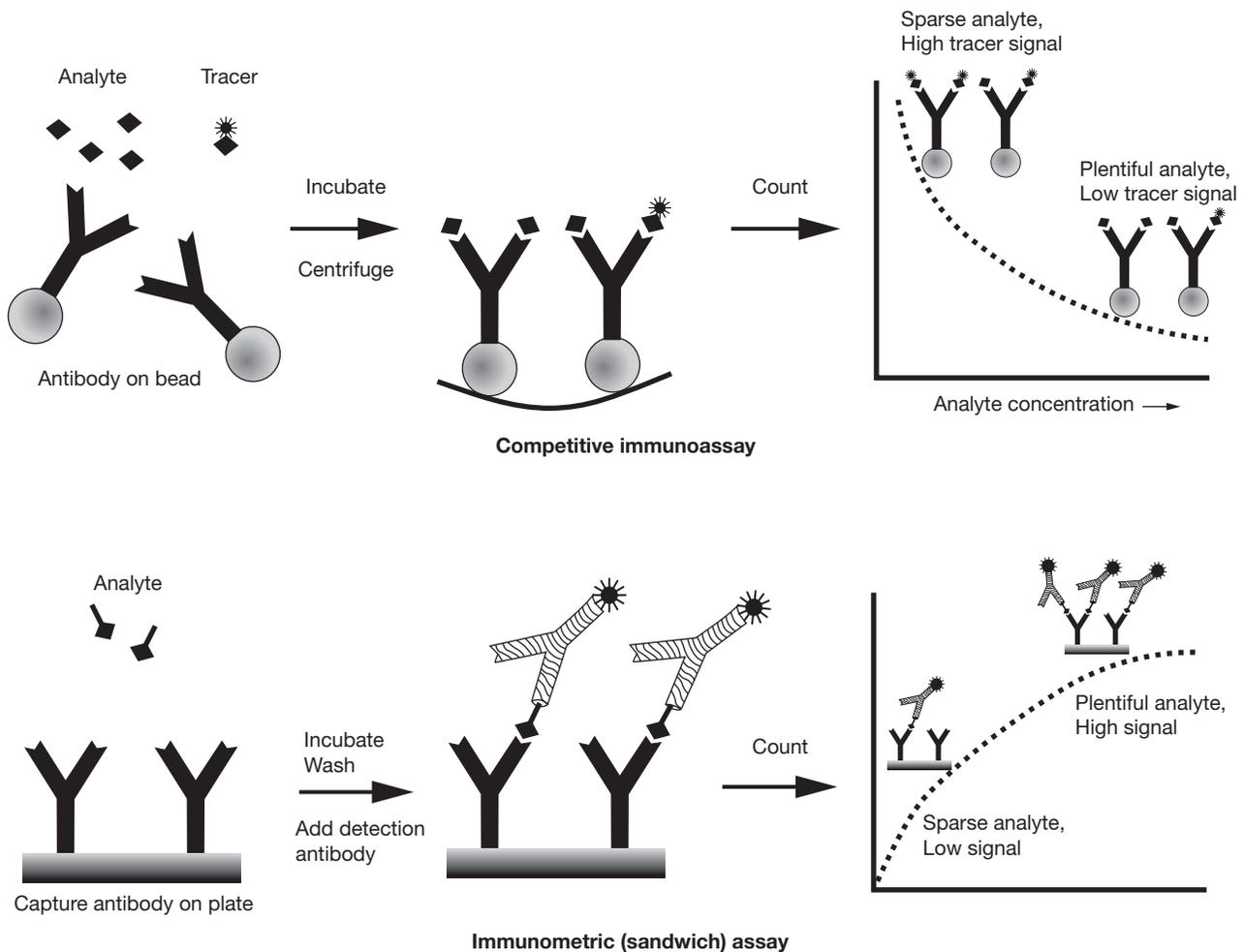


Fig. 4.7 Comparison of competitive versus immunometric (sandwich) assays. In competitive immunoassay (*top*), the labeled tracer (labeled analyte) and unlabeled analyte compete to bind to the assay antibodies. In the two-site immunometric method (*bottom*), the analyte of interest is sandwiched between the capture and signal antibodies.

higher activity signal, which is more easily detected even when analyte concentrations are very low. As signal detection technology continues to improve, increasingly sensitive immunometric assays can be developed. A third advantage of immunometric assays arises from the ability to use monoclonal antibodies, which can be more easily produced in quantity and have more predictable characteristics than polyclonal antibodies.

Limitations of Immunoassays

The ability of an antibody to bind a specific target with high affinity is remarkable, yet there are limitations. The small size and relatively poor immunogenicity of steroid molecules make it difficult to obtain an antibody that can clearly distinguish a specific steroid from other similar steroids. For example, an antibody raised against testosterone may show significant cross-reactivity with a conjugated form, such as testosterone glucuronide, or with the structurally similar dihydrotestosterone molecule. A solvent extraction step to remove water-soluble conjugates, such as glucuronides, coupled with a chromatographic step to separate out structurally similar molecules, will greatly improve the analytic specificity of the assay⁵ (Fig. 4.8A). The extraction step also separates testosterone from binding proteins that can interfere with accurate measurement.

Testosterone immunoassays that do not involve such extraction and chromatography steps, including the majority of assays found on most automated laboratory platforms, may

perform adequately at the higher levels found in adult males (>300 ng/dL) but will frequently measure inappropriately high values in the range most pertinent to women and prepubertal children (Fig. 4.8B). This overestimation (and poor low-end analytical sensitivity) is seen as well for other steroids, such as estradiol or 17-hydroxyprogesterone. Because in practice the lower values are more concordant with the clinical picture, endocrinologists caring for women and children have traditionally relied on immunoassays that involve extraction and chromatography steps, now largely replaced by assays involving chromatography and tandem mass spectrometry.

Free Hormone Assays

The concept that the “free” (not attached to any binding proteins) hormone level best reflects the endocrine status of a patient is still referred to as a *hypothesis*, because the clinical utility of free hormone assays varies from certain to speculative. A free T4 by equilibrium dialysis is clearly valuable for the evaluation of a patient with thyroxine-binding globulin (TBG) deficiency; a free testosterone may be helpful (although not always required) in some patients with findings not fully explained by the total testosterone level; and theoretically a free 25-hydroxyvitamin D (25-OHD) level might help explain why black Americans have lower average 25-OHD levels than white Americans, despite having a lower incidence of osteoporosis.⁶

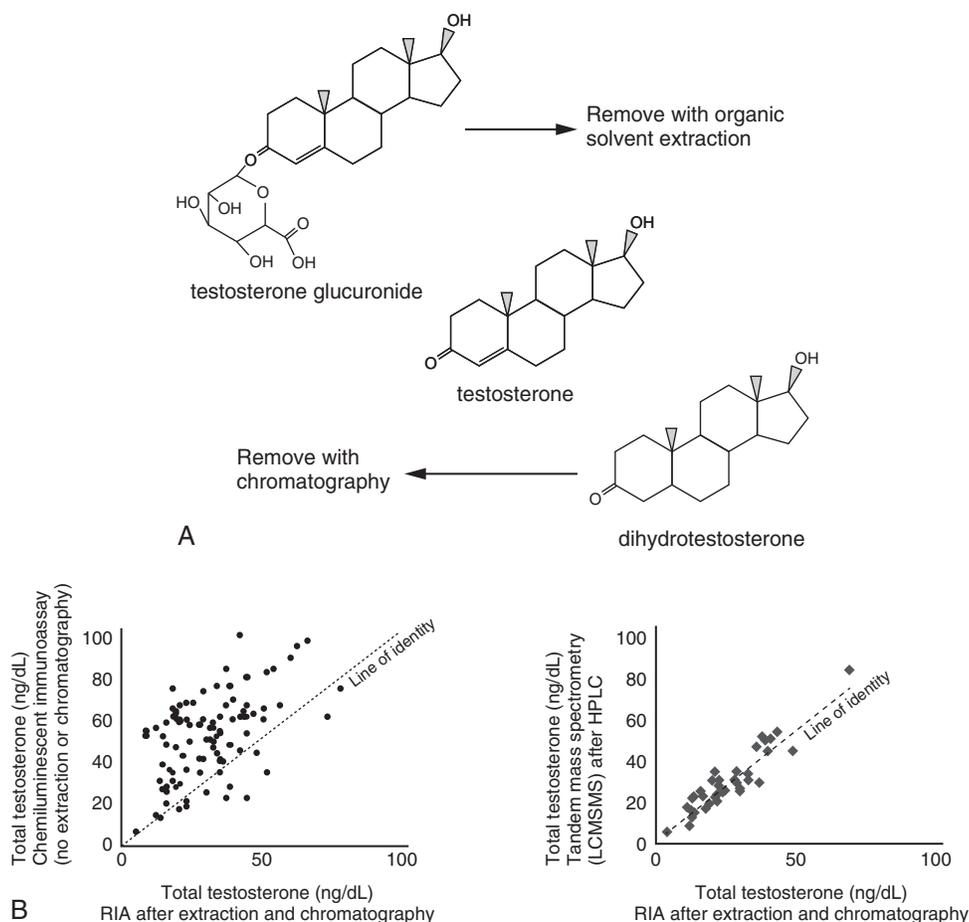


Fig. 4.8 A, Increasing analytical specificity via extraction and chromatography. After organic solvent extraction, water-soluble conjugates remain in the aqueous phase whereas steroid molecules generally remain in the organic phase. Similar steroid molecules can be separated by an additional chromatography step. **B**, Correlation of testosterone assays (extraction/chromatography/radioimmunoassay [RIA] versus nonextraction immunoassay or tandem mass spectrometry). Left panel: Steroid immunoassays without additional preparation steps before assay tend to give higher values than immunoassays after extraction and chromatography, likely because of steroid conjugates and compounds of similar structure (occasional lower values may reflect issues with incompletely removed steroid binding proteins). Right panel: Measurement of steroids by high-performance liquid chromatography [HPLC]-tandem mass spectrometry (MS) (which also involves extraction and chromatography) produces testosterone results very similar to those obtained with immunoassay after extraction and chromatography.

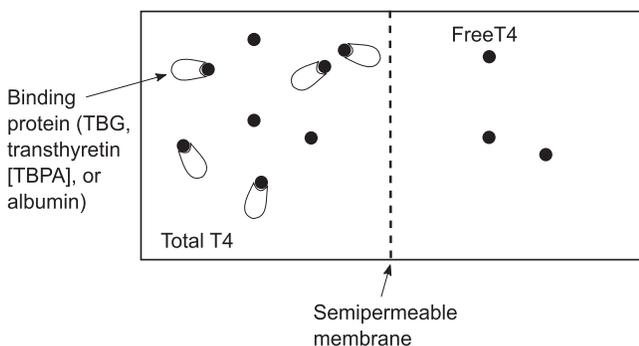


Fig. 4.9 Free T4 by direct measurement after equilibrium dialysis. See text for details.

Free T4 by direct measurement after equilibrium dialysis is the archetypal free hormone assay. As shown in Fig. 4.9, it involves incubation of a patient sample on one side of a dialysis buffer-filled chamber separated by a semipermeable membrane which free (but not bound) T4 can cross. After a period of time sufficient to reach equilibrium, the amount of free T4 can be directly measured by a highly sensitive detection

method (immunoassay using a very high-affinity antibody, or tandem mass spectrometry). This assay performs well at extremes of TBG (deficiency or excess), likely better than some of the alternative analogue-based assays described later. Disadvantages include a longer waiting time (turnaround time) for results, expense because of the additional labor required, and sensitivity to substances like nonesterified (free) fatty acids (NEFA) that can displace T4 from its binding proteins. The last issue can be seen when samples for free T4 are drawn through heparinized lines; heparin in the sample tube can accelerate the release of NEFA from triglycerides in the sample, artifactually elevating the measured free T4 level. Even in the absence of a heparin effect, there may be subtle to significant effects of NEFA on the free T4 by dialysis assay in patients who have hypertriglyceridemia, particularly if they have low thyroid hormone binding capacity (low TBG or very low albumin).

Free T4 and free serum cortisol are amenable to direct measurement after dialysis, because total T4 and total cortisol circulate in the micrograms per deciliter range. In contrast, total testosterone and total triiodothyronine (T3) are measured in the nanogram per deciliter range, with the resultant free hormone levels below what can be measured dependably by current immunoassays or mass spectrometry. A different approach (indirect measurement by tracer equilibrium dialysis) is thus

required. As shown in Fig. 4.10, the patient sample is placed in the dialysis chamber along with a known amount of radioactively-labeled hormone (the tracer). Some tracer is bound and some remains free. After equilibrium, the amount of radioactivity that has crossed the dialysis membrane (representing the fraction of tracer which remains free) can be measured to determine the “percent free hormone.” The total hormone (total T3 or total testosterone) level can be multiplied by this percent age of free hormone to get the resultant calculated free hormone level.

Because the dialysis-based free hormone assays are labor intensive and not amenable to automation, alternative “direct” approaches have been developed. The most common is free T4 by the labeled analogue method (Fig. 4.11). A labeled T4 analogue that does not bind to any of the TBGs (TBG, TBPA, albumin) is added to the patient sample; this analogue competes with the patient’s free T4 for the T4 antibodies (chosen to bind only free T4 molecules), with the resultant free T4 determined as with any competitive immunoassay (more signal from the bound analogue corresponds to less free T4 in the patient sample).

A clever variation involves labeling the antibody rather than an analog of free T4 (Fig. 4.12). T4 immobilized on a tube wall or plate well competes with the patient’s free T4 for the labeled

antibody. After incubation and a wash step, only the antibody bound to the immobilized T4 remains and the signal measured (as with any competitive immunoassay, more signal corresponds to less free T4 in the patient sample).

These direct assays are fast, relatively inexpensive, and easily automated. They have therefore become the dominant free T4 assays, displacing older approaches, such as calculation of the free T4 index. One concern is that the performance of the analogue assay is dependent upon the assumption that the analogue does not interact at all with TBG or other binding proteins, an assumption that may not be completely true, especially when TBG levels are high, or binding protein affinities are increased. These assays may therefore perform poorly at the extremes of thyroid hormone binding capacity (e.g., very low or very high TBG levels, extremely low albumin levels as in nephrotic syndrome, or in patients with altered thyroid hormone binding protein affinity). In general, the direct assays tend to underestimate free T4 when TBG or albumin levels are low⁷ and overestimate when binding protein levels are high. Nevertheless, analogue-based direct free T4 assays are widely accepted for general use, in contrast to the analogue free testosterone assays, which are specifically not recommended by the Endocrine Society.⁸

Mass Spectrometry

Mass spectrometry (MS) represents a detection paradigm completely different from antibody-based methods. It is a well-established method to achieve very high analytical sensitivity and specificity and accurate quantitation for small molecules like drugs, steroids, catecholamines, and various inorganic compounds. Using MS, proteins can be quantitated by measuring the amount of a “signature” peptide fragment after proteolysis, or (with more recent technological advances) by direct measurement of intact proteins up to about 20 kDa in size.

A typical mass spectrometry assay (Fig. 4.13) might include the following steps:

- Initial purification: most commonly an extraction step to remove binding proteins or other interfering substances from

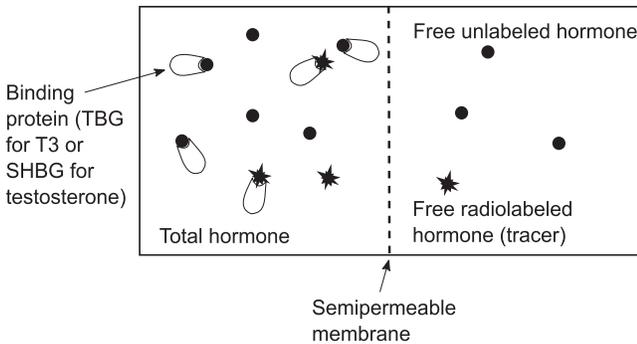


Fig. 4.10 Free hormone (e.g., free T3 or free testosterone) by tracer equilibrium dialysis. See text for details.

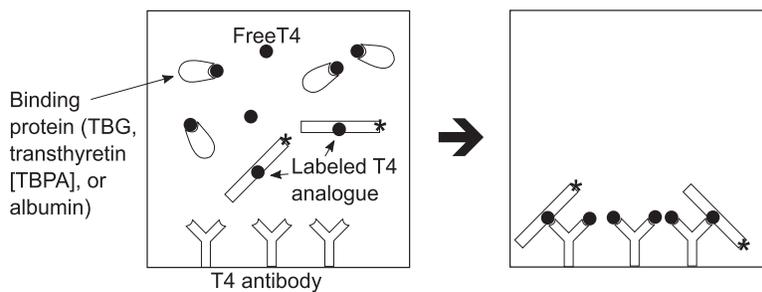


Fig. 4.11 Free T4 (nondialysis) by labeled analogue method. This method depends upon a T4 analogue that binds to the assay T4 antibody, but not to thyroxine-binding globulin (TBG). See text for further details.

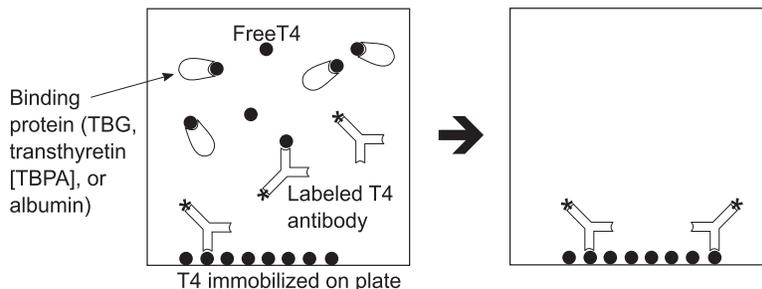


Fig. 4.12 Free T4 (nondialysis) by labeled antibody method. See text for details.

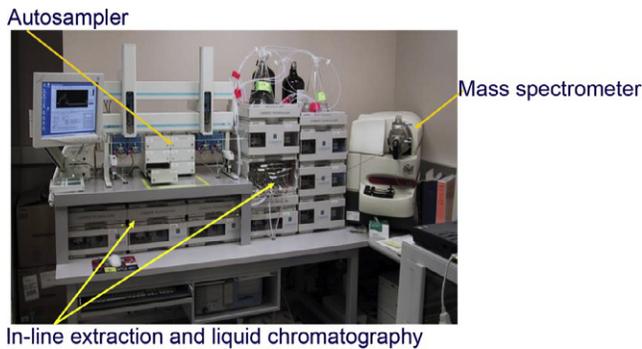


Fig. 4.13 An older but still representative setup for a quantitative high-performance liquid chromatography-tandem mass spectrometry assay. Note the extensive front-end equipment for the extraction and chromatography steps, with the mass spectrometer taking up only the small space at the extreme right.

the target analyte. Many clever techniques have been used to reduce the complexity of the overall sample and enrich the concentration of the target analyte.

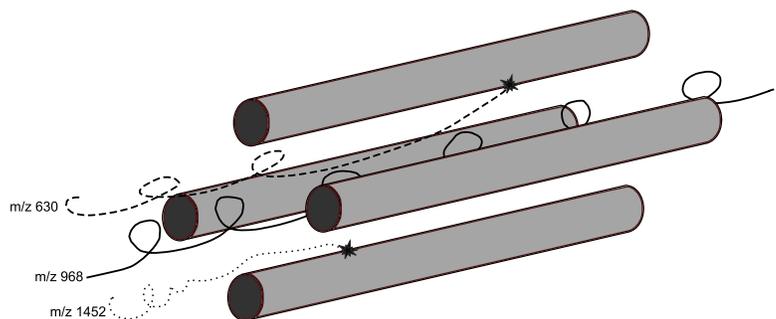
- Derivatization is a chemical modification of the target analyte that can make it easier to analyze accurately during the later detection step.
- Chromatographic separation, for example, gas chromatography (GC) or liquid chromatography (LC). GC typically uses very long columns and has the ability to resolve many different analytes with great accuracy, although it is both slow and generally not automation-friendly. LC is both faster and automatable, with high-performance liquid chromatography (HPLC) or ultraperformance liquid chromatography (UPLC) most commonly used. The target analyte has a typical retention time, which is the time between injection of the sample into the chromatography system and the detection of the analyte peak.
- Ionization of molecules in the partially purified mixture, typically using one or a combination of the following: heat, ion bombardment, high voltage, laser bombardment, or chemical ion transfer. The target analyte will have a characteristic mass to charge (m/z) ratio after ionization. The ionization techniques most commonly used in the clinical laboratory (because of compatibility with liquid chromatography systems) are electrospray ionization (ESI) for larger or polar molecules like peptides and proteins, and atmospheric pressure chemical ionization (APCI) for smaller, nonpolar molecules like steroids. Matrix-assisted laser desorption ionization (MALDI) is less often seen in the clinical chemistry/endocrinology laboratory because of difficulties in automation and achieving precise quantitation, but it has been invaluable in the microbiology laboratory to produce peptide fingerprints characteristic of different microorganisms.

- Mass analyzer separation of the ions produced earlier according to their m/z ratio. This can be accomplished by first accelerating the ions in an electrical field and then separating out the desired analyte ions using any one of several techniques. For endocrine testing, the most commonly encountered techniques are:
 - Quadrupole mass filtration: ions trace a complex path in an oscillating electrical field between four cylindrical rods (Fig. 4.14). The frequency of the oscillation can be tuned so that only ions with the desired m/z ratio make it to the end (where they will proceed to the detection step) without colliding with one of the rods, ending its journey.
 - Ion traps: ions are corralled in an oscillating electrical field that can be tuned to eject ions of a specific m/z ratio so that they reach the detector. They may allow higher resolution of different molecular weights than can be achieved by the typical quadrupole system.
 - Time of flight (TOF): all ions are subjected to the same electrical field, with lower molecular weight ions reaching the detector earlier than their heavier counterparts. These systems may allow analysis of larger mass ions than can be easily handled with the other approaches.
- Detection of ions (ion intensity) corresponding to analytes of interest within the desired m/z range (Fig. 4.15). Compounds can be further distinguished based on when they elute from the chromatography column (retention time).
- Data processing to convert the ion intensity data into quantitative laboratory results.

Further improvements in analytical specificity result when two mass analyzers are placed in tandem (Fig. 4.16). In a triple quadrupole tandem mass spectrometer (MS/MS), the first quadrupole mass analyzer/filter separates the initial molecular ions, of which only the ions with the desired m/z ratio are allowed to proceed onward. In the second chamber, where a quadrupole accelerates the ions but does not filter them, the selected ions collide with molecules of an inert gas, such as argon, breaking up these "precursor ions" predictably into characteristic fragments ("daughter ions"), which act like a molecular fingerprint for the analyte of interest. These daughter ions proceed to the third chamber, where a second mass analyzer/filter allows only ion fragments with a particular m/z ratio to pass onward to the detector.

Although the most important feature of MS for endocrinologists is the high analytical specificity, there are additional capabilities of interest. One of these is the ability to measure multiple analytes simultaneously, known as *multiplexing*. Immunoassays can be multiplexed as well; however, such assays tend to be much more difficult to design and often show undesirable interactions among the multiple different antibody-antigen reactions taking place in the same specimen tube. In contrast, provided that the analytes tolerate reasonably similar chromatographic and ionization conditions, multiple MS measurements can be taken from the

Fig. 4.14 Quadrupole mass filtration. An oscillating electric current is chosen to allow only ions with a certain mass to charge (m/z) ratio to pass through the quadrupole. Other ions trace an unstable path and collide with the quadrupoles, ending their journey.



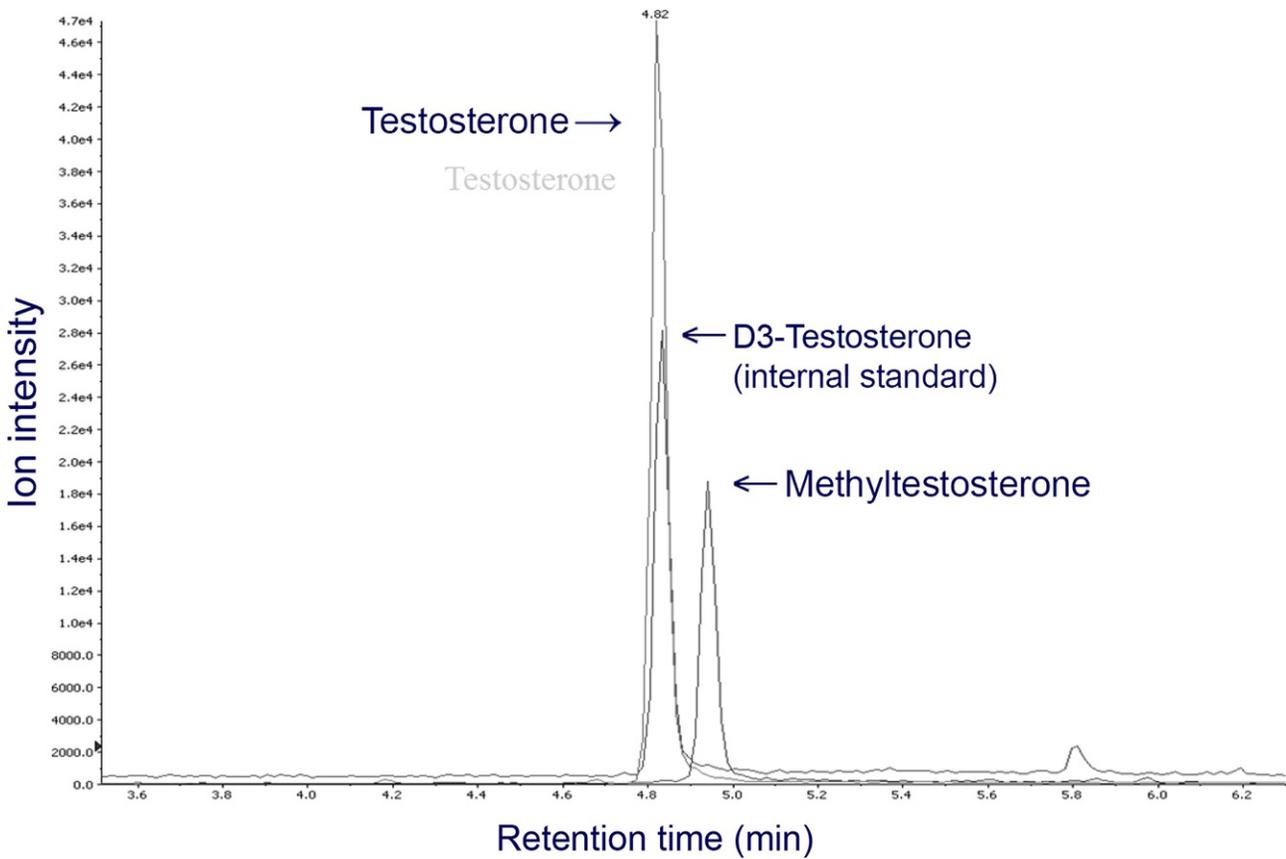


Fig. 4.15 Mass spectrometric quantitation of testosterone. Testosterone can be measured quantitatively by comparison to a known amount of an internal standard (deuterium-labeled testosterone that has a slightly higher mass and retention time compared with native testosterone) diluted into the sample. Methyltestosterone can also be measured separately, because of its distinctly higher mass and longer retention time on the chromatographic column before it reaches the mass spectrometer.

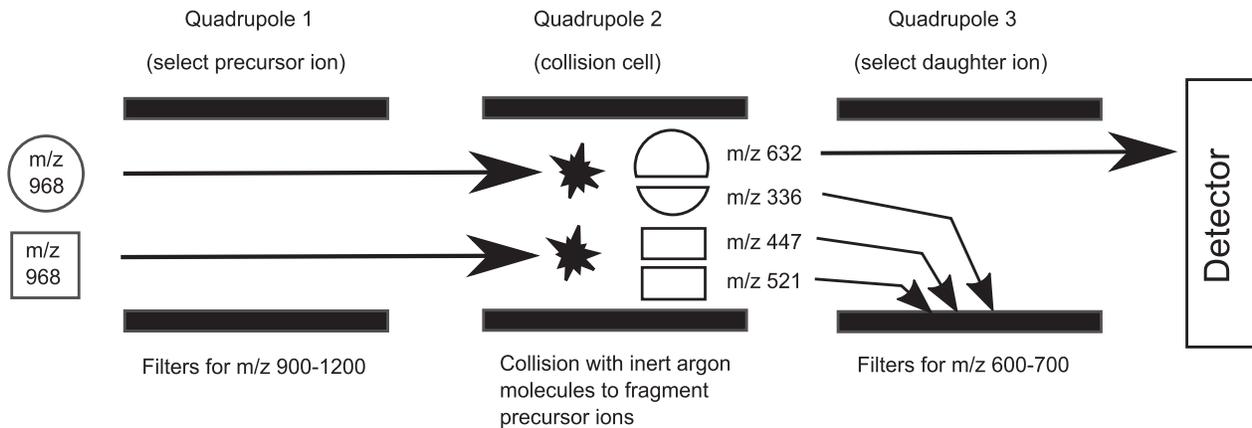


Fig. 4.16 Triple quadrupole configuration to achieve high analytical specificity. Even if two compounds have identical mass to charge (m/z) ratios and similar chromatographic retention times, they can be distinguished by measuring their unique “daughter ions” after a fragmentation step.

same sample without cross-interferences, greatly reducing the sample volume required, for example, for a panel of adrenal steroids.

Over the past several years, mass spectrometers have become increasingly able to handle larger and larger molecules at increasingly high resolution. Accurate quantitation of many peptide and protein hormones is now available. The earliest approach, which is still required for very large proteins, such as thyroglobulin (Tg, 660 kDa), involves thorough proteolysis and quantitation of an invariant “fingerprint” peptide fragment. This method, known informally as *bottom-up* proteomics,

is complemented by the more recent use of top-down proteomics,⁹ where smaller proteins (up to 20 kDa, with the upper limit continually increasing) can be directly analyzed and quantitated in their intact state (no proteolysis required). This top-down proteomic approach is particularly exciting, given that it allows examination of proteins in their native states, including their posttranslational modifications (e.g., glycosylation, sialylation, etc.). Thus there is the possibility of defining specific protein isoforms (perhaps the most bioactive ones?) for quantitation, if endocrine researchers can define which forms should be measured.

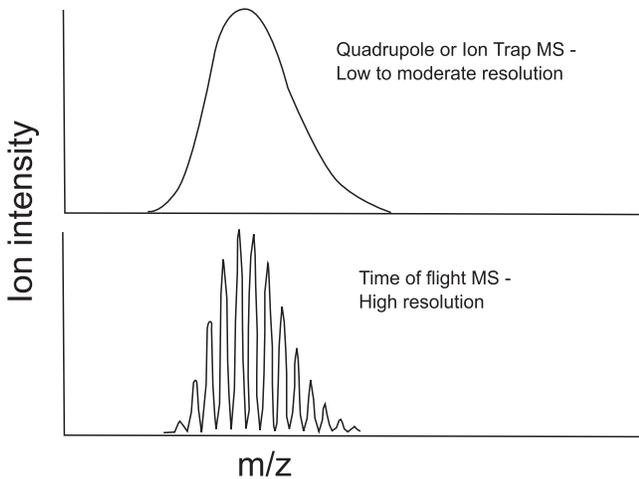


Fig. 4.17 High-resolution mass spectrometry reveals multiple peaks corresponding to proteins with different amounts of naturally occurring stable isotopes.

Top-down proteomics requires the use of mass spectrometers with high resolution, because the method of identifying a protein relies on an “isotopic fingerprint.” With a lower resolution instrument, a small protein like insulin (5.8 kDa) might appear as a single gently rounded peak whose identity as insulin is not 100% certain. As the resolution of the mass spectrometer increases, this peak is revealed to be composed of multiple sharper peaks, all with slightly different m/z ratios (Fig. 4.17). The reason that insulin does not have a single peak at one m/z ratio is because some of the insulin molecules contain naturally occurring stable isotopes like ^{13}C rather than ^{12}C and thus have a slightly higher mass. Based on the knowledge that about 1.1% of all carbon atoms are ^{13}C , and that insulin has 257 carbon atoms, one can use probabilities to calculate how many insulin molecules are likely to have 0, 1, 2, 3, or more ^{13}C . One can do the same for less abundant natural isotopes, such as ^{15}N , ^{18}O , and ^{34}S , and thus predict mathematically the distribution and ion intensity of insulin peaks at various m/z ratios, with experimental profiles matching quite closely (Fig. 4.18). These profiles act like fingerprints that identify the molecule definitively as insulin.

Isotope	Mass [Da]	% Abundance	Isotope	Mass [Da]	% Abundance
^1H	1.007825	99.985	^{16}O	15.994915	99.76
^2H	2.014102	0.015	^{17}O	16.999131	0.038
^{12}C	12. (exact)	98.90	^{18}O	17.999159	0.20
^{13}C	13.003355	1.10	^{31}P	30.973763	100.
^{14}N	14.003074	99.63	^{32}S	31.972072	95.02
^{15}N	15.000109	0.37	^{33}S	32.971459	0.75
			^{34}S	33.967868	4.21

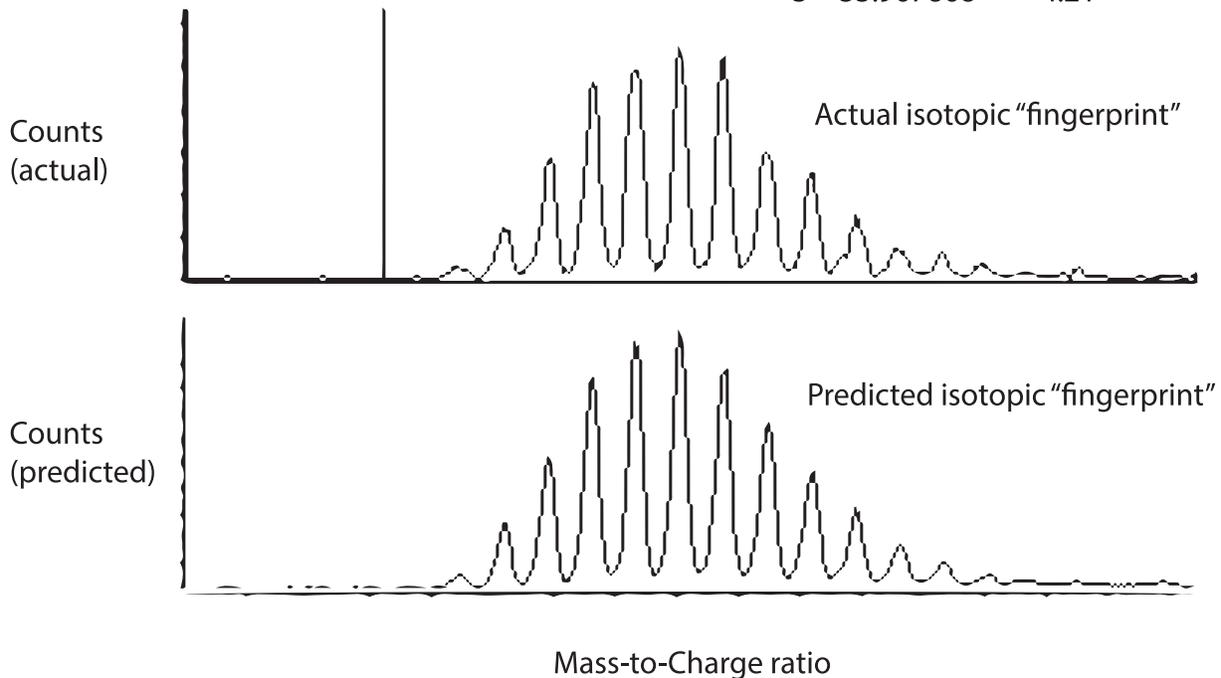


Fig. 4.18 Predicted versus actual high-resolution mass spectrometry “isotopic fingerprint” of human insulin.

WHAT CAN GO WRONG? ERRORS, INTERFERENCE, AND THE LIKE

Errors can occur at any point in the diagnostic testing cycle, even before the sample is actually collected.

- “Preanalytical” refers to anything occurring before the actual testing of a sample (complemented by “analytical” and “postanalytical”). Because an estimated half to two-thirds of errors affecting laboratory test results occur in the preanalytical phase,¹⁰ it is important for the clinician to understand the many things that can go wrong even before testing starts.

One of the first questions to ask is whether the right test was ordered for the right patient at the right time. For example, a serum free cortisol drawn at 4 PM on a patient with suspected adrenal insufficiency has a high probability of being uninterpretable, both because of the odds of a noninformative low value (could easily be a normal finding) and because normative data using free cortisol is much more limited than that for total cortisol (particularly in pediatrics). This might be marginally more useful for a patient with suspected Cushing syndrome/disease, but even then better testing approaches exist. For some tests, there is no technical/physiological reason that an afternoon sample cannot be used—except that, if all normative data available are based on fasting morning samples or first morning urine voids, then interpretation will be that much more difficult.

The right test issue is particularly important for pediatric endocrinologists, who fortunately are usually well trained to seek out assays (at least for the most commonly used core tests) that most accurately answer their clinical questions. The issues surrounding accuracy of testosterone or estradiol assays in the low ranges typical for children has been well known by subspecialists for decades, yet to date, there is little or nothing in the U.S. Food and Drug Administration guidance or the College of American Pathologists checklists (used to assess laboratory quality) to discourage the use of testosterone immunoassays that do not involve extraction and chromatography steps (see Methodology section) to ensure accuracy of results in the lower ranges typically seen in children (note: this also applies to measurement of testosterone in adult women, or estradiol in men, so it is not a uniquely pediatric concern). A clinician must know enough about the laboratory tests ordered to know when to ask for something different than the standard offering on a laboratory form, and knowing the right test to order may not always involve asking for the gold standard version of a test; for example, when

screening a basically healthy obese patient for thyroid dysfunction, measuring a free T4 by equilibrium dialysis may be overkill, when the faster, more readily available, and likely much cheaper nondialysis free T4 may suffice (assuming that the endocrinologist knows the limitations of this test).

Was the correct sample type/collection tube type used? For many analytes, there are significant differences in serum versus plasma values, or between EDTA versus sodium or lithium heparin plasma values. A serum sample inadvertently sent for measurement of plasma renin activity (a surprisingly common occurrence) will show apparently suppressed values (one can easily test for this possibility by asking the laboratory to check the calcium level in the sample, which should be low in the proper EDTA plasma sample). Another very common issue is the use of serum separator (SST) tubes, which may cause interferences or affect measured levels of some steroids (e.g., estradiol and testosterone),¹¹ fat-soluble vitamins (vitamin A and vitamin D metabolites), and hydrophobic drugs. Because SST tubes are much more convenient for collection purposes (less waiting time for clotting of the sample, and much less labor involved in separating the serum from the cell mass), they tend to be the “default” tube type for serum collection; therefore those collecting the sample must pay close attention to specimen requirements when the tube can potentially affect test accuracy.

Were there factors during the sample collection process that could affect the results? Examples include hemolysis from a difficult blood draw (releasing insulin-degrading enzyme from red cells) or contamination of an antecubital puncture site by topical testosterone (potentially raising testosterone levels to many hundreds or even thousands of ng/dL). Another example would be elevation of free T4 by equilibrium dialysis results when samples are drawn through a heparinized line; heparin stimulates lipoprotein lipase to degrade triglycerides into NEFA + glycerol, with the NEFA displacing T4 from TBG in the sample tube.

Were there any issues in the processing, storage, and transport of samples after collection? For example, serum phosphoethanolamine (PEA) levels can be increased 2.7-fold in samples where the serum remains in contact with blood cells for 6 hours, instead of being separated within an hour; PEA levels can drop by 80% if properly separated serum is allowed to remain at room temperature for 24 hours.¹² Serum potassium increases dramatically when whole blood samples are refrigerated for as short a time as 6 hours before being spun and processed (Fig. 4.19), as compared with samples left at room temperature for the same period of time.

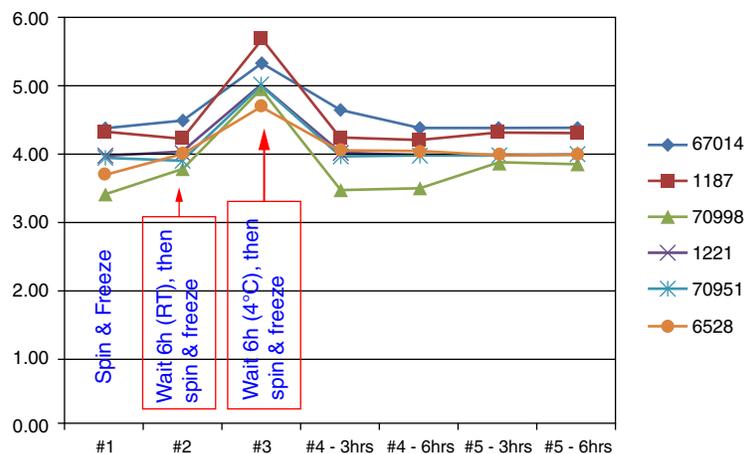


Fig. 4.19 Effect of refrigeration on release of potassium from intact red blood cells into serum. Six healthy volunteers had blood drawn into plain red top (no additives) tubes, with one aliquot (#1) centrifuged immediately and the serum frozen right away. Aliquot #2 was allowed to stand for 6 hours at room temperature (20° C) before centrifugation and frozen storage of serum. Aliquot #3 was placed in the refrigerator (4° C) for 6 hours before centrifugation/serum storage. All subjects showed a significantly higher serum potassium in aliquot #3. (Data from Quest Diagnostics Nichols Institute, San Juan Capistrano.)

Is there any chance of a sample switch/mix-up anywhere along the process from collection to reporting of a result? The implications of such an event on test accuracy are obvious.

Potential Confounders in Immunoassays

Sandwich immunometric assays are potentially vulnerable to what has been termed the *hook* or *prozone* effect that can lead to falsely normal or low values in the presence of large amounts of the analyte (Fig. 4.20). In general, as analyte concentration increases, more antibody-analyte-antibody sandwiches are formed, leading to more signal detected. At extremely high analyte concentrations, however, it is possible for both capture and detection antibodies to be saturated with analyte before formation of a sandwich complex, leading to the detection antibody being washed away and the low signal being misinterpreted as a low concentration of analyte. This phenomenon is not seen with competitive immunoassays. To minimize the chances of a hook effect, most immunoassays have now modified their methods as follows: analyte is allowed to bind to capture antibody, a thorough wash step removes excess unbound analyte, and only then is the detection antibody added. In the now extremely rare situation where a hook effect is still suspected, serial dilutions of the sample may be performed to see if the

apparent concentration of the analyte actually rises with increasing sample dilution.

There is some inconsistency in the literature around the terms *heterophilic antibody* and *human antianimal antibody* (HAAA) or *human antimouse antibody* (HAMA). Some authors use these terms interchangeably; others reserve the term *heterophilic* for low-affinity antibodies that spontaneously arise against multiple, poorly defined antigens and use HAMA or HAAA only for those patients who develop specific high-affinity antibodies because of treatment with a murine monoclonal antibody or because of recurrent exposure to a particular animal species (e.g., scratches endured by a rabbit handler). Regardless of the term used, any given patient may have antibodies present that bind to and interfere with animal (mouse monoclonal; rabbit, goat, or donkey polyclonal) antibodies used in a particular immunoassay,¹³ even without previous exposure to that particular animal. These antibodies can bridge the capture and detection antibodies in an immunoassay, as shown in Fig. 4.21 (left panel), creating a sandwich and a high signal (false elevation) even in the absence of analyte. Alternatively, they could attach at the binding site of the capture antibody and prevent binding of the analyte (Fig. 4.21, right panel), eliminating formation of the sandwich, and causing a falsely low value. Competitive immunoassays are less often affected, although

Fig. 4.20 Hook (prozone) effect leading to falsely low results. Extremely high analyte concentration leads to occupancy of all binding sites without creating the assay “sandwich” configuration. Signal antibody is washed away and the low signal is interpreted as a low concentration of analytes (*dotted line*). Dilution of sample may lead to increasing signal and an apparent increase in the measured analyte concentration.

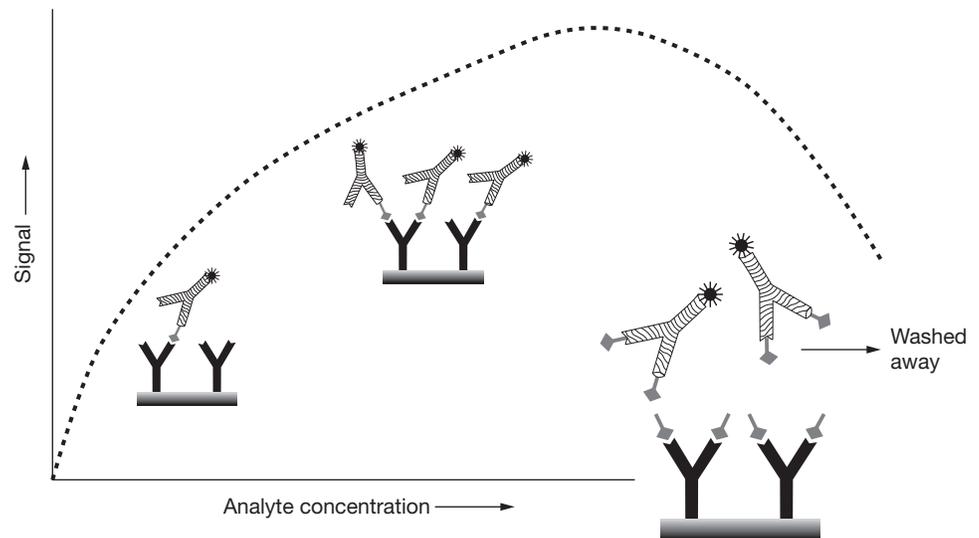
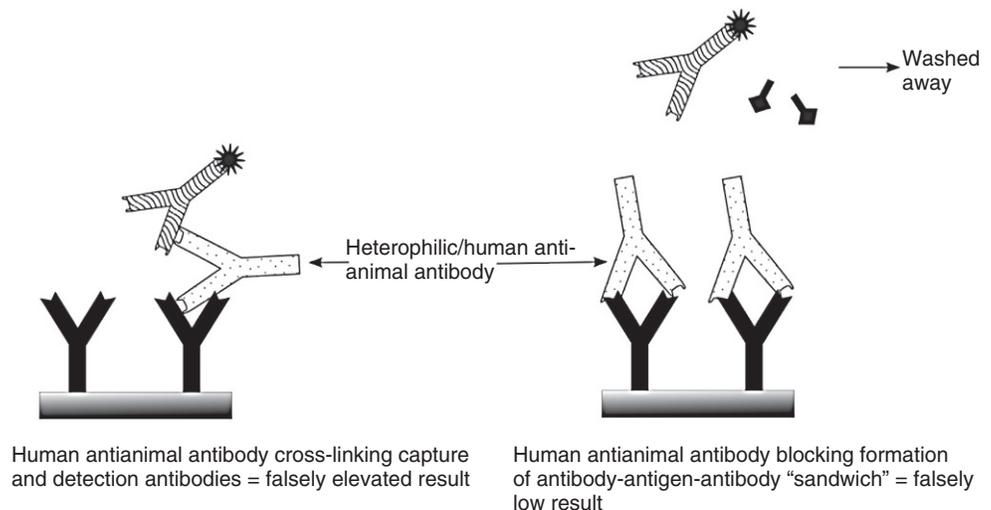


Fig. 4.21 Mechanism of immunoassay interference by heterophilic or human antianimal antibodies. See text for details.



they may show falsely high values if the interfering antibody outcompetes the assay antibody for tracer, or falsely low values if they bind preferentially to the analyte over the tracer.

When a heterophilic/HAAA/HAMA effect is suspected, the sample should be assayed on a different immunoassay platform because the interference is often specific for one manufacturer's assay but not another's. Only rarely¹⁴ does a heterophilic antibody interference affect multiple assays or different platforms. Such a maneuver is often the quickest to accomplish, but the sending laboratory must ensure that the second laboratory is truly using a different platform. Another option is to use heterophilic antibody blocking agents, which are a mixture of animal immunoglobulin fragments or other proprietary agents that soak up the interfering antibodies. A disadvantage of this approach for pediatrics is that a significantly larger volume of sample is required to assay both with and without use of the blocking agent. A third, more labor-intensive option is to assay the sample on serial dilutions, as those showing antibody interference effects tend not to show linear responses on dilution. Of note, the last option also requires previous proof (validation) that the assay continues to show a linear response on diluted samples, proof that is not always available.

Specific autoantibody interferences appear to be rare compared with the heterophilic/HAAA/HAMA interferences described in the previous section, with the notable exception of Tg antibody (TgAb) in patients with thyroid cancer. TgAb is present in about 20% to 25% of thyroid cancer patients and leads to false depression of Tg levels by immunometric assay (e.g., Tg-ICMA). In addition, different Tg antibody assays may report discordant (negative vs. positive) results in some cases. Although a competitive assay, such as Tg-RIA, is less often affected by TgAb interference than is the Tg-ICMA, if the autoantibody is of sufficiently high affinity to outcompete the assay antibody, a Tg-RIA may show false elevation in the presence of such TgAb.¹⁵ Overall, any Tg immunoassay result, whether performed by Tg-ICMA or Tg-RIA, must be viewed as potentially affected in a patient with TgAb present (and even possibly in patients whose TgAb is reportedly negative). Helpful proxies for Tg include following TgAb titer, which should fall over time in patients without cancer recurrence.¹⁶ Measurement of Tg concentration by LCMS is the method of choice for profiling Tg levels for thyroid cancer monitoring in the presence of anti-Tg antibodies. Imaging studies may also help assess the probability of recurrence. Although rare, autoantibodies may be of sufficiently high affinity to affect both competitive and immunometric assays alike. Consequently, they would be less likely to show differences when assayed on different platforms and are not affected by heterophilic antibody blocking agents. In these scenarios, diagnosis may require

measurement of specific autoantibody (e.g., a parathyroid hormone [PTH] antibody assay in someone with a discordantly high or low PTH level).

STANDARDIZATION/HARMONIZATION: CLINICALLY RELEVANT AND CRUCIAL

A frustrating reality is that different versions of a test with the same name more often than not give different results, sometimes surprisingly so. Sometimes the issue is methodological, related to differences in sample purity and analytical specificity, as we saw with testosterone immunoassays run directly on serum, versus mass spectrometric measurement of testosterone after extraction and chromatography. In other cases, it may relate to the complex biology of an analyte, such as human chorionic gonadotropin hormone (hCG), with at least seven different molecular isoforms (intact, nicked, hyperglycosylated, free subunits, beta core fragments, and more); the result of an hCG immunoassay will therefore depend upon what epitopes the assay antibodies recognize and what reaction conditions were used. In some cases, differences appear because of assay design: for example, an assay designed specifically to detect only full-length osteocalcin will be much more vulnerable to differences in processing, handling, and storage, as opposed to an assay targeting the more stable midmolecule region.

A premiere example of assay differences relevant to pediatric endocrinology is that of GH. Many of the early studies performed to establish diagnostic cutoffs for GHD used a competitive immunoassay with polyclonal antibodies. When a new monoclonal antibody-based immunometric (IRMA) GH assay was introduced by Hybritech, GH results were typically 60% of those seen in the original assay, leading to more patients diagnosed with GHD if the same diagnostic cutoffs were used. Although it was assumed that the lower values were caused by the higher analytical specificity of an immunometric format and monoclonal antibodies, this was not the entire answer, as another immunometric/monoclonal antibody GH assay (Wellcome IRMA) actually gave results about 30% higher than the original competitive/polyclonal GH assay,¹⁷ showing that other factors (e.g., use of animal versus human serum in the assay diluents, ionic strength of assay solutions, temperature and time of incubation) made a significant difference as well.

Another factor that contributes (even today) to GH assay differences is varying detection of alternate growth hormone isoforms.¹⁸ Although 22-kDa GH is the dominant isoform (90% of total GH), the other 10% includes 20-kDa GH and a small amount of other forms. To put this into perspective schematically, imagine that you are asked to count the number of stars in the box shown in Fig. 4.22. Unless everyone agrees on

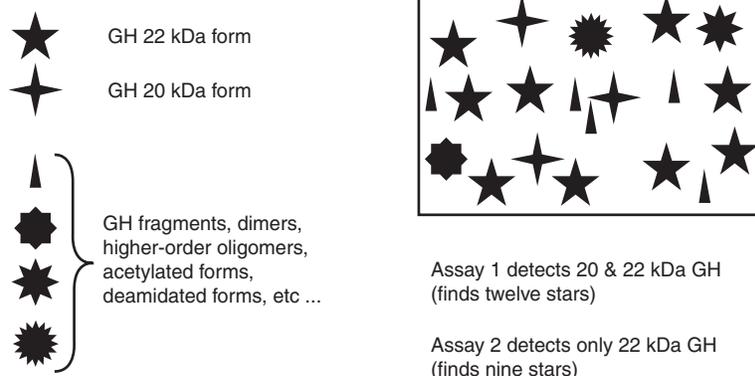


Fig. 4.22 How many stars are in the box? This shows the futility of quantitation if definitions are not first agreed upon.

the definition of a star (5-pointed only?) before counting, different observers may get different totals. The take-home lesson is that endocrinologists as a group need to define exactly what they want to measure, if there is any hope for getting assays to agree more closely in the future.

To convert a laboratory assay measurement (e.g., counts per minute, relative light units, or peak heights) to a quantifiable amount of hormone requires use of calibrators—known concentrations of the hormone being measured that can be measured along with patient samples. But how can we make certain that calibrators are similar among different assays?

The best available solution at present is to provide laboratories and diagnostic kit/instrument manufacturers with well-characterized reference materials. These reference materials should themselves be traceable back to a standard reference material (SRM) or international reference preparation (IRP), which is ideally as pure as possible and whose concentration is as exact as possible. The SRMs are often provided by organizations, such as the National Institute of Standards and Technology or the World Health Organization (WHO); SRMs from the WHO are often called *international standards* (ISs).

Several years ago, almost all GH assays switched to calibrators composed of pure recombinant 22-kDa GH (WHO International Reference Preparation 98/574). It was hoped that this would bring assays more closely in agreement. This did help somewhat, but it is not a definitive solution. For example, looking at three common GH assays all using IRP 98/574 as the reference standard for assay calibrators, there are still substantial differences. The GH assay manufactured by Siemens on the Immulite platform has varying degrees (40%–77%) of cross-reactivity with 20-kDa GH. The Beckman Coulter GH assay on the Access platform has essentially no 20-kDa cross-reactivity and gives results about 76% of those seen on the Immulite. The Roche Elecsys assay, with substantial cross-reactivity with 20-kDa GH runs about 11% higher than the Immulite.¹⁹

If GH assays do not agree, how can we establish universal diagnostic thresholds? One European group has proposed different platform-specific GH assay cutoffs (e.g., peak GH 7.77 ng/mL for the Immulite and 5.15 ng/mL for the Beckman Coulter Access/DxI platform) for GH stimulation testing, an approach that has not yet gained wide traction.²⁰

An alternative to assay-specific cutoffs is to standardize or harmonize the different assays:

- Standardization means getting assays to agree by clearly defining the analyte to be measured (e.g., 22 kDa GH rather than all GH isoforms), having a well-defined pure reference preparation (e.g., IRP 98/574), and a gold standard method to which all other methods can be compared. Only a handful of assays can meet these criteria.²¹
- Harmonization is a less stringent approach to improve assay agreement by choosing arbitrary reference materials (even if not pure and well defined) and designating a specific assay as the reference standard, even if not truly a gold standard method. Luteinizing hormone would be a good candidate for harmonization, but not standardization, given that it has dozens of different isoforms (differing by degree of sialylation, glycosylation, or sulfation),²² no pure reference preparation available, and no gold standard assay. Typically for a harmonization effort, a central laboratory prepares pools of sample with a defined value and distributes aliquots of this pool to other laboratories, who can then adjust their calibration to match these values.

There are active working groups within the international clinical chemistry community seeking to standardize assays for insulin (working with the American Diabetes Association), vitamin D metabolites, free T₄/TSH, and some bone markers

(C-telopeptide and procollagen type 1 N-terminal propeptide). The difficulty in accomplishing these tasks is illustrated by the Insulin Standardization Workgroup's greater than 15 years of working on this project²³ (badly needed given that insulin assays can vary easily by up to twofold at the high end and perhaps much more at the low end).²⁴ Current standardization and harmonization efforts can also be tracked at the website of the International Consortium for Harmonization of Clinical Laboratory Results (www.harmonization.net). Currently listed are:

- Alkaline phosphatase, alpha fetoprotein, C-peptide, ionized calcium, cortisol, fructosamine, glucose, GH, hemoglobin A1c, hCG, insulin, lipoproteins/triglycerides, magnesium, PTH, phosphate, T₄, free T₄, and free T₃.

There are also less-formal initiatives for harmonization of common steroid assays (testosterone and estradiol). Pediatric endocrinologists with clinical or research interests in any particular analyte may want to monitor the progress and decision-making of these working groups.

REFERENCE INTERVALS

Reference interval (RI) is the term preferred by laboratory professionals for what is more commonly referred to as a *reference range*, preferred because of the fact that range is formally defined in statistics as a single number (serum sodium may have an RI of 135–145 mmol/L, but the range is 10 mmol/L). Whatever terminology is chosen (as long as one does not use the term *normal range*!), the importance of this concept is obvious for the practice of pediatric endocrinology. What may not be as obvious is how surprisingly fragile are many of the existing RIs for frequently used tests, many of which are based on small numbers of subjects, older/different methodologies, or literature references that may or may not be applicable to the test in question. Also not apparent to most, are the number of subjective decisions that must be made when planning RI studies, analyzing the data, and deciding on the final RI citations. While good clinical judgment can compensate for the current limitations of RIs, it is critical that the pediatric endocrinology community participate with laboratorians, statisticians, and even regulators to ensure that the RI situation constantly improves, rather than worsens.

As a quick review, the first (and likely most obvious) point about RIs is that they apply to a reference sample group (chosen from what is hopefully an accurately defined reference population) and not necessarily to the patient in question. An obvious example would be a severely hypoglycemic individual whose insulin value of 9 uIU/mL is technically within the RI of less than 23 uIU/mL but is abnormal in that clinical context. The RI applies only to healthy fasting (and presumably normoglycemic) subjects, not hypoglycemic patients. A more difficult example would be applying a TSH RI generated from subjects of "normal" weight to obese children referred to the endocrine clinic, who clearly have a higher (than the reference sample group) incidence of TSH values above the upper RI limit. Most of those with slightly high TSH values will have normal free T₄ results and negative thyroid antibodies.

This leads to the importance of establishing clinical decision limits beyond the standard RIs provided on laboratory reports. Using the example earlier of TSH in obese children, some pediatric endocrinologists through experience may decide that obese children with TSH values only slightly above the upper RI cutoff should not be referred or have additional laboratory testing, particularly if growth and development will be followed regularly. However, subspecialists would likely agree that a child with a TSH above 10 mIU/L deserves formal evaluation and additional laboratory testing.

The generation of RIs is simple in theory:

- Obtain results from a random sample of healthy individuals drawn from a representative population.
- Exclude statistical outliers.
- Stratify and subgroup by appropriate dimensions (e.g., age, sex) as indicated.
- Analyze the data and generate a reference interval citation; on laboratory reports, flag anything that falls outside of the reference interval.

In practice, the choice of a representative reference population is surprisingly difficult/subjective. When deciding upon exclusion criteria for a testosterone RI, should we exclude slightly heavy Hispanic girls, who will have somewhat higher testosterone levels than, say, Asian girls with average body mass index? Should we insist that subjects participating in an RI study for C-peptide and insulin responses to an oral glucose tolerance test (OGTT) all be carbohydrate-loaded for the days before the study—when in clinical practice almost no one has this preparation before an OGTT? Ultimately, the investigators will have to make arbitrary decisions that will affect the RI citation.

A similar issue arises when deciding what represents a statistical outlier (and whether or not to exclude these). Fig. 4.23 shows the results of an osteocalcin and bone-specific alkaline RI study, where the point circled in red represents the same individual. This point is clearly a statistical outlier but represents a healthy individual who just happened to be at his peak growth velocity when the study was conducted. If we exclude this outlier, will we end up flagging as high many healthy teens at their peak growth spurts? If we include this point, will the RI be too wide?

A valid reply might be that the RI study is better stratified by Tanner stage of puberty, rather than age, given the wide variability of the age of pubertal onset. There are caveats, of course. First of all, getting accurately Tanner-staged subjects is difficult in practice, because of the frequent reluctance of subjects, parents, and Institutional Review Boards to allow breast palpation or external genital examinations of otherwise healthy children. Self-assessment of pubertal staging can be performed, but it is well documented to result in some significant inaccuracies.²⁴ Second of all, even when Tanner staging can be conducted by well-trained investigators, there are certain stages that can be surprisingly difficult to distinguish (e.g., male genital stage II vs. stage III), leading to some investigators preferring to use testicular volume or length for evaluating sexual maturity in boys, even though testicular size is not part of the original Tanner staging system. Even Tanner staging of breast development can be difficult at times; although the original Tanner staging was based solely on inspection, it is clear that in overweight

girls the examiner must actually palpate the breast in search of glandular tissue to distinguish true pubertal development from increased breast size because of fat accumulation. Breast Tanner stage II can be difficult to define, and there may even be confusion between Tanner III development in a large-breasted individual versus Tanner V development in a young woman with smaller breasts (although breast papillae/nipple diameter >1 cm might help more clearly define Tanner V development). Some women may always retain the secondary mound of the nipple and areola that defines Tanner IV, and thus never officially reach Tanner V breast staging.

Additional issues with Tanner staging include discordance of breast/genital development (driven by pituitary-gonadal activation) with pubic hair development (driven by both gonadal and adrenal activation). It is not uncommon for a girl to have Tanner III pubic hair but only Tanner II breast development. Ideally, we should be able to define the subject as “B II, PH III,” but to date, such a system has not been used for Tanner-based RIs. The pediatric endocrinology and clinical chemistry communities may want to come to an agreement that some hormones (e.g., estradiol) should be based on Tanner breast stage, whereas others (e.g., dehydroepiandrosterone [DHEAS]) should be based primarily on Tanner pubic hair staging.

One last point about Tanner stage stratification is that some stages are actually not at all homogeneous. An infant, toddler, preschooler, and a 7-year-old child may all be Tanner I by breast/genital/pubertal hair development, and yet have very different hormonal profiles. It is clear, for example, that adrenal activation (adrenarche) and rising DHEAS start even before age 6 years, at least 3 to 4 years before the appearance of pubic hair. Therefore a study that included Tanner I children age 1 to 7 years would have a much lower average DHEAS than a study that only used Tanner I children age 5 to 7 years. At present, there is no standardized definition of which subjects should be included in a Tanner I subgroup.

Even the much simpler stratification by age requires decision-making that can affect the final RI citation. A typical approach to generating age-related RIs is to take observed results, such as those shown in Fig. 4.24, divide them into age subgroups (e.g., 1- or 2-year-wide bins) that contain reasonably similar values and calculate individual RIs for each subgroup. For analytes that change rapidly with age, however, this tends to result in small subgroups that show significant variability from sampling error alone because of the small number.

An extreme hypothetical example is shown in Fig. 4.25. Here, the values vary continuously with age, and subgrouping these data would either result in untenably low n if the subgroups were small; subgrouping by a wider range of ages would

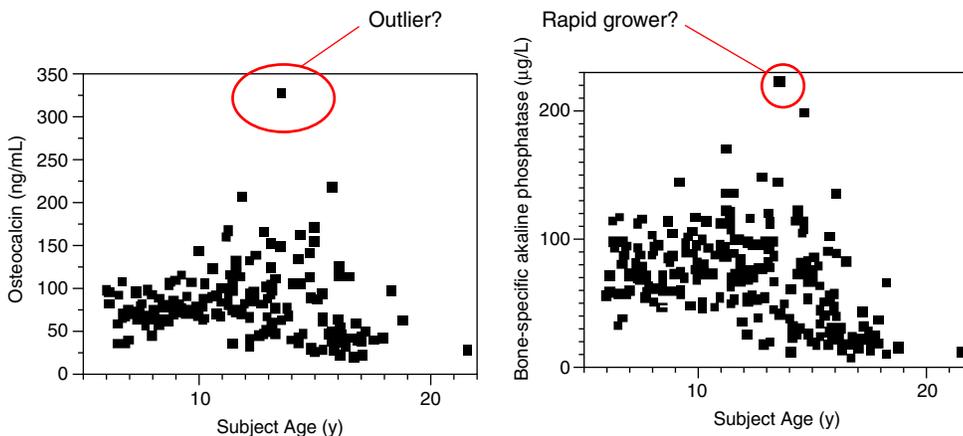


Fig. 4.23 Reference interval study of two bone formation markers (osteocalcin and bone-specific alkaline phosphatase). The same subject had results on both assays that were statistical outliers (circled in red), but clinical history revealed that this individual was at his peak growth velocity when the sample was drawn.

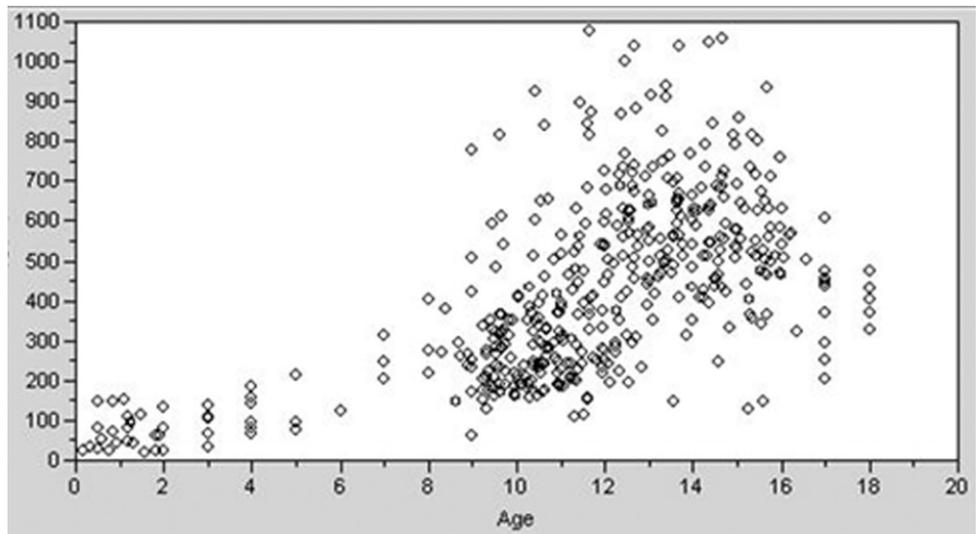


Fig. 4.24 Reference interval study for a hormone (insulin-like growth factor 1 [IGF-I]), which changes rapidly during the pediatric life cycle.

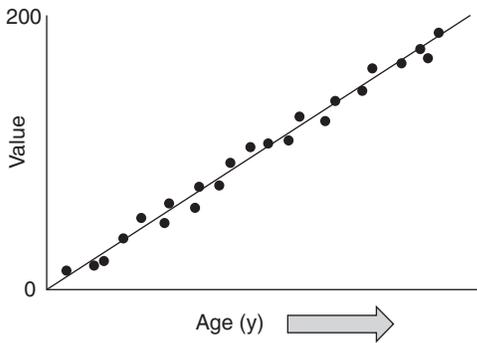


Fig. 4.25 Hypothetical example of an analyte that changes rapidly by age, demonstrating the need to do curve-fitting rather than dividing all of the data into tiny (e.g., 1 year) age subgroups.

lead to RIs that would overestimate values for the young end of the subgroup and underestimate values for those at the older end. In this case, it is obvious that a far better approach would be to fit a curve (in this case a straight line) to the data and use this to produce the RI based on age.

A more realistic example of this curve-fitting approach is shown in Fig. 4.26. Not only does this result in smoother, more physiological representations of the changes in value related to age, but it allows calculation of SD, or z-scores. Note that the z-score curves are not symmetrical about the mean value (the distance between the mean and +2 SD is more than the distance between the mean and -2 SD), which reflects the reality that most endocrine hormone values do not follow a normal/Gaussian (bell-shaped curve) distribution. Thus RI tables that provide a mean value and a single value for the SD may be misleading.

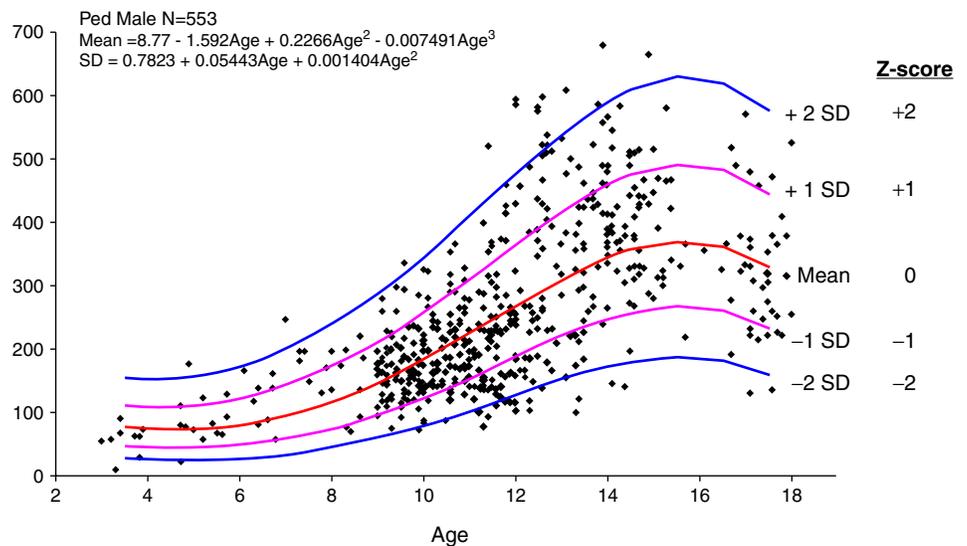


Fig. 4.26 Example of insulin-like growth factor 1 (IGF-I) z-scores derived after Box-Cox data transformation and curve-fitting.

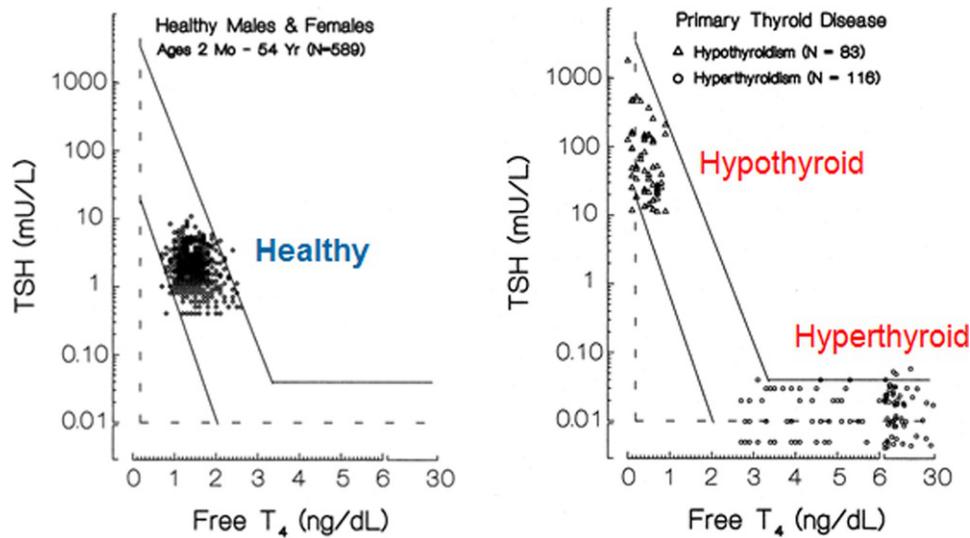


Fig. 4.27 Bivariate reference interval presentation: thyroid-stimulating hormone (TSH) versus free T₄ by equilibrium dialysis, in patients with an intact hypothalamic-pituitary axis.

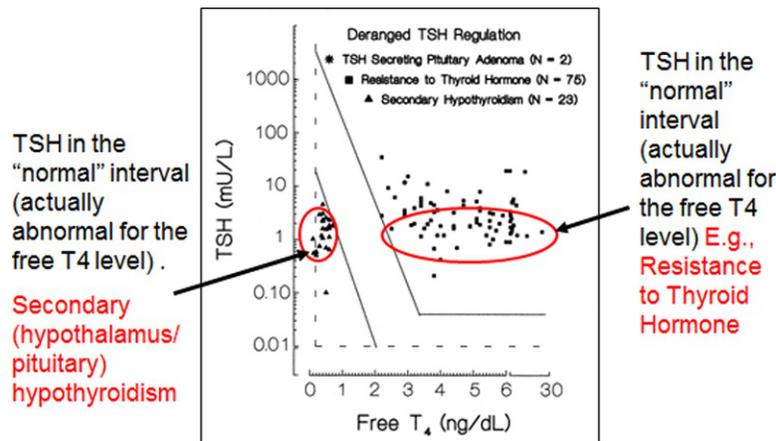


Fig. 4.28 Thyroid-stimulating hormone (TSH) versus free T₄ by equilibrium dialysis, in patients with secondary/tertiary hypothyroidism or resistance to thyroid hormone.

Another underused aid to improve understanding of laboratory results is to move from single (univariate) RIs to a bivariate presentation. For example, one can look at plots of TSH versus free T₄ for healthy individuals and for various disease conditions (Fig. 4.27). For any diseases that affect the thyroid but leave the hypothalamic-pituitary axis intact, the points will generally fall within the channel defined by the solid lines.

Diseases that affect the hypothalamic-pituitary axis will result in points falling outside of this channel (Fig. 4.28). Note that all of the points circled in red have TSH values within the RI, but when analyzed relative to the free T₄ level, these TSH values are inappropriately normal.

SUMMARY

Although it is not necessary for a pediatric endocrinologist to master all of the aspects of laboratory medicine mentioned in this chapter, having at least basic knowledge of the topics covered will help one make better test-ordering decisions and avoid interpretation errors. Knowing some of the “language of the lab” allows for better discussions with laboratory experts and makes it much easier to understand relevant publications

from the clinical chemistry community. Learning more about the statistics behind diagnostic decision-making, understanding how tests are validated for quality purposes, and understanding the various methods available (including the limitations and possible sources of error) are valuable for specialists at all levels of experience. An additional goal of this chapter has been to reveal some of the uncertainties involved with laboratory testing, to assuage some of the frustration experienced when one sees widely different results for the same test done at different laboratories, or to realize the current gaps in RI information. Hopefully, the pediatric endocrinology community will participate with the laboratory community to help harmonize testing and fill critical reference interval gaps. Most importantly, connecting with laboratory experts for consults involving diagnostic testing will help clinical subspecialists make the best decisions for their pediatric endocrinology patients.

REFERENCES

1. Greenspan SL, Klibanski A, Schoenfeld D, Rigway EC. Pulsatile secretion of thyrotropin in man. *J Clin Endocrinol Metab.* 1986;63:661–668.

2. Newman T. Dichotomous tests. In: Newman T, Kohn M, eds. *Evidence-Based Diagnosis*. 2nd ed. Cambridge, UK: Cambridge University Press; 2019.
3. Yu Z, Kastenmüller G, He Y, Belcred P, Möller G, Prehn C, et al. Differences between human plasma and serum metabolite profiles. *PLoS One*. 2011;6. e21230.
4. Davies C. Introduction to immunoassay principles. In: Wild O, ed. *The Immunoassay Handbook* 3rd ed. Boston: Elsevier; 2005.
5. Fuqua JS, Sher ES, Migeon CJ, Berkovitz GD. Assay of plasma testosterone during the first six months of life: importance of chromatographic purification of steroids. *Clin Chem*. 1995;41(8 Pt1): 1146–1149.
6. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of Black Americans and White Americans. *N Engl J Med*. 2013;369(21): 1991–2000.
7. Nelson JC, Weiss RM, Wilcox RB. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. *J Clin Endocrinol Metab*. 1994;79(1):76–79.
8. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab*. 2007;92: 405–413.
9. Aebbersol R, Mann M. Mass-spectrometric exploration of proteome structure and function. *Nature*. 2016;537:347–355.
10. Plebani M. Errors in clinical laboratories or errors in laboratory medicine? *Clin Chem Lab Med*. 2006;44:750–759.
11. Zungun C, Yilmaz FM, Boru EG, Topcuoglu C. Comparison of Improvacuter™ tubes with BD Vacutainer™ tubes for various hormones in the aspects of stability and influence of gel separators. *Clin Chem Lab Med*. 2015;53:231–238.
12. Kamlage B, Neuber S, Bethan B, Maldonado SG, Wagner-Golbs A, Peter E, et al. Impact of prolonged blood incubation and extended serum storage at room temperature on the human serum metabolome. *Metabolites*. 2018;8:6.
13. Klee GG. Interferences in hormone immunoassays. *Clin Lab Med*. 2004;24(1):1–18.
14. Gulbahar O, Degertekin CK, Akturk M, Yalcin MM, Kalan I, Atikeler GF, et al. A case with immunoassay interferences in the measurement of multiple hormones. *J Clin Endocrinol Metab*. 2015;100:2147–2153.
15. Spencer CA, Wang C. Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls. *Endocrinol Metab Clin N Am*. 1995;24(4):841–863.
16. Spencer CA. Clinical review: clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). *J Clin Endocrinol Metab*. 2011;96(12):3615–3627.
17. Levin PA, Chalew SA, Martin L, Kowarski AA. Comparison of assays for growth hormone using monoclonal or polyclonal antibodies for diagnosis of growth disorders. *J Lab Clin Med*. 1987;109:85–88.
18. Ribeiro de Oliveira Longo Schweizer, J, Ribeiro-Oliveira Jr A, Bidlingmaier M. Growth hormone: isoforms, clinical aspects and assays interference. *Clin Diabetes Endocrinol*. 2018;4:18.
19. Davidson J. Harmonisation of growth hormone assays in Australasia. *Clin Biochem Rev*. 2012;33:101–102.
20. Wagner IV, Paetzold C, Gausche R, Vogel M, Koerner A, Thiery J, et al. Clinical evidence-based cutoff limits for GH stimulation tests in children with a backup of results with reference to mass spectrometry. *Eur J Endocrinol*. 2014;171:389–397.
21. Vesper HW, Myers GL, Miller WG. Current practices and challenges in the standardization and harmonization of clinical laboratory tests. *Am J Clin Nutr*. 2016;104(suppl 3):907S–912S.
22. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. *Mol Cell Endocrinol*. 2014;383:203–313.
23. Staten MA, Stern MP, Miller WG, Steffes MW, Campbell SE. Insulin assay standardization. Leading to measures of insulin sensitivity and secretion for practical clinical care. *Diabetes Care*. 2010;33: 205–206.
24. Manley SE, Stratton IM, Clark PM, Luzio SD. Comparison of 11 human insulin assays: implications for clinical investigation and research. *Clin Chem*. 2007;53:922–932.
25. Bonat S. Self-assessment of pubertal stage in overweight children. *Pediatrics*. 2002;110:743–747.

5 Fetal-Maternal Endocrinology and Parturition

Sam Mesiano, Helen N. Jones, Heide Aungst, Tani Malhotra, Louis J. Muglia

CHAPTER OUTLINE

OVERVIEW	106
Evolution of Human Pregnancy and Birth	106
Establishment of Pregnancy	107
Endocrine Placenta: Structure and Function	108
Fetal Neuroendocrine Development	110
Fetal Maturation and Parturition	113
The Hormonal Control of Human Parturition	114
Genetic and Genomic Approaches to Reveal Novel Endocrine Pathways Related to Birth Timing	116
GENOME-WIDE ASSOCIATION STUDIES	117
RARE VARIANT ANALYSIS	117
MENDELIAN RANDOMIZATION AND CAUSATIVE PATHWAYS	117
FETAL GENOME	118
CONCLUSION	118
References	118

OVERVIEW

Pregnancy is a complex physiological state consisting of a symbiotic relationship between two genetically distinct, but related, individuals: the mother and the fetus. The success of pregnancy requires dramatic alterations of maternal physiology to accept, protect, house, and nurture the fetal allograft. A successful pregnancy involves implantation of the developing embryo into the endometrium; its avoidance of immunological rejection by the maternal immune system; adaptation of the maternal uterus to sustain a pregnancy; and specific changes in maternal physiology to meet the nutritional, metabolic, and physical needs of the growing conceptus—along with the proper timing of parturition, so that birth occurs when the fetus is mature enough to survive outside the uterus and the mother is able to nurture her newborn child. Hormonal interactions between the fetal and maternal compartments control these processes. A unique and extremely important endocrine organ of pregnancy is the placenta. The hemochorial anatomy of the human placenta allows direct access of the fetal syncytiotrophoblast to the maternal circulation for hormonal secretion. Placental hormones flood the maternal systems and act on maternal target cells to adjust maternal physiology in favor of maintaining pregnancy and meeting fetal metabolic needs. Through this endocrine relationship, the fetus and mother maintain homeostasis and allows the fetus necessary time for growth and functional development. Defects in placentation, placental function, and aberrations in placental hormone production cause maternal and newborn pathophysiology, such as life-threatening preeclampsia and fetal growth restriction. The end of pregnancy usually occurs through the process of parturition and, in most cases, at a time, referred to as *term*, when the

fetus is sufficiently mature to survive as a neonate. Term for human gestation is between the 37th and 42nd completed week of gestation (measured from the last menstrual period). Preterm birth, defined as less than 37 completed weeks of gestation, is a major worldwide socioeconomic problem that accounts for the majority of neonatal morbidity and mortality. The hormonal control of parturition—such that it occurs at term—is therefore a major determinant on neonatal wellness and the success of pregnancy. This chapter addresses current understanding of the endocrinology of pregnancy and parturition from the perspective of fetal/placental wellness and the process and timing of parturition, including recent advances in unraveling the genetics of human gestation length and birth timing. The discussion will be prefaced by a contextual overview of the evolutionary biology of human pregnancy and birth timing.

Evolution of Human Pregnancy and Birth

Pregnancy is both a personal event between mother and baby and a sociological and public health concern, as is evidenced by high infant and maternal mortality rates, even in developed countries like the United States. Although scientific advances have allowed us to learn about potential causes of preterm birth and pregnancy complications, it is critical to look back, through an evolutionary lens, to more fully understand the process of pregnancy and birth.

Evolution optimizes reproductive fitness across generations—maximizing transmission of genes to the next generation. Pregnancy and parturition are key events in the reproduction of viviparous species and, as such, would have been subjected to strong selective pressure through evolution. Advances in genetic technologies allow in-depth examination of the influence of evolutionary dynamics on pregnancy—including the impact of genetics and hormones on conception, pregnancy maintenance, and triggers for parturition. Ultimately, genetic makeup reflects natural selection and, as such, evolutionary adaptations may contribute to extant pregnancy complications, because the current genetic makeup of the mother, or even the baby, may not be ideal for current environmental conditions. This mismatch may be the reason for diseases of pregnancy, such as preeclampsia and gestational diabetes—and, in fact, likely influences health throughout the life span.¹

“Nothing in biology makes sense except in light of evolution,” said geneticist and evolutionary biologist Theodosius Dobzhansky.² This concept is certainly reflected in the comparative biology of pregnancy and parturition among viviparous species. The common theme among viviparous species is that the fetal development requires a minimum gestation time and a supply of nutrients for the fetus to achieve functional maturity needed to survive as a neonate. Fetal organ systems (especially the pulmonary, renal, and gastrointestinal) and neuroendocrine axes (especially the hypothalamic-pituitary-adrenal [HPA] and thyroid systems) must be sufficiently developed at birth for the newborn to achieve and maintain homeostasis. Similarly, maternal physiology, which has been modified to provision pregnancy, must be prepared to nurture and protect the

newborn. Certain processes are common (e.g., the requirement for progesterone to establish and maintain pregnancy; the promotion of fetal organ maturation by glucocorticoids) to all species, but subtle differences exist that relate to specific selective pressures on pregnancy-related traits that improved reproductive efficiency against the backdrop of general habitus, environmental niche, and overall reproductive strategy.

Viewing conception through an evolutionary lens begs the question: *Why doesn't the mother's immune system attack the fetus?* The "inflammation paradox" hypothesis posits that inflammation may be a necessary process that evolved to aid implantation, rather than attack the fetus. This is just one part of the equilibrium that must be established in a healthy pregnancy between the needs of the mother and the needs of the fetus. This concept has been described as maternal-fetal "cross-talk," "tug-of-war," or a "modulation" of gene regulation or cell networks. This often-conflicting cadence between mother and fetus continues throughout pregnancy, highlighting nutritional needs and hormonal fluctuations that must remain balanced for a successful pregnancy to carry to term. Recent studies of individual cells at the maternal-fetal interface have identified regulatory interactions at the cell level between the mother and fetus that prevent immune cell attack of the conceptus.³ Through an evolutionary framework, preeclampsia, for example, could be the result of shallow trophoblast invasion and inadequate spiral artery remodeling—forcing the fetus to increase maternal blood flow to the placenta by increasing maternal peripheral vascular resistance and providing greater blood flow to the fetal interface.

Another "obstetric dilemma" is conferred by the combined traits of encephalization: the increase in brain mass relative to body mass that is unique to hominid lineages, and obligate bipedalism. Bipedalism evolved between 3 to 5 million years ago, leading to an increase in hominid brain size, estimated at about 1 million years ago. The size of the human brain has remained relatively constant for at least the last 100,000 years.⁴ Obligate bipedalism required changes in the pelvic anatomy that decreased the size of the obstetric outlet. This would have limited the extent of intrauterine encephalization and required that parturition occur before the fetal head becomes larger than the pelvic outlet. This trait appears to be unique among human lineages. No other primates face this level of cephalopelvic disproportion, as they have pelvic openings that are larger than the fetal head diameter. Women, however, must pass a large-headed fetus through a relatively small pelvic opening. This means that the fetus cannot grow too large, or it risks death for the baby and the mother. Evolutionary selection of ancestral lineages must have been at work to develop a gestation length optimal for fetal development, especially lung maturity, before parturition. In addition, recent research has found regional and ancestral variability in the shape of birth canal. Strict evolutionary theory might surmise that the birth canal should be similarly shaped. Instead, this new work shows that other evolutionary forces, such as genetic drift, migration patterns, or even climatic adaptation also may have been at work. Study authors Lia Betti and Andrea Manica state that thoroughly understanding this has implications for obstetric practice in multiethnic societies.⁵

Rapid fetal brain growth requires substantial energy, and therefore traits that increase energy transfer from the mother to the fetus would have been favored. This may explain the production of placental lactogen and placenta growth hormone (GH) that promote maternal insulin resistance, providing more glucose for fetal consumption. However, traits that favored fetal brain growth would have conflicted with the problem of birthing a large-headed fetus through a relatively small outlet. This problem may have been solved by altering the parturition timing mechanism to shorten gestation thereby avoiding

the *obstetric dilemma*. Allometric analyses of neonatal brain size to body ratios across primate species, and the fact that human neonates are altricial (referred to as *secondary altriciality*), whereas other extant primates are precocial, support the hypothesis that gestation was shortened in modern-day hominids.⁶ This is a reasonable hypothesis that explains much of the unique characteristics of human pregnancy and parturition. As natural selection generally operates at the population level and over many generations, traits that increase reproductive efficiency of the population (such as encephalization; fetal neuroendocrine plasticity) and are beneficial over the long term, may impart costs to an individual, such as increased risk for preterm birth or cardiovascular disease over the short term.⁷

Consideration of these traits through an evolutionary lens expands our perspective of pregnancy complications, such as preeclampsia, fetal growth restriction, gestational diabetes, and preterm birth, and normal processes, such as the plasticity of fetal neuroendocrine development in response to environmental cues.

Establishment of Pregnancy

Under normal circumstances, implantation of a viable embryo into a receptive uterine endometrium establishes pregnancy. During the menstrual cycle, the ovarian steroid hormones, estradiol and progesterone, induce structural and functional changes in the endometrium essential for the establishment of pregnancy. During the luteal phase, the endometrium converts to a secretory phenotype in response primarily to progesterone produced by the corpus luteum (CL). The secretory endometrium is spongy and highly vascularized and has a glandular epithelium that secretes factors into the uterus that favors embryo survival. At the same time, epithelial cells in the endometrium produce chemokines, growth factors, and cell adhesion molecules that attract the embryo to specific docking sites for implantation and, in addition, increased vascularization of the endometrial stroma provides an optimal substrate for placentation.

After fertilization, the zygote undergoes an intrinsic program of cell division and differentiation that is not dependent on the hormonal milieu of the fallopian tube or the uterus. At around the fourth day after fertilization, the embryo is a solid cluster of cells encapsulated by the remnant of the oocyte zona pellucida. In the next 24 to 48 hours, as the embryo moves through the fallopian tube toward the uterus, it develops a fluid-filled cavity, the blastocoel, and is referred to as a *blastocyst*. The outer layer of blastocyst cells, known as the *trophoblast*, will give rise to the placenta and chorionic membrane. An inner cell mass of the blastocyst will produce the fetus, amnion, and mesenchymal and vascular components of the placenta. The human blastocyst enters the uterus at around the fifth day after fertilization and floats freely in the uterine cavity for 2 to 3 days. By this stage, the zona pellucida degenerates, leaving the nascent blastocyst embryo in an optimal condition to implant into the endometrium.

Embryo implantation into the endometrium has temporal and spatial limits within the receptive uterus.^{8–11} Physical interaction of the blastocyst with the endometrial epithelium occurs at dome-like structures known as *pinopodes* that express chemokines and cell adhesion molecules that attract the embryo and appear to be the preferred site for embryo adherence and subsequent implantation.^{12,13} Embryo adherence and implantation is most successful between days 21 and 24 of the menstrual cycle (~85% success rate), whereas implantation before or after this optimal window has a low (~11%) success rate.¹⁴ After day 24 to 25, the endometrial stroma undergoes morphologic and functional changes collectively referred to as *decidualization*, which is dependent on progesterone and occurs independently of conception and implantation.^{11,15,16} The decidualized endometrium, composed of large polyhedral

cells, containing high levels of glycogen and lipids that secrete a tough pericellular capsule, is hostile to implantation. Therefore successful implantation must occur before the establishment of the decidual barrier. In an infertile cycle, menstruation occurs to eliminate the nonreceptive decidualized endometrium. In the subsequent cycle, the endometrium then renews to a receptive state.

For implantation, trophoblast cells proliferate and secrete proteases that degrade the extracellular matrix between endometrial cells forming a path for the blastocyst to enter the uterine stroma. The invading trophoblast cells are referred to as *cytotrophoblasts* (CTBs) and form columns penetrating to the basal membrane beneath the decidual cells and into the myometrium. Eventually, the entire embryo embeds in the uterine stroma, anchored by CTB columns. Some extravillous CTBs invade into maternal spiral arterioles, displacing endothelial cells and smooth muscle tissue, and dilating the vessels to create a low-resistance arteriolar system. This system directs maternal blood into spaces, known as *lacunae*, between the invading columns of CTB cells. During this time, some CTBs fuse their plasma membranes to become a *syncytiotrophoblast*. This becomes the outer layer of the functional placenta. Eventually, the syncytiotrophoblast forms chorionic villi that are bathed in maternal blood within the lacunae.

The embryo is semiallogeneic with respect to the mother, and therefore its invasion into the endometrium represents a major breach of the maternal immune system. For successful pregnancy, the placental syncytiotrophoblast and CTBs at the maternal-fetal interface must avoid attack and destruction by activated maternal immune cells. To this end, the trophoblast cells produce an immunogenic camouflage in the form of human leukocyte antigen (HLA)-G that has reduced polymorphism; therefore maternal immune cells do not recognize it as foreign.^{17–19} CTBs also express multiple factors: Fas ligand that induces apoptosis in immune cells that carry Fas receptor,²⁰ early pregnancy factor,²¹ and the progesterone-induced blocking factor²² that blocks lytic natural killer (NK) cell activity and several antiinflammatory cytokines (such as transforming growth factor [TGF]- β , interleukin [IL]-10, IL-4).²³

For the establishment of pregnancy, menstruation that normally occurs during a nonfertile cycle must be prevented. This happens when the trophoblastic cells of the early embryo secrete chorionic gonadotropin (CG) that prevents CL regression and maintains its secretion of progesterone. In nonfertile cycles, the CL usually regresses at about the second week after ovulation, and the subsequent decline in progesterone leads to menstruation. Thus one of the first endocrine interactions between the conceptus and the mother involves early embryo signaling to promote intrauterine conditions that will allow implantation and the establishment of pregnancy via maintenance of CL progesterone secretion.

Endocrine Placenta: Structure and Function

The placenta fulfills a variety of essential functions during prenatal life. Its global role is to maintain a protected environment that facilitates optimal growth and development of the embryo and fetus. The human, hemochorial placenta includes a chorionic plate and chorionic villi. The chorionic villi consist of stem villi (types 1 to 3) either anchored to the decidua basalis or floating free. Type 3 villi branch out into intermediate and finally terminal villi.²⁴ The surface of the terminal villi is covered in CTB progenitor cells, derived from the trophoblast of the early blastocyst, and anchored to a basal lamina. The low oxygen environment of the early placenta protects organogenesis and favors CTB proliferation.²⁵ However, as development progresses, differentiation of these trophoblast progenitor cells produces different cell phenotypes.

CTBs differentiate into an invasive phenotype, and extravillous trophoblasts (EVTs) invade the uterine wall anchoring some chorionic villi to the decidua basalis entering the maternal blood vessels. These invading CTBs plug maternal arterioles maintaining the state of physiological hypoxia.²⁵ EVT cells continue to move further up maternal spiral arterioles, replacing maternal endothelial cells, remodeling, and increasing the diameter of the arterioles to accommodate the massive increase in blood supply required for fetal growth. Furthermore, several maternal cell types maintain the balance between inflammation and tolerance in the decidua. In particular, innate lymphoid cells, such as NK cells, interact with trophoblasts, stromal cells, and neutrophils to play a key role in the induction and maintenance of pregnancy. Decidual NKs represent 50% to 70% of infiltrating lymphocytes during the first trimester, but numbers diminish throughout gestation. Decidual NK cells are involved in early remodeling of the maternal spiral arteries before trophoblast invasion, as well as secreting chemotactic factors critical for EVT migration and invasion (such as IL-8, CXCL10, and IL-6). An association exists between impaired trophoblast chemotaxis and improper spiral artery remodeling and abnormal placentation. Abnormal decidual NK (dNK) function also may lead to a loss of control of trophoblast invasion.

During this time, some CTBs fuse their plasma membranes to become a single multinucleated cell known as the *syncytiotrophoblast*, which becomes the outer layer of the functional placenta. By 10 to 12 weeks of gestation, the CTB plugs are broken down and maternal blood reaches the intervillous spaces (lacunae), resulting in a change in oxygen tension in the placenta and the syncytiotrophoblast comes into direct contact with maternal blood in the lacunae. Remodeling of the placental villi and their associated underlying vasculature, which connects to the umbilical cord, occurs throughout gestation and can be responsive to maternal signals/environmental exposures impacting placental function, fetal growth, and development, and potentially, disease development later in childhood and adulthood (a process known as *fetal programming*).

The placental syncytiotrophoblast facilitates oxygen and nutrient transfer from the maternal circulation to the fetus and disposes of fetal waste products. It synthesizes and secretes hormones, growth factors, cytokines, and other bioactive molecules mainly into the maternal compartment. It also metabolizes maternal hormones (such as glucocorticoid and insulin) to prevent fetal exposure, and thus, separates components of the maternal and fetal endocrine systems. Importantly, the hemochorial arrangement allows hormones produced by the syncytiotrophoblast to directly access the maternal circulation. In contrast, the syncytiotrophoblast prevents most maternal hormones from entering the fetal compartment. Most hormones the placenta produces are identical, or close structural and functional homologues, of existing maternal hormones. As such, they interact with cognate receptors on maternal cells. Consequently, placental hormones that are secreted in relatively large amounts to achieve high levels (compared with levels in the nonpregnant women) in the maternal circulation override maternal counterparts and have profound effects on maternal physiology. Placental hormones include members of the prolactin and GH family, steroid hormones, and neuroactive hormones.

Prolactin-Growth Hormone Family

The prolactin-growth hormone (PRL-GH) family is one of the major groups of hormones the placenta secretes during gestation. Members of this family consist of prolactin,²⁶ placental lactogens, and GH.²⁷ Their roles include mediating maternal metabolic adaptations to pregnancy. The placenta's

production of the PRL-GH family of hormones appears to be important in regulating both insulin production and the sensitivity of the mother in response to pregnancy. The PRL-GH family also is implicated in the regulation of appetite and body weight.

Steroid Hormones

The placenta is the primary source of steroid hormones during gestation. Placental steroid hormones include estrogens and progesterone.²⁸ Both estrogen and progesterone play roles in regulating insulin and glucose homeostasis, lipid handling, and appetite regulation, which may be important in promoting metabolic changes and mobilizing nutritional stores in the mother during pregnancy. Steroid hormones are implicated in pregnancy complications, such as gestational diabetes and preeclampsia. High progesterone and estrogen concentrations have been reported for women with gestational diabetes, whereas levels are reduced in preeclamptic pregnancies compared with healthy pregnancies.²⁹

Neuroactive Hormones

Major targets of placental hormones include the maternal brain, hypothalamus, and pituitary glands. These neuroendocrine effects enable the mother to respond to her environment and adapt to avoid adverse effects of stress and maintain homeostasis.³⁰ Neuroactive hormones also prepare and enable the future mother to adequately care for her offspring.³¹ Melatonin and its precursor, serotonin, are tryptophan-derived hormones with well-known neuroendocrine impacts. During gestation, circulating levels of melatonin and serotonin increase.^{32,33} The placenta also secretes neuroactive hormones, such as thyrotropin-releasing hormone (TRH) and kisspeptin, which may function in adapting maternal physiology to support pregnancy.^{34,35} The placenta secretes oxytocin and expresses its receptor. Both increase gradually in the late stage of pregnancy in the normal placenta.³⁶

Corticotropin-Releasing Hormone

The human placenta expresses the gene encoding corticotropin-releasing hormone (CRH) starting around the sixth to eighth week of gestation. Placental CRH can be detected in the maternal blood by the 15th week of gestation with levels increasing exponentially up to the time of delivery.^{37,38} For most of pregnancy, CRH circulates in association with a binding protein, which sequesters CRH and prevents its biological activity. At the end of pregnancy (4–5 weeks before parturition), levels of the CRH binding protein in the maternal blood fall. This is associated with an exponential increase in placental CRH production, resulting in increased CRH biological activity. Placental CRH production (based on in vitro studies of placental explants) is increased by prostaglandins (PGs) E₂ and F_{2 α} , norepinephrine, acetylcholine, vasopressin, angiotensin-II, oxytocin, IL-I, glucocorticoids, and neuropeptide-Y, and decreased by progesterone and nitric oxide donors.^{39–41}

Growth Factors

The human placenta secretes multiple growth factors and cytokines.^{42,43} Growth of the placenta involves trophoblast proliferation, migration, differentiation, and fusion, and as such, growth factors are likely to be involved in these processes. Development of the placenta also involves extensive angiogenesis and vascularization at the implantation site, and modulation of the maternal immune system to prevent rejection of the

allogeneic fetal tissue. These processes likely involve a complex autocrine/paracrine communication that involves a plethora of growth factors, angiogenic factors, and cytokines.

Insulin-Like Growth Factors

The human placenta produces insulin-like growth factor (IGF)-1 and IGF-2 from as early as the eighth week of gestation.⁴⁴ IGF-1 is present in syncytiotrophoblast and CTBs at all stages in gestation.⁴⁵ IGF-2, plays a role in modulating maternal sensitivity to glucose,^{46,47} insulin, and glucose levels.⁴⁸ Abnormal IGF-1 expression, levels, and signaling are highly associated with intrauterine growth retardation in humans.⁴⁹

Vascular Endothelial Growth Factor Family

CTBs, the syncytiotrophoblast, and villous stromal cells in the placenta express members of the vascular endothelial growth factor (VEGF) family of peptides.⁵⁰ Placental growth factor (PGF) is a member of this family, which shares about 50% homology with VEGF-A. It is expressed by villous and extravillous trophoblast cells, and in contrast to VEGF expression levels, are positively correlated with advancing gestation. Imbalance in circulating maternal levels of these peptides and their soluble receptor, soluble FMS-like tyrosine kinase (sFLT), is strongly, but not exclusively, associated with the etiology of preeclampsia.⁵¹ However, the contribution to these levels from maternal endothelium is unclear. Moreover, CTB and villous stromal expression of these peptides regulates villous vascular formation and remodeling throughout pregnancy, and if disrupted, impacts transfer of nutrients and oxygen into the fetal circulation.

Fibroblast Growth Factor Family

Villous trophoblasts and villous mesenchyme in the human placenta express members of the fibroblast growth factor (FGF) family,⁵² and placental mononuclear phagocytes (also known as *Hofbauer cells*) express FGF receptors.⁵³ FGF induces Hofbauer cells to produce multiple growth factors and cytokine involved in tissue repair.

Adipokines

The human placenta expresses adipokines, leptin, adiponectin, resistin, ghrelin, and visfatin, and secretes them into the maternal circulation. They are thought to regulate maternal metabolic adaptation to pregnancy, especially increased insulin resistance.^{54,55} In placental cells, leptin and adiponectin modulate trophoblast invasiveness and the nutrient supply.⁵⁶ Leptin is inversely correlated with birth weight in small-for-gestational-age newborns. Positive correlation of adiponectin with leptin and ghrelin expression suggests an interaction between these hormones in the placenta.⁵⁷

Transforming Growth Factor- β Family

TGF- β influences both the proliferation and differentiation of trophoblast cells acting through receptors that include endoglin (ENG), a coreceptor for TGF- β 1 and TGF- β 3, which the syncytiotrophoblast and endothelial cells express. Trophoblast cells produce a soluble truncated ENG (sENG) consisting of the extracellular domain. sENG expression is upregulated in preeclampsia and may act as a decoy to sequester and block TGF- β 1 binding to its endothelial cell receptor, preventing vasodilation. sENG appears to augment the endothelial dysfunction caused by elevated sFLT1.⁵³

Activins and inhibins are disulfide-linked homo- and heterodimeric members of the TGF- β family that derive their names from their ability to activate or inhibit, respectively,

pituitary follicle-stimulating hormone (FSH) secretion.⁵⁸ The syncytiotrophoblast expresses each of the activin/inhibin subunits and follistatin, and the levels of expression do not change with advancing gestation.⁵⁹ These factors are secreted into the maternal and fetal circulations and amniotic fluid and their production varies with stage of gestation.^{60,61} Activins and inhibins may affect placental CG production.⁶² In cultured trophoblast cells, inhibin suppresses gonadotropin-releasing hormone (GnRH)-induced CG expression, whereas activin augments the GnRH-induced release of CG. Thus at least in vitro, activin and inhibin via paracrine effects on placental GnRH production may contribute to the regulation of CG secretion in a manner similar to their effect on hypothalamic-pituitary gonadotropin secretion.⁶² Interestingly, levels of inhibin-A and activin-A in the maternal circulation have been reported to have predictive value for pathologies, such as placental tumors, hypertensive disorders of pregnancy, intrauterine growth restriction, fetal hypoxia, Down syndrome, fetal demise, preterm delivery, and intrauterine growth restriction.^{59,60,63,64}

Epidermal Growth Factor Family

CTBs and the syncytiotrophoblast in the human placenta express epidermal growth factors (EGFs) early in pregnancy, and the level of expression decreases with advancing gestation.⁶⁵ EGF is thought to promote trophoblast invasion during implantation and deficiency in EGF expression and/or signaling is associated with preeclampsia and fetal growth restriction.^{66,67}

Extracellular Vesicles

The placenta produces extracellular vesicles (EVs) in large quantities in both healthy and pathological pregnancies. EVs now are being recognized as important carriers for proteins, lipids, and nucleic acids, which may play a crucial role in fetomaternal communication and maternal adaptation to pregnancy.⁶⁸ Micro- and nanovesicles from both first trimester and term human placentae carried Flt-1, and levels significantly increased in EVs from severe, but not mild, preeclampsia compared with normotensive placentae.^{68,69} Placental exosomes can be detected in the maternal circulation from the sixth week of gestation, and their levels increase gradually with advancing gestation in proportion to the increased size of the placenta.⁷⁰ Microribonucleic acid (microRNAs) in placental exosomes affect the function of local immune cells to boost resistance to viral infection.^{71–74} Levels of exosomes (placental and non-placental) in the maternal circulation, and the composition of their cargo, have been associated with pregnancy complications, such as preeclampsia, preterm birth, intrauterine growth restriction, and gestational diabetes.⁷⁵

Cell-Free Fetal Nucleic Acids

Cell-free deoxyribonucleic acid (cfDNA) originates in trophoblast cells and can be assayed in the maternal blood as biomarkers of placental health and function.^{76,77} From around the seventh week of gestation, cfDNA is detectable in the maternal circulation, and the amount increases with advancing gestation, such that late in pregnancy, it represents around 4% of the cfDNA in maternal blood.⁷⁸ After delivery of the placenta, the amount of cfDNA in maternal blood rapidly decreases. The physiologic role of cfDNA—and why it is shed from trophoblast cells into the maternal compartment—is not known. The amount of cfDNA in maternal blood has been used as a biomarker of placental health, with increases in cfDNA noted in nonviable pregnancies, preeclampsia, and intrauterine growth restriction-associated with placental insufficiency.

Because it can be isolated and sequenced, cfDNA is used for noninvasive prenatal diagnosis and testing for conditions, such as X-linked genetic disorders and aneuploidies, as well as fetal sex determination.⁷⁹

Cell-free messenger RNA (mRNA) derived from the placenta has been detected in maternal blood by eight weeks of gestation.⁸⁰ The cell-free mRNA has been evaluated quantitatively for specific transcripts, particularly those encoding proteins expressed uniquely by the placenta (such as CG β -subunit, placental lactogen, placenta enriched -1 14). It has been found that some of these transcript levels in maternal blood are elevated in preeclampsia and intrauterine growth restriction.

MicroRNAs, short noncoding single-strand RNA molecules, are known to be epigenetic factors regulating gene expression by targeting specific sequences in mRNAs and destabilizing the transcripts and/or inhibiting or reducing translation. The microRNAs released from the placenta may be incorporated into microvesicles, including exosomes, in apoptotic bodies, or protein-bound. The placenta-derived microRNAs are thought to influence maternal gene expression, particularly in vascular endothelial cells. The placental-specific microRNAs include those encoded in clusters on chromosomes 14 and 19, with the best studied being the microRNAs from the chromosome 19 cluster (C19MC).⁸¹ Alterations in levels of specific microRNAs in maternal blood have been reported in nonviable pregnancies and preeclampsia.⁸²

Fetal Neuroendocrine Development

The neuroendocrine system, including the hypothalamus, anterior pituitary, and the major endocrine glands, is essential for physiologic homeostasis, growth, response to stress, and reproduction. Appropriate development of the neuroendocrine systems during fetal life is therefore critical for postnatal health and wellness.

The neuroendocrine system functionally develops by mid-gestation. The hypothalamus originates on the inner surface of the diencephalic neural canal at around the sixth week of gestation. By the tenth week, the median eminence can be distinguished and specific cells in the hypothalamus begin producing GnRH, TRH, CRH, GH-releasing hormone (GHRH), and somatostatin (SS). The primordium of the anterior pituitary appears at around the fourth week of gestation as an evagination of Rathke's pouch, in front of the buccopharyngeal membrane at the roof of the developing buccal cavity. By the seventh week, the floor of the sella turcica is in place and separates the anterior pituitary from its epithelial origins. Beginning during week 8, capillaries interdigitate among the mesenchymal tissue of Rathke's pouch and the median eminence of the hypothalamus, and by the 12th to 15th week of gestation, these vessels form the hypothalamic-hypophyseal vascular system. The mature anterior pituitary gland includes lactotropes that produce prolactin (PRL), somatotropes that produce GH, corticotropes that produce proopiomelanocortin (POMC) and that give rise to adrenocorticotrophic hormone (ACTH), β -lipotropin and β -endorphin, thyrotropes that produce thyroid-stimulating hormone (TSH), and gonadotropes that produce luteinizing hormone (LH) and FSH. Thus the fetal hypothalamic-pituitary axis is anatomically and functionally developed by midgestation and, for the remainder of pregnancy, the fetal anterior pituitary, under the control of the hypothalamus, produces tropic hormones and affects the fetal thyroid glands, adrenal glands, and gonads. Although, each endocrine axis exhibits varying levels of activity, the fetal HPA axis is remarkably active in preparation for its essential role postnatally.

Integrative Steroidogenesis During Pregnancy: Fetal Adrenal-Placental Crosstalk

The human placenta produces large amounts of progesterone and estrogens, especially after the first trimester. At 6 to 10 weeks of gestation, placental trophoblast cells and the syncytiotrophoblast gain the capacity to convert maternal cholesterol to progesterone. Placental cells also have high aromatase and 17β hydroxysteroid dehydrogenase (17βHSD) activity and convert C19 androgens to C18 estrogens (Fig. 5.1). However, the placenta cannot produce estrogens de novo from cholesterol, or from pregnenolone or progesterone because the cells lack the 17α-hydroxylase/17,20-lyase enzyme. Instead, the fetal adrenal cortex supplies C19 androgens. Thus the human placenta produces progesterone from maternal cholesterol (referred to as the *maternal-placental endocrine unit*) and estrogens from fetal adrenal C19 precursors (referred to as the *feto-placental endocrine unit*) (Fig. 5.2). Deficiency in placental aromatase may not affect implantation, but women report virilization in the third trimester, resulting in facial hair and acne. Female newborns had varying degrees of pseudohermaphroditism with clitoromegaly and hypospadias—resulting from the inability of the placenta to convert dehydroepiandrosterone (DHEA) to estrogen. Male offspring developed tall stature because of failure of epiphyseal fusion, along with delayed bone age, causing osteopenia and undermineralization, along with several other issues.^{83,84}

The human adrenal glands derive from a thickening of the celomic epithelium between the urogenital ridges that occurs at around the fourth week of gestation. Approximately 1 week later, the primordial adrenal cortical cells migrate toward the mesonephros where they aggregate and proliferate to form the anlage of the adrenal cortex.^{85–87} For most of pregnancy, the fetal-adrenal cortex displays two morphologically distinct zones: the large inner fetal zone and the narrow outer definitive zone. A third zone, the transitional zone, distinguished by ultrastructural and functional characteristics, exists between the fetal and definitive zones and is apparent early in the third trimester (Fig. 5.3). Thus late in pregnancy, the fetal-adrenal cortex resembles a rudimentary form of the adult-adrenal cortex. Soon after birth, the fetal zone involutes to become the

zona reticularis, the transitional zone becomes the zona fasciculata, and the definitive zone becomes the zona glomerulosa. Between the innermost fetal zone cells—that eventually will aggregate to form the adrenal medulla late in the third trimester—are clusters of immature neuroblasts.

At around week 12 of gestation, steroidogenesis in response to ACTH initiates in the fetal zone cells. This increase coincides with the initiation of fetal zone enlargement and the fetal pituitary gland secreting ACTH.⁸⁸ At midgestation, the fetal zone occupies 80% to 90% of the cortex and is the primary site of growth and steroidogenesis production. Remarkably, the adrenal glands are disproportionately enlarged because of fetal zone hypertrophy, and at 20 weeks, are larger than their adjacent kidney. Fetal zone cells are highly steroidogenic and in response to ACTH produce 100 to 200 mg/day of dehydroepiandrosterone sulfate (DHEA-S), a C19 steroid with weak androgenic activity, during the third trimester. The fetal adrenals produce DHEA-S that is used as substrate for placental estrogen synthesis. In the placenta, the steryl-sulfatase enzyme removes the sulfate and the 3βHSD, 17βHSD, and aromatase enzymes convert DHEA to estradiol and estrone. The fetal liver expresses the 16-hydroxylase enzyme and converts some DHEA-S to 16-hydroxy-DHEA-S (16OH-DHEA-S), which is then converted to estriol. Because 16-hydroxylase is not expressed postnatally, the amount of estriol in the maternal circulation indirectly reflects activity of the fetal HPA axis. For this reason, maternal estriol previously was used as an endocrine marker to evaluate fetal well-being. The fetal adrenal cortex continues to produce DHEA-S in response to ACTH for the remainder of pregnancy, increasing considerably during the second and third trimesters. By term, the human fetal adrenal produces around 200 mg DHEA-S per day. Thus the fetoplacental endocrine unit constitutes a cooperative interaction between the incomplete steroidogenic pathways in the fetal HPA axis and the placenta to produce a complete estrogen synthetic unit. Initial reports of steroid sulfatase deficiency suggested delayed onset of labor and lack of cervical dilation. However, subsequent research has shown normal gestation length with the possibility of spontaneous vaginal delivery.^{89,90}

The placenta’s production of CRH also influences the fetal HPA axis. A unique feature of primate pregnancy is that the

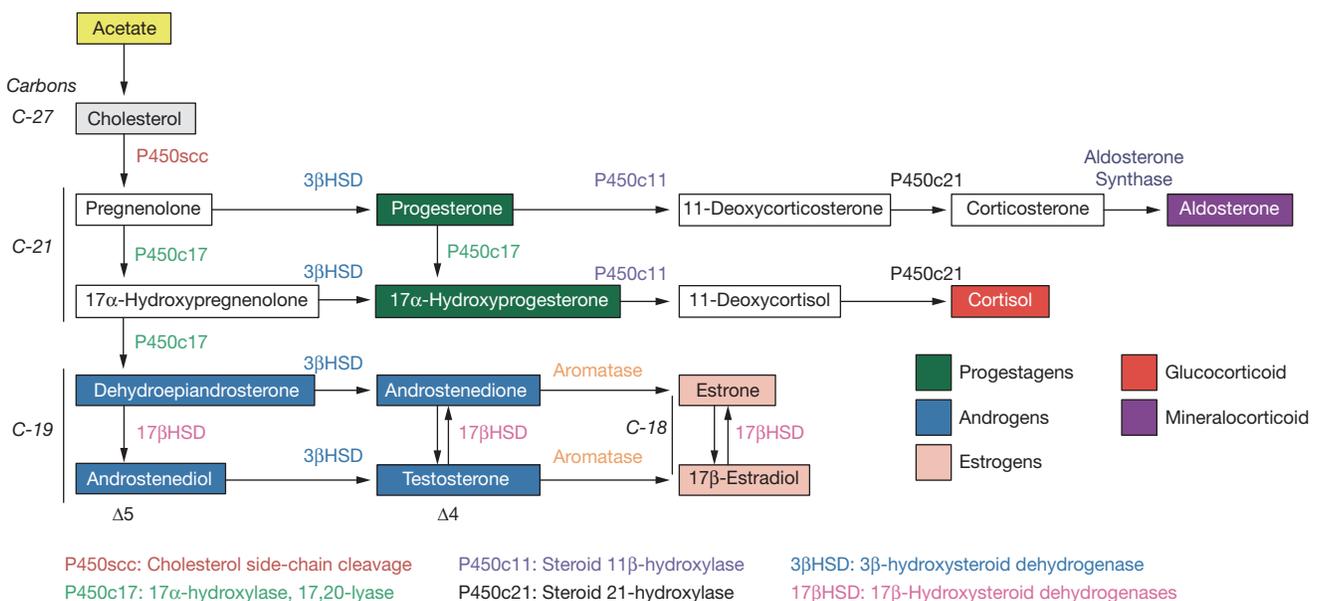
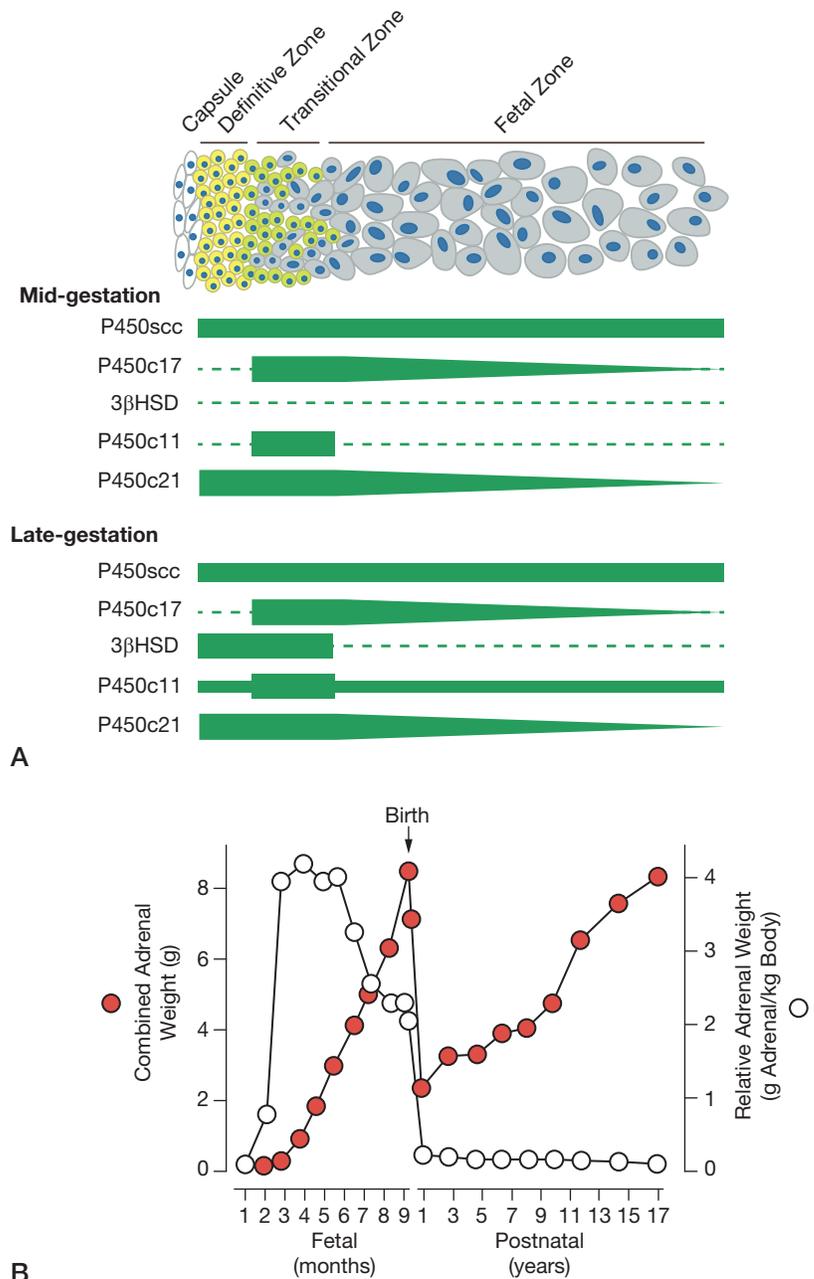


Fig. 5.1 Schematic diagram of steroid biosynthesis. Enzymatic steps and steroid intermediates progestogens, mineralocorticoids, glucocorticoids, androgens, and estrogens are shown.

Fig. 5.3 Morphologic zonation and steroidogenesis in the fetal adrenal and adrenal growth during development. A, Steroidogenic enzymatic activity by zones during mid or late gestation. B, Absolute adrenal weight and adrenal weight relative to body size during fetal and postnatal life. (Modified from Mesiano, S., Jaffe, R.B. (1997). Development and functional biology of the primate fetal adrenal context. *Endocr Rev*, 18, 378–403.)



negative feedback on pituitary ACTH secretion during the first trimester. Thus indirect data suggest that the fetal adrenals produce cortisol before 12 to 15 weeks, which then declines until 25 to 30 weeks' gestation, after which transitional zone cells express steroidogenic enzymes needed for cortisol synthesis de novo from cholesterol. Mineralocorticoid production by the human fetal adrenal cortex is low early in gestation but increases during the third trimester. At term, 80% of the aldosterone in human and rhesus monkey fetal blood appears to originate from the fetal adrenal.⁹⁷ In 18- to 21-week human fetal adrenals, the mineralocorticoid metabolic pathway localizes to the definitive zone, but its activity is low and unresponsive to secretagogues.^{98,99} Nonetheless, under normal conditions the steroidogenic capacity of the fetal adrenals to produce the cortisol and aldosterone is in place by the middle of the third trimester in preparation for the HPA axis to perform its vital function after birth.

Fetal Maturation and Parturition

At birth the fetus, as a newborn, must abruptly adapt to the extrauterine environment and independently establish and maintain physiological homeostasis. Success of this transition requires that organ systems needed for extrauterine life (such as lungs, gut, adrenals, thyroids, kidneys, liver, pancreas, immune system) are functionally mature before, or at least soon after, parturition. Maturation of the fetal lungs with the capacity for gas exchange is especially important. Cortisol is a key hormonal regulator of this process and promotes the functional maturation of multiple fetal organ systems before parturition. Interestingly, in some species, the cortisol responsible for fetal maturation also triggers parturition. For example, in sheep, activity of the fetal HPA axis increases during the week before parturition so that the fetal adrenals secrete a surge of cortisol that not only stimulates fetal organ maturation (especially

surfactant production by the lungs) but also triggers parturition by inducing the metabolism of placental progesterone to androstenedione, leading to a systemic decrease in maternal progesterone levels that induces labor.^{100,101} Thus in the central role, the fetal HPA axis produces cortisol to ensure that fetal maturation precedes parturition and that the two processes are coordinated. The discovery that cortisol promotes fetal lung maturation led to the widespread clinical uses of synthetic glucocorticoids that cross the placenta to promote fetal lung maturation in women with threatened preterm birth. This therapy increases the survival rates of preterm infants mainly by decreasing the severity of respiratory distress syndrome because of pulmonary insufficiency in premature infants. Thus as with most species, glucocorticoid stimulates maturation of the human fetus, preparing it for life outside the uterus. However, unlike the sheep, neither glucocorticoid or activity of the fetal HPA axis induces human parturition.

In sheep, the cortisol needed to induce fetal maturation and trigger parturition originates in the fetus, whereas maternal cortisol or cortisol therapy to the maternal side only, has no effect. The reason for this finding is that the sheep placenta inactivates maternal cortisol by expressing the 11β -HSD-2 enzyme that converts cortisol to the inactive cortisone.¹⁰² This is likely a safeguard to protect the developing fetal brain from adverse effects of glucocorticoid on neuronal development. Similarly, the human placenta prevents maternal cortisol from entering the fetal compartment throughout most of human pregnancy. This is why synthetic glucocorticoids that the placenta does not metabolize are used to treat women in preterm labor. This barrier, however, appears to collapse late in the third trimester. Evidence for this is that fetuses that have impaired cortisol production because of 21-hydroxylase deficiency are usually born at term without any apparent signs of organ immaturity.¹⁰³ This suggests that another source of glucocorticoid, possibly from the maternal adrenals, could contribute to fetal organ maturation. If this is the case, maternal cortisol must cross the placenta. Late in human pregnancy, the placental barrier to maternal cortisol appears to weaken. The evidence for this is that estriol levels in the maternal circulation during late pregnancy are inversely related to the circadian changes in circulating maternal cortisol levels,¹⁰⁴ suggesting that maternal cortisol crosses the placenta and exerts negative feedback on the fetal HPA axis, leading to a decrease in ACTH stimulation of DHEA-S production that, in turn, causes a decrease in placenta estriol production. Increased transfer of maternal cortisol to the fetus may represent a backup mechanism to ensure fetal lung maturation before term parturition.

The Hormonal Control of Human Parturition

The gravid uterus accommodates the growing conceptus physically and immunologically. For most of pregnancy the extracellular matrix of the uterine cervix is rigid and noncompliant, which effectively closes the uterine outlet. In contrast, the myometrium of the uterine wall relaxes, becoming compliant, growing mainly by cellular hypertrophy, and distending in response to the growing conceptus and amniotic fluid. The maternal immune system is also suppressed, especially at the maternal-fetal interface in the decidua, to prevent rejection of the semiallogenic fetal tissue. At parturition, the gravid uterus undergoes a series of temporally coordinated transformations, such that the myometrium becomes highly contractile and excitable and contracts rhythmically and forcefully to become the engine for birth, and the extracellular matrix of the cervix softens to allow dilation and opening of the gateway for birth. In addition, the fetal membranes weaken and rupture to release the fetus.¹⁰⁵ The process also is associated with immune activation in the decidua that produces extensive

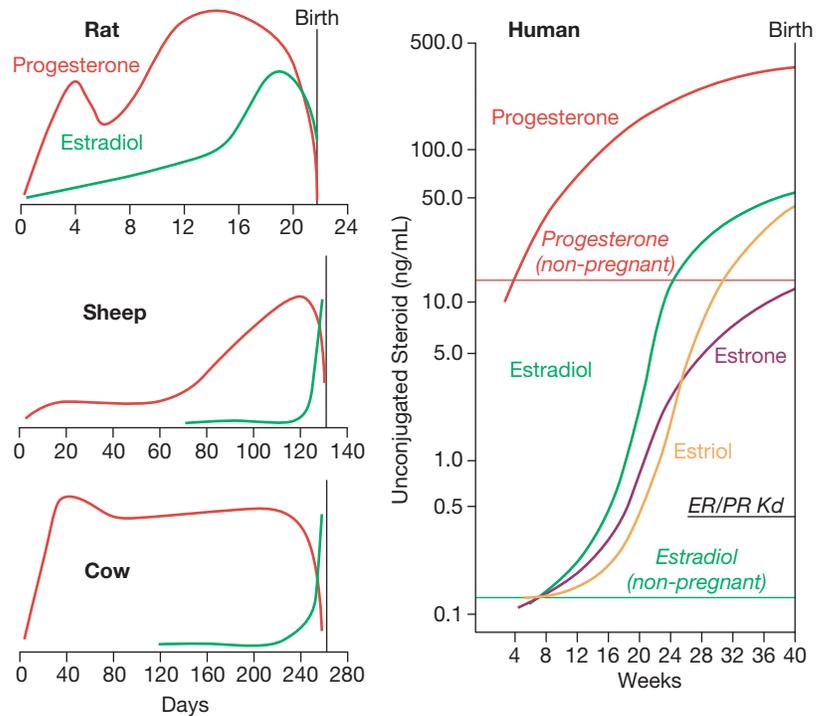
tissue-level inflammation and the local production of pro-labor uterotonic factors, such as prostaglandins. These events make up what we call *labor*, which is clinically defined as rhythmic and forceful contractions with softening and dilation of the cervix and rupture of the fetal membranes. The process culminates in the forceful expulsion of the fetus, placenta, and fetal membranes, including the decidua, and through hemostasis, involution, and endometrial regeneration, the uterus reverts to the nonpregnant state to resume menstrual cyclicality.

Hormones control the process and timing of parturition. Progesterone and estrogens affect the growth and contractility of the myometrium, the immunologic/inflammatory state of the decidua, and the mechanical integrity of the cervix. In this regard, the balance between the pro-pregnancy actions of progesterone that dominate for most of pregnancy, and the pro-labor actions of estrogens that occur as part of the parturition process, is critical. For most of pregnancy, progesterone and estrogen exert uterotrophic effects to promote myometrial cell hypertrophy and increase blood flow to the uterus and cervix. Progesterone exerts a relaxatory and antiinflammatory effect on the uterine tissues to block labor by maintaining closure of the cervix and quiescence of the myometrium. At parturition, the effects of progesterone are lost and estrogens dominate to promote the expression of genes in myometrial and cervical cells whose products increase uterine contractility and cervical softening.¹⁰⁶ Thus a general characteristic of viviparous species is that the withdrawal of the progesterone block triggers parturition, which permits stimulatory estrogenic actions to increase myometrial contractility and excitability and promote cervical softening and membrane weakening. Cortisol from fetal or maternal adrenals, does not appear to contribute to the hormone control of human parturition. Evidence for this is that parturition occurs normally at term in pregnancies where the fetus has CAH because of the inability to produce cortisol,¹⁰³ and glucocorticoid therapy does not hasten the parturition process.

Placental CRH may be involved in the regulation of human parturition. Maternal plasma CRH concentrations—reflecting CRH mainly of placental origin—increase exponentially with advancing gestation, and the rate of change as gestation progresses is predictive of the gestation length.⁹² The more rapid the increase in maternal CRH from midgestation, the earlier parturition occurs. A slower rate of increase is associated with postterm birth. The physiology and molecular biology underlying this association is not understood. In vitro experiments suggest some possibilities. CRH receptors have been identified in myometrial cells and fetal membranes.^{107–110} CRH stimulates PG secretion from decidua and amnion,^{111,112} and increases myometrial contractility by augmenting responsiveness to oxytocin and $PGF_2\alpha$.^{113–115} Paradoxically, placental CRH increases adenylate cyclase activity in myometrial cells, which promotes relaxation, but this effect is lost late in pregnancy, possibly because of changes in CRH receptor isoforms in myometrial cells.¹¹⁶

How progesterone withdrawal happens to achieve parturition differs between species, depending on the source of progesterone that maintains pregnancy. For example, in rodents the maternal CL produces progesterone during pregnancy and CL regression triggers parturition, leading to systemic progesterone withdrawal. In sheep, the placenta exclusively produces progesterone and its withdrawal is induced by a prepartum cortisol surge that induces the expression of the 17α -hydroxylase enzyme in placental cells that diverts progesterone from secretion to conversion to androstenedione, which is converted to estradiol. This enzymatic induction leads to a systemic decrease in maternal progesterone levels and a concomitant increase in estrogen levels that triggers parturition.¹¹⁷

Fig. 5.4 Patterns of maternal serum progesterone and estrogen concentrations through gestation in mammals. The rat, sheep, and cow all demonstrate systemic progesterone withdrawal preceding labor, whereas women do not. Estrogens increases in mammals as parturition approaches. In women the rapid increase in placental estrogen production is during the first trimester.



In contrast to rodents and sheep, parturition in women occurs without a systemic progesterone withdrawal (Fig. 5.4).¹¹⁸ Instead, the gravid uterus receives high levels of progesterone and estrogens—because of activity of the fetoplacental unit—for most of pregnancy and during labor and delivery. Although this suggests that progesterone withdrawal does not trigger human parturition, clinical studies show that disruption of progesterone signaling with agents, such as mifepristone, a selective progesterone receptor modulator/antagonist, increases myometrial contractility, and induces labor at all stages of pregnancy.¹¹⁹ That observation suggests that a functional, rather than systemic, progesterone withdrawal triggers human parturition, whereby uterine cells become refractory to progesterone.

Progesterone maintains pregnancy via its interaction with the nuclear progesterone receptor PR isoforms, PR-A and PR-B, expressed by myometrial, cervical, and decidual cells (Fig. 5.5).¹²⁰ The PRs function primarily as ligand-activated modulators of transcription by directly interacting with cis-elements in gene promoters or by modulating the activity of other transcription factors. Changes in PR transcriptional activity likely mediate functional progesterone withdrawal. Possible mechanisms include: (1) increased abundance and transrepressive activity of PR-A in uterine cells that decreases net progesterone-induced PR transcriptional activity^{121–131}; (2) decreased levels and/or activity of key transcriptional coactivators that modulate PR transcriptional activity^{132–134}; and (3) posttranslational modifications, especially site-specific serine phosphorylation, which suppress PR transcription activity.¹³⁵ Any, or all, of these PR-mediated functional progesterone withdrawal mechanisms may be responsive to prolabor parturition triggers. It is also possible that uterine cells metabolize progesterone to a form that does not bind the PR, leading to generation of unliganded PRs that may have prolabor actions.¹³⁴

Clinical^{136–143} and animal^{144–148} studies show that tissue-level inflammation within the uterine tissue compartments (myometrium, cervix, and decidua) is a major prolabor stimulus that plays a causal role in the process and timing of

parturition.^{149–154} Labor is also associated with increased production of uterotonic PGs by the decidua and fetal membranes; likely as a sequelae of tissue-level inflammation.¹⁵⁴ Increased levels of PGs (especially $\text{PGF}_2\alpha$ and PGE_2) in the uterine tissue have the potential to transform the myometrium to the laboring state and promote softening and dilation of the cervix. Thus a leading hypothesis is that parturition ensues from a local autocrine/paracrine process that involves tissue-level inflammation. By implication, this hypothesis predicts that antiinflammatory effects on the uterine and gestational tissues maintains pregnancy and blocks labor. Progesterone may mediate this via the PRs in myometrial, cervical, and decidual cells, as in vitro studies show that progesterone/PR signaling inhibits the response of uterine cells to prolabor/proinflammatory stimuli.^{126,135,155–159} Loss of progesterone/PR antiinflammatory activity would allow proinflammatory stimuli to prevail and induce tissue-level inflammation with the concomitant production of PGs that transform the myometrium and cervix to the laboring state. According to this model, physiological and/or pathological conditions that affect the capacity for PRs to mediate antiinflammatory actions of progesterone in uterine cells could trigger parturition. Studies suggest that in myometrial cells, prolabor/proinflammatory stimuli block progesterone/PR antiinflammatory activity by inducing site-specific serine phosphorylation of PR-A.^{135,160}

Throughout pregnancy, various physiologically relevant proinflammatory/prolabor stimuli affect the gravid uterus. Some may be intrinsic (such as fetal or maternal stress, placental senescence, uterine distention) and some may be extrinsic (such as intrauterine infection, maternal nutritional, and environmental psychosocial stress). The net effect is that the gravid uterus is exposed to an inflammatory load that increases as pregnancy advances. It is proposed¹³⁵ that for most of pregnancy progesterone/PR inhibits response of myometrial, decidual, and cervical cells to inflammatory load stimuli and that an inflammatory load threshold exists, above which specific protein kinase activity catalyzes PR phosphorylation and/or other mechanisms that

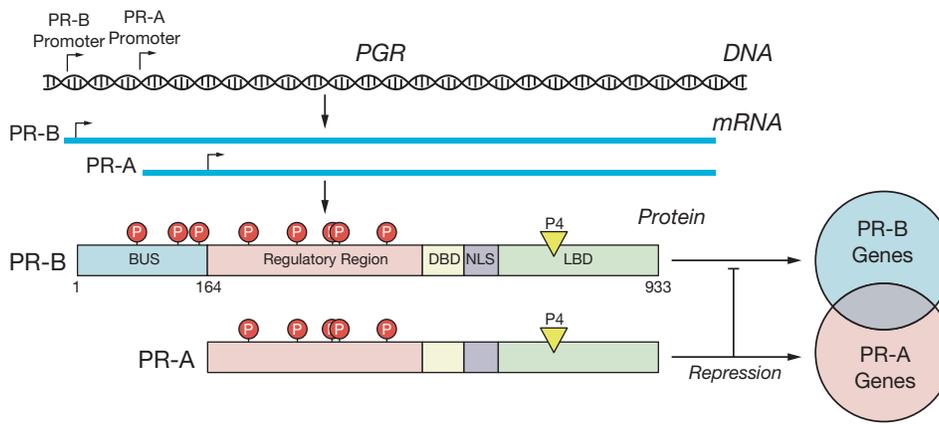


Fig. 5.5 Progesterone receptor isoform production. The progesterone receptor gene (*PGR*) generates two primary translation products, PR-A and PR-B. PR-A lacks N-terminal sequences encoding a transcriptional activation domain. Both isoforms undergo extensive posttranslational modifications with phosphorylation. The PRs function as ligand activated transcription factors to affect the expression of specific and common target genes. At some gene promoters PR-A represses that transcriptional activity of PR-B. *BUS*, B upstream segment; *DBD*, DNA binding domain; *LBD*, ligand binding domain; *NLS*, nuclear localization sequence.

cause PR-mediated functional progesterone withdrawal. The loss of progesterone/PR antiinflammatory activity would predispose the development of a positive-feedback proinflammatory state within the uterine tissues, leading to the production of PGs that activate the myometrium and dilate the cervix. This paradigm suggests that birth timing results from the inflammatory load trajectory, the set point for the inflammatory load threshold, and the capacity to induce PR-mediated functional progesterone withdrawal. Any of these parameters could be amenable to therapeutic strategies to clinically control birth timing and prevent preterm birth. Progestin prophylaxis in the form of 17-hydroxyprogesterone-caproate (intramuscular weekly) or progesterone (vaginal suppository daily) may decrease the risk for preterm birth in a subset of at-risk women by affecting the inflammatory load trajectory and/or augmenting the PR-mediated antiinflammatory activity.

Genetic and Genomic Approaches to Reveal Novel Endocrine Pathways Related to Birth Timing

As highlighted throughout this chapter, estrogen and progesterone are essential to the establishment, maintenance, and termination of pregnancy. These key reproductive steroid hormones have received considerable attention and certainly form a portion of the conserved signaling molecules in pregnancy, along with prostaglandins, across species. In attempting to understand the regulation of progesterone and estrogen synthesis, and activity at the end of pregnancy that allows term parturition to take place, it has been challenging to define molecular mechanisms. Typical animal models differ significantly from women in terms of progesterone and estrogen regulation associated with the initiation of parturition, as has been reviewed extensively elsewhere.¹⁰⁶ In women, circulating serum progesterone rises through gestation and does not decline with the onset of labor. Over the end of pregnancy, estrogen concentrations also rise, a pattern observed in most species. Various hypotheses regarding mechanisms of “functional” progesterone withdrawal in women have been put forward, including variation in progesterone receptor isoforms, progesterone receptor cofactors, posttranslational modification of the progesterone receptor, and increased estrogen-to-progesterone ratio, but no mechanism has been definitively proven.

In the last two decades, the ability to gain mechanistic insights into human physiology through nonbiased, genome-wide

analytic techniques has provided the opportunity to elucidate the control of human birth timing. Experimental manipulation of human pregnancy is not ethically acceptable, as it could result in harm to the mother or fetus. Genotype-based studies in mothers and infants, whether seeking common or rare variants, have the potential to produce new discoveries on the control of birth timing, including modulation of known endocrine signaling pathways and identification of new pathways. The ability to use genotypic information from mothers, fathers, and their offspring to identify loci associated with the length of gestation, as a continuous variable, or risk for preterm birth, as a dichotomous trait (term vs. preterm), is an especially important strategy, as human pregnancy has had to accommodate several species-specific constraints to optimize outcomes for both the infants and mothers.

Studying the genetics and genomics of pregnancy has several inherent challenges. The most unique of these is the potential for regulatory information to reside in two separate, but related, genomes, those of the mother and of the fetus. Moreover, although genetic factors affect birth timing and preterm birth, so, too, does a variety of environmental exposures, such as infection, psychosocial stress, nutrition, and health behaviors, that may act through similar or divergent mechanisms and pathways.

Abundant evidence has accumulated that genetic factors, particularly in the mother, contribute to determining gestational length for human pregnancy. Classic twin studies, investigating the concordance of birth timing for offspring of identical twins compared with dizygotic twins or other siblings, has suggested that maternal genetic factors contribute 30% to 40% of the variation in birth timing, with little influence from the paternal genome.¹⁶¹ Family-based segregation analysis findings come to a similar conclusion.^{162,163} Very large epidemiological studies also conclude that the heritability of preterm birth risk resides in the maternal genome, acting either in the mother or maternally transmitted information to the fetus.¹⁶¹ It should be noted that 60% to 70% of the risk for preterm birth resides in environmental factors, and that loci contributing to this common complex syndrome likely individually contribute risk of small effect size (odds ratio [OR] 1.10–1.25). Although these data support the utility of a genome-wide approach, they herald that large sample sizes will be required to detect loci that are statistically significant and associated with birth timing at the genome-wide level of $P < 5 \times 10^{-8}$.

GENOME-WIDE ASSOCIATION STUDIES

Studies have overcome the limitation of inadequate sample sizes for detection of this heterogeneous phenotype with presumably multiple loci of modest effect size. Important new findings have emerged from research, using a large discovery cohort assembled through a commercial direct-to-consumer genotyping company that included gestation duration information in one of their surveys, in conjunction with carefully phenotyped Northern European cohorts for birth timing and spontaneous preterm birth.

In 2017 Zhang and colleagues published the first well-powered, validated, and replicated study of the maternal genome that identified six loci that achieved genome-wide significance.¹⁶⁴ This study involved approximately 44,000 women in the discovery cohort and an additional 8000 in the replication cohort. The power of genome-wide association studies (GWAS) to explore preterm birth genetics has finally approached the magnitude of other complex common phenotypes, such as type 2 diabetes, obesity, hypertension, and other adult metabolic and cardiovascular disorders. As would be anticipated, retrospectively, logical associations of these gene regions with gestational duration are evident, but they were not previously a focus for targeted analysis. Of the six regions identified—those near the *EBF1*, *EEFSEC*, *AGTR2*, *WNT4*, *ADCY5*, and *RAP2C* genes—four of these have immediate endocrine signaling ramifications.

AGTR2 encodes the angiotensin II type 2 receptor. Responsive to the hormone angiotensin 2, this receptor is implicated in cardiovascular development and regulation and is, therefore, a plausible candidate for harboring variants that determine birth timing. In addition to its known cardiovascular effects, the endometrium upregulates *AGTR2* expression late in the decidualization process. Its specific actions at this time are not known, but the finding—taken in the context of *WNT4* ramifications to be discussed—raises the visibility and impact of the endometrium and the decidualization process as key factors in not only the initiation of pregnancy, but also the termination of pregnancy.

WNT4 and the *WNT* signaling pathway are part of the essential toolbox of general mammalian development. This paracrine signaling pathway is prominently involved in the development of the female reproductive tract, and also plays an essential role for proper decidualization. The single nucleotide polymorphism (SNP) identified in the GWAS study of birth timing was in the same linkage disequilibrium block as SNPs associated with risk for endometriosis, again suggesting the importance of the endometrial stromal cell. Functional studies of this locus revealed a putative causative variant in the nonreference allele that generated a new estrogen receptor binding site. Here, it is not the generation of estrogen that results in human variation in birth timing, but its action on pregnancy-relevant target tissues.

ADCY5 that encodes a member of the adenylyl cyclase family has been previously associated with variation in birth weight in the fetal genome. The gene region in the fetal genome, however, is not strongly linked to the region revealed in birth timing in the maternal genome. Adenylyl cyclases generate cyclic adenosine monophosphate, which plays an important role in promoting myometrial relaxation. The endocrine signals that activate *ADCY5* in pregnancy, if any, have not been determined and provide an important avenue for future research.

Perhaps the most intriguing locus emerging from the maternal GWAS analysis was *EEFSEC*, which encodes eukaryotic elongation factor for selenocysteine. Selenocysteine, the “21st amino acid,” is incorporated into a group of 25 human selenoproteins by a complex molecular machinery that identifies a translation stop codon instead as a selenocysteine insertion site

by adjacent selenocysteine insertion sequences. These selenoproteins exert antioxidant and antiinflammatory functions, and again could contribute to promoting or restricting signals that enhance uterine contractility and decidual function. In addition, iodothyronine deiodinase is a selenoprotein, and altered thyroid activity could affect myometrial contractility and birth timing. Perhaps most relevant in the identification of this locus in risk for preterm birth is the potential role for selenium itself, an essential micronutrient, in shaping preterm birth risk. Data suggest that selenium deficiency may predispose women to preterm birth, with the suggestion that selenium supplementation in selenium-deficient individuals could improve their pregnancy outcomes.¹⁶⁵

EBF1 demonstrated the strongest association with risk for preterm birth. This locus also was associated with birth weight in the maternal genome, likely caused by its effects on gestational duration. *EBF1* encodes early B-cell factor 1 that is involved in B-cell function, which has been recently linked to preterm birth risk,¹⁶⁶ and also is associated with a variety of cardiovascular and metabolic traits potentially influencing birth timing.

Lastly, *RAP2C*, which encodes RAS-related protein-2c and is a member of the RAS oncogene family, is most highly expressed in the uterus among other profiled tissues. Its role remains to be determined.

RARE VARIANT ANALYSIS

The GWAS described earlier analyzes the relationship of common human genomic variants with adverse pregnancy outcomes. The question subsequently arises as to whether rare, more highly penetrant mutations exist that also could contribute to preterm birth risk. Rare variants often can be shared with close family members and provide an additional avenue for genome sequencing to reveal new loci associated with birth timing. Until now, there have been few family-based rare variant and birth timing studies. Hallman and colleagues used linkage analysis to identify variants around the *IGF-1* receptor with risk for preterm birth in the infant genome.¹⁶⁷ Subsequently, McElroy et al. revealed an overrepresentation of variants associated with coagulation and complement pathways with risk for preterm birth.¹⁶⁸ The largest rare variant study to date, by Huusko and colleagues, demonstrated again the importance of steroid signaling variants and particularly found shared risk around variants in *HSPA1L*, which modulates steroid signaling.¹⁶⁹ This next-generation sequencing approach should also be useful for revealing many pathogenic variants related to adverse pregnancy outcomes beyond preterm birth.

MENDELIAN RANDOMIZATION AND CAUSATIVE PATHWAYS

One new avenue for investigation of genetic variants associated with birth timing is application of Mendelian randomization approaches to pregnancy outcomes. This approach seeks to determine the causative relationship of traits associated with a specific phenotype, in this case preterm birth. Zhang and colleagues reported in 2015 that maternal height was associated with risk for preterm birth.¹⁷⁰ They went on to show that construction of a genetic risk score based on known height-associated SNPs predicted maternal height and duration of gestation. Intriguingly, although transmission of growth promoting alleles determined fetal growth parameters from the mother to the fetus, the duration of gestation was most strongly shaped by maternal nontransmitted height variants, suggesting that maternal height is a causative factor for preterm birth risk and that genetic programming of maternal environment shapes pregnancy outcomes.

FETAL GENOME

Most genetic evidence suggests that the majority of the genetic risk for preterm birth resides within the maternal genome. The study mentioned earlier by Hallman and colleagues revealed the *IGF1R* locus had a predominantly fetal effect on preterm birth risk.¹⁶⁷ Recently, a preprint of a GWAS of the fetal genome has been deposited that implicates the proinflammatory locus around the *IL-1* gene as being involved in preterm birth risk in the fetus.¹⁷¹

This highly plausible target is supported with biochemical data. Although the evidence for the *IL-1* region is robust, it should be noted that in the cohort of approximately 80,000 pregnancies, only this single locus was substantiated. This finding is again consistent with the majority of risk for preterm birth being in the maternal genome.

CONCLUSION

Despite years of studying the symbiotic relationship between mother and fetus, there remains much that we do not completely understand about pregnancy and birth. Hormonally, estrogen and progesterone establish, maintain, and end pregnancy. By studying the birth process in other animals, changes refined by evolution throughout centuries, and new genetic knowledge, we can begin to decipher the mystery of what causes pregnancy complications and what triggers birth around 40 weeks in most women and prematurely in others. For example, we know the role of the fetal HPA axis and the abrupt fall of circulating maternal serum progesterone that sheep experience is not duplicated in women. In fact, shifts in progesterone during the birth process seem to be decidedly species specific. We do know, however, that despite the different roles in animals and humans, hormones and their function in maternal-fetal cross-talk, tug-of-war, or modulation of signaling throughout human pregnancy remain a key focus. Equilibrium must be established in pregnancy between the needs of the mother and the needs of the fetus, balancing the nutritional requirements and hormonal fluctuations that must endure for a healthy pregnancy and delivery at term.

We also recognize the critical importance of viewing pregnancy through an evolutionary frame: hormonal interactions have evolved through natural selection to increase a woman's chance of a successful pregnancy. Physiologically, encephalization and bipedalism are clear reasons a pregnancy should not continue past 40 weeks or risk the baby is too large to make it through the birth canal. Yet, some complications, such as gestational diabetes, may result from a mismatch between our ancestral and modern diets and environments. And, in fact, some researchers believe this disparity may contribute to the growing obesity epidemic, as well.

Finally, as we turn our focus on understanding contributions of the maternal and fetal genomes (and paternal contribution, although so far, that appears to not have an impact), GWAS have uncovered four genes in the maternal genome that have endocrine signaling ramifications: *AGTR2*, *WNT4*, *ADCY5*, and *EEFSEC*. Rare variant analysis and Mendelian randomization are two other tools researchers are using to discover causative pathways leading to preterm birth.

Preterm birth is the leading cause of infant mortality throughout the world, often affecting those infants who do survive with a lifetime of costly health issues. Some urban areas in the United States have premature birth rates higher than some developing countries. Maternal mortality—defined by the World Health Organization as the death of the mother within 42 days of giving birth—is also a growing concern because many deaths occur from preventable causes, such as hemorrhage or preeclampsia. It remains critical that we continue

working on understanding the causes of pregnancy complications and parturition timing. With emerging technologies and high-dimensional omics platforms, there has never been greater opportunity to study human pregnancy directly and further elucidate mechanisms.

None of these have major effects on birth timing. Evidence that pregnancy is prolonged in anencephaly and sulfatase deficiency is sketchy, and most steroidogenic enzyme deficiencies (placental aromatase and fetal adrenal enzymes) do not affect birth timing. Preeclampsia may increase risk for preterm birth via indirect mechanisms related to fetal/maternal stress. This is all speculation, however. Regarding placental aromatase deficiency, the major effect is to virilize the female fetus and mother.

REFERENCES

- Godfrey KM, Gluckman PD, Hanson MA. Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends Endocrinol Metab*. 2010;21(4):199–205.
- Dobzhansky T. Nothing in biology makes sense except in the light of evolution. *Am Biol Teacher*. 1973;35:3125–3129.
- Vento-Tormo R, et al. Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature*. 2018;563(7731):347.
- Rightmire GP. Brain size and encephalization in early to mid-Pleistocene Homo. *Am J Phys Anthropol*. 2004;124(2):109–123.
- Betti L, Manica A. Human variation in the shape of the birth canal is significant and geographically structured. *Proc Royal Soc Biol Sci*. 2018;285(1889).
- Plunkett J, et al. An evolutionary genomic approach to identify genes involved in human birth timing. *PLoS Genet*. 2011;7(4):e1001365.
- Rosenberg KR, Trevathan WR. The evolution of human birth. *Sci Am*. 2001;285(5):72–77.
- Kammerer U, von Wolff M, Markert UR. Immunology of human endometrium. *Immunobiology*. 2004;209(7):569–574.
- Dimitriadis E, et al. Cytokines, chemokines and growth factors in endometrium related to implantation. *Hum Reprod Update*. 2005;11(6):613–630.
- Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. *J Endocrinol*. 2011;210(1):5–14.
- Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev*. 2014;35(6):851–905.
- Bentin-Ley U. Relevance of endometrial pinopodes for human blastocyst implantation. *Hum Reprod*. 2000;15(6):67–73.
- Rarani FZ, Borhani F, Rashidi B. Endometrial pinopode biomarkers: Molecules and microRNAs. *J Cell Physiol*. 2018;233(12):9145–9158.
- Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med*. 1999;340(23):1796–1799.
- Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. In: *Seminars in Reproductive Medicine*. © Thieme Medical Publishers; 2007.
- Herington JL, Bany BM. Do molecular signals from the conceptus influence endometrium decidualization in rodents? *J Exp Zool (B)*. 2009;312(8):797–816.
- Le-Bouteiller P. HLA-G in the human placenta: expression and potential functions. *Biochem Soc Trans*. 2000;28(2):208–212.
- van Beekhuizen H, et al. Natural killer cells and HLA-G expression in the basal decidua of human placenta adhesiva. *Placenta*. 2010;31(12):1078–1084.
- Liu K-J, et al. Surface expression of HLA-G is involved in mediating immunomodulatory effects of placenta-derived multipotent cells (PDMCs) towards natural killer lymphocytes. *Cell Transplant*. 2011;20(11-12):1721–1730.
- Jerzak M, Bischof P. Apoptosis in the first trimester human placenta: the role in maintaining immune privilege at the maternal-foetal interface and in the trophoblast remodelling. *Eur J Obstet Gynecol Reprod Biol*. 2002;100(2):138–142.

21. Corrao S, et al. Human Hsp10 and early pregnancy factor (EPF) and their relationship and involvement in cancer and immunity: current knowledge and perspectives. *Life Sci*. 2010;86(5-6):145–152.
22. Anderle C, et al. Human trophoblast cells express the immunomodulator progesterone-induced blocking factor. *J Reprod Immunol*. 2008;79(1):26–36.
23. von Rango U, et al. Cytokine microenvironments in human first trimester decidua are dependent on trophoblast cells. *Fertil Steril*. 2003;79(5):1176–1186.
24. Demir R, et al. Classification of human placental stem villi: review of structural and functional aspects. *Microsc Res Tech*. 1997;38(1-2):29–41.
25. Red-Horse K, et al. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *J Clin Invest*. 2004;114(6):744–754.
26. Handwerger S, Richards RG, Markoff E. The physiology of decidual prolactin and other decidual protein hormones. *Trends Endocrinol Metab*. 1992;3(3):91–95.
27. Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes*. 2011;18(6):409–416.
28. Costa MA. The endocrine function of human placenta: an overview. *Reprod Biomed Online*. 2016;32(1):14–43.
29. Acikgoz S, et al. Levels of oxidized LDL, estrogens, and progesterone in placenta tissues and serum paraoxonase activity in pre-eclampsia. *Mediators Inflamm*. 2013;2013: 862982.
30. Voltolini C, Petraglia F. Neuroendocrinology of pregnancy and parturition. *Handb Clin Neurol*. 2014;124:17–36.
31. Levy F. Neuroendocrine control of maternal behavior in non-human and human mammals. *Ann Endocrinol (Paris)*. 2016;77(2): 114–125.
32. Lin B, Zhu S, Shao B. Changes of plasma levels of monoamines in normal pregnancy and pregnancy-induced hypertension women and their significance. *Zhonghua Fu Chan Ke Za Zhi*. 1996;31(11):670–672.
33. Nakamura Y, et al. Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. *J Pineal Res*. 2001;30(1):29–33.
34. Bajoria R, Babawale M. Ontogeny of endogenous secretion of immunoreactive-thyrotropin releasing hormone by the human placenta. *J Clin Endocrinol Metab*. 1998;83(11):4148–4155.
35. de Pedro MA, et al. Circadian Kisspeptin expression in human term placenta. *Placenta*. 2015;36(11):1337–1339.
36. Kim SC, et al. The regulation of oxytocin and oxytocin receptor in human placenta according to gestational age. *J Mol Endocrinol*. 2017;59(3):235–243.
37. Goland RS, et al. High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. *J Clin Endocrinol Metab*. 1986;63:1199–1203.
38. Frim DM, et al. Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. *J Clin Invest*. 1988;82:287–292.
39. Petraglia F, Sutton S, Vale W. Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. *Am J Obstet Gynecol*. 1989;160(1):247–251.
40. Ni X, et al. Nitric oxide inhibits corticotropin-releasing hormone exocytosis but not synthesis by cultured human trophoblasts. *J Clin Endocrinol Metab*. 1997;82(12):4171–4175.
41. Robinson BG, et al. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci*. 1988;85(14):5244–5248.
42. Deal CL, et al. Ontogeny of growth factor receptors in the human placenta. *Pediatr Res*. 1982;16(10):820–826.
43. Bowen JM, et al. Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition. *Placenta*. 2002;23(4):257–273.
44. Demendi C, et al. Gene expression patterns of insulin-like growth factor 1, 2 (IGF-1, IGF-2) and insulin-like growth factor binding protein 3 (IGFBP-3) in human placenta from preterm deliveries: influence of additional factors. *Eur J Obstet Gynecol Reprod Biol*. 2012;160(1):40–44.
45. Hiden U, et al. Insulin and the IGF system in the human placenta of normal and diabetic pregnancies. *J Anat*. 2009;215(1):60–68.
46. Casellas A, et al. Insulin-like growth factor 2 overexpression induces beta-cell dysfunction and increases beta-cell susceptibility to damage. *J Biol Chem*. 2015;290(27):16772–16785.
47. Modi H, et al. Autocrine action of IGF2 regulates adult β cell mass and function. *Diabetes*. 2015;64(12):4148–4157.
48. Sferruzzi-Perri AN, et al. Hormonal and nutritional drivers of intrauterine growth. *Curr Opin Clin Nutr Metab Care*. 2013;16(3): 298–309.
49. Martin-Estal I, de la Garza RG, Castilla-Cortazar I. Intrauterine growth retardation (IUGR) as a novel condition of insulin-like growth factor-1 (IGF-1) deficiency. *Rev Physiol Biochem Pharmacol*. 2016;170:1–35.
50. Clark DE, et al. Localization of VEGF and expression of its receptors flt and KDR in human placenta throughout pregnancy. *Hum Reprod (Oxford, England)*. 1996;11(5):1090–1098.
51. Huppertz B. An updated view on the origin and use of angiogenic biomarkers for preeclampsia. *Expert Rev Mol Diagn*. 2018;18(12): 1053–1061.
52. Di Blasio AM, et al. Basic fibroblast growth factor messenger ribonucleic acid levels in human placentas from normal and pathological pregnancies. *Mol Hum Reprod*. 1997;3(12):1119–1123.
53. Arany E, Hill DJ. Fibroblast growth factor-2 and fibroblast growth factor receptor-1 mRNA expression and peptide localization in placentae from normal and diabetic pregnancies. *Placenta*. 1998;19(2-3):133–142.
54. Aye IL, Powell TL, Jansson T. Review: adiponectin—the missing link between maternal adiposity, placental transport and fetal growth? *Placenta*. 2013;34:S40–S45.
55. Toro AR, et al. Leptin is an anti-apoptotic effector in placental cells involving p53 downregulation. *PLoS ONE*. 2014;9(6): e99187.
56. Dos Santos E, et al. The roles of leptin and adiponectin at the fetal-maternal interface in humans. *Horm Mol Biol Clin Investig*. 2015;24(1):47–63.
57. Struwe E, et al. Gene expression of placental hormones regulating energy balance in small for gestational age neonates. *Eur J Obstet Gynecol Reprod Biol*. 2009;142(1):38–42.
58. Dye RB, Rabinovici J, Jaffe RB. Inhibin and activin in reproductive biology. *Obstet Gynecol Surv*. 1992;47(3):173–185.
59. Rabinovici J, et al. Localization and regulation of the activin-A dimer in human placental cells. *J Clin Endocrinol Metab*. 1992;75(2):571–576.
60. Petraglia F, et al. High levels of fetal membrane activin β A and activin receptor IIB mRNAs and augmented concentration of amniotic fluid activin A in women in term or preterm labor. *J Endocrinol*. 1997;154(1):95.
61. Florio P, et al. Inhibins and activins in pregnancy. *Mol Cell Endocrinol*. 2004;225(1-2):93–100.
62. Petraglia F, Vaughan J, Vale W. Inhibin and activin modulate the release of gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone from cultured human placental cells. *Proc Natl Acad Sci*. 1989;86(13):5114–5117.
63. Yokoyama Y, et al. Identification of activins and follistatin proteins in human follicular fluid and placenta. *J Clin Endocrinol Metab*. 1995;80(3):915–921.
64. Florio P, et al. Changes in inhibins and activin secretion in healthy and pathological pregnancies. *Mol Cell Endocrinol*. 2001;180(1-2): 123–130.
65. Jessmon P, Leach RE, Armant DR. Diverse functions of HBEGF during pregnancy. *Mol Reprod Dev*. 2009;76(12):1116–1127.
66. Leach RE, et al. Pre-eclampsia and expression of heparin-binding EGF-like growth factor. *Lancet*. 2002;360(9341):1215–1219.
67. Moll SJ, et al. Epidermal growth factor rescues trophoblast apoptosis induced by reactive oxygen species. *Apoptosis*. 2007;12(9): 1611–1622.
68. Tong M, Chamley LW. Placental extracellular vesicles and fetomaternal communication. *Cold Spring Harb Perspect Med*. 2015; 5(3):a023028.
69. Tong M, et al. Micro- and nano-vesicles from first trimester human placentae carry Flt-1 and levels are increased in severe pre-eclampsia. *Front Endocrinol*. 2017;8(174).
70. Mitchell MD, et al. Placental exosomes in normal and complicated pregnancy. *Am J Obstet Gynecol*. 2015;213(4 Suppl): S173–S181.

71. Southcombe J, et al. The immunomodulatory role of syncytiotrophoblast microvesicles. *PLoS One*. 2011;6(5). e20245.
72. Abumaree MH, et al. Trophoblast debris modulates the expression of immune proteins in macrophages: a key to maternal tolerance of the fetal allograft? *J Reprod Immunol*. 2012;94(2):131–141.
73. Delorme-Axford E, et al. Human placental trophoblasts confer viral resistance to recipient cells. *Proc Natl Acad Sci U S A*. 2013;110(29):12048–12053.
74. Mincheva-Nilsson L, Baranov V. Placenta-derived exosomes and syncytiotrophoblast microparticles and their role in human reproduction: immune modulation for pregnancy success. *Am J Reprod Immunol*. 2014;72(5):440–457.
75. Ezrin AM, et al. Circulating serum-derived microparticles provide novel proteomic biomarkers of spontaneous preterm birth. *Am J Perinatol*. 2015;32(6):605–614.
76. Gupta AK, et al. Detection of fetal DNA and RNA in placenta-derived syncytiotrophoblast microparticles generated in vitro. *Clin Chem*. 2004;50(11):2187–2190.
77. Alberry M, et al. Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenat Diagn*. 2007;27(5):415–418.
78. Lo YM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet*. 1998;62(4):768–775.
79. Mackie FL, et al. The accuracy of cell-free fetal DNA-based noninvasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *BJOG*; 2017;124(1):32–46.
80. Heung MM, et al. Placenta-derived fetal specific mRNA is more readily detectable in maternal plasma than in whole blood. *PLoS One*. 2009;4(6). e5858.
81. Mouillet JF, et al. MicroRNAs in placental health and disease. *Am J Obstet Gynecol*. 2015;213(4 Suppl):S163–S172.
82. Manokhina I, Wilson SL, Robinson WP. Noninvasive nucleic acid-based approaches to monitor placental health and predict pregnancy-related complications. *Am J Obstet Gynecol*. 2015;213(4 Suppl):S197–S206.
83. Simpson ER. Genetic mutations resulting in loss of aromatase activity in humans and mice. *J Soc Gynecol Invest*. 2007;7(1 Suppl):S18–S21.
84. Simpson ER, et al. Aromatase—A brief overview. *Annual Rev Physiol*. 2002;64(1):93–127.
85. Pepe GJ, Albrecht ED. Regulation of the primate fetal adrenal cortex. *Endocr Rev*. 1990;11:151–176.
86. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*. 1997;18(3):378–403.
87. Ishimoto H, Jaffe RB. Development and function of the human fetal adrenal cortex: a key component in the fetoplacental unit. *Endocr Rev*. 2011;32(3):317–355.
88. Baker BL, Jaffe RB. The genesis of cell types in the adenohipophysis of the human fetus as observed with immunocytochemistry. *Am J Anat*. 1975;143(2):137–161.
89. Bedin M, et al. Incidence of placental sulfatase deficiency on the mode of termination of pregnancy. *Gynecol Obstet Invest*. 1987;24(2):86–91.
90. Shapiro LJ, et al. Steroid sulfatase deficiency. *Pediatr Res*. 1977;11(8):894–897.
91. Smith R, et al. Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. *J Clin Endocrinol Metab*. 1999;84:2820–2825.
92. McLean M, et al. A placental clock controlling the length of human pregnancy. *Nat Med*. 1995;1:460–463.
93. Rehman KS, et al. The regulation of adrenocorticotrophic hormone receptor by corticotropin-releasing hormone in human fetal adrenal definitive/transitional zone cells. *Reprod Sci*. 2007;14(6):578–587.
94. Miller WL. The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab*. 2012;97(1):59–67.
95. Mesiano S, Coulter CL, Jaffe RB. Localization of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17 alpha-hydroxylase/17, 20-lyase, and 3 beta-hydroxysteroid dehydrogenase isomerase steroidogenic enzymes in human and rhesus monkey fetal adrenal glands: reappraisal of functional zonation. *J Clin Endocrinol Metab*. 1993;77(5):1184–1189.
96. Johnston ZC, et al. The human fetal adrenal produces cortisol but no detectable aldosterone throughout the second trimester. *BMC Med*. 2018;16(1):23.
97. Bayard F, et al. Transplacental passage and fetal secretion of aldosterone. *J Clin Invest*. 1970;49:1389–1393.
98. Nelson H, et al. Human fetal adrenal definitive and fetal zone metabolism of pregnenolone and corticosterone: Alternate biosynthetic pathways and absence of detectable aldosterone synthesis. *J Clin Endocrinol Metab*. 1990;70:693–698.
99. Serón-Ferré M, Biglieri EG, Jaffe RB. Regulation of mineralocorticoid secretion by the superfused fetal monkey adrenal gland: lack of stimulation of aldosterone by ACTH. *J Dev Physiol*. 1990;13:33–36.
100. Liggins GC. The role of cortisol in preparing the fetus for birth. *Reprod Fertil Dev*. 1994;6(2):141–150.
101. Liggins GC. Premature delivery of foetal lambs infused with glucocorticoids. *J Endocrinol*. 1969;45(4):515–523.
102. Yang K. Placental 11 β hydroxysteroid dehydrogenase: Barrier to maternal glucocorticoids. *Rev Reprod*. 1997;2:129–132.
103. Price HV, Cone BR, Keogh M. Length of gestation in congenital adrenal hyperplasia. *J Obstet Gynecol Br Comm*. 1971;78:430–434.
104. Patrick J, et al. Circadian rhythms in maternal plasma cortisol, estrone, estradiol, and estriol at 34 to 35 weeks' gestation. *Am J Obstet Gynecol*. 1979;135(6):791–798.
105. Casey ML, MacDonald PC, Simpson ER. Endocrinological changes of pregnancy. In: Wilson JD, Foster DW, eds. *Williams Textbook of Endocrinology*. Philadelphia: W.B. Saunders Company; 1992:977–991.
106. Young IR, et al. The comparative physiology of parturition in mammals: Hormones and parturition in mammals. In: Norris D, Lopez K, eds. *Hormones and Reproduction in Vertebrates*. London: Academic Press; 2010.
107. Grammatopoulos D, et al. Human corticotropin-releasing hormone receptor: differences in subtype expression between pregnant and nonpregnant myometria. *J Clin Endocrinol Metab*. 1998;83(7):2539–2544.
108. Grammatopoulos D, Hillhouse EW. Solubilization and biochemical characterization of the human myometrial corticotrophin-releasing hormone receptor. *Mol Cell Endocrinol*. 1998;138(1-2):185–198.
109. Grammatopoulos DK. The role of CRH receptors and their agonists in myometrial contractility and quiescence during pregnancy and labour. *Front Biosci*. 2007;12:561–571.
110. Jin D, et al. Expression of corticotropin-releasing hormone receptor type 1 and type 2 in human pregnant myometrium. *Reprod Sci*. 2007;14(6):568–577.
111. Jones SA, Challis JR. Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. *Biochem Biophys Res Comm*. 1989;159(1):192–199.
112. Jones SA, Challis JR. Effects of corticotropin-releasing hormone and adrenocorticotropin on prostaglandin output by human placenta and fetal membranes. *Gynecol Obstet Invest*. 1990;29(3):165–168.
113. Quartero HW, Fry CH. Placental corticotrophin releasing factor may modulate human parturition. *Placenta*. 1989;10(5):439–443.
114. Quartero HW, et al. Role of prostaglandins and leukotrienes in the synergistic effect of oxytocin and corticotropin-releasing hormone (CRH) on the contraction force in human gestational myometrium. *Prostaglandins*. 1991;42(2):137–150.
115. Benedetto C, et al. Corticotropin-releasing hormone increases prostaglandin F2 alpha activity on human myometrium in vitro. *Am J Obstet Gynecol*. 1994;171:126–131.
116. Grammatopoulos D, et al. The biological activity of the corticotropin-releasing hormone receptor-adenylate cyclase complex in human myometrium is reduced at the end of pregnancy. *J Clin Endocrinol Metab*. 1996;81(2):745–751.
117. Flint APP, et al. The mechanism by which foetal cortisol controls the onset of parturition in the sheep. *Biochem Soc Trans*. 1975;3:1189–1194.
118. Walsh S, Kittinger G, Novy M. Maternal peripheral concentrations of estradiol, estrone, cortisol, and progesterone during late pregnancy in rhesus monkeys (*Macaca mulatta*) and after

- experimental fetal anencephaly and fetal death. *Am J Obstet Gynecol.* 1979;135:37–42.
119. Neilson JP. Mifepristone for induction of labour. *Cochrane Database Syst Rev.* 2000;4. CD002865.
 120. Merlino A, et al. Nuclear progesterone receptor expression in the human fetal membranes and decidua at term before and after labor. *Reprod Sci.* 2009;16(4):357–363.
 121. Tung L, et al. Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. *Mol Endocrinol.* 1993;7(10):1256–1265.
 122. Vegeto E, et al. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol.* 1993;7(10):1244–1255.
 123. Giangrande PH, et al. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol.* 2000;20(9):3102–3115.
 124. Pieber D, et al. Interaction between progesterone receptor isoforms in myometrial cells in human labour. *Mol Hum Reprod.* 2001;7(9):875–879.
 125. Condon JC, et al. Up-regulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor-kappaB may contribute to the onset of labor through inhibition of PR function. *Mol Endocrinol.* 2006;20(4):764–775.
 126. Hardy DB, et al. Progesterone receptor plays a major anti-inflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. *Mol Endocrinol.* 2006;20(11):2724–2733.
 127. Merlino AA, et al. Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. *J Clin Endocrinol Metab.* 2007;92(5):1927–1933.
 128. Mesiano S, et al. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab.* 2002;87(6):2924–2930.
 129. Oh SY, et al. Progesterone receptor isoform (A/B) ratio of human fetal membranes increases during term parturition. *Am J Obstet Gynecol.* 2005;193(3 Pt 2):1156–1160.
 130. Chai SY, et al. Increased progesterone receptor A expression in labouring human myometrium is associated with decreased promoter occupancy by the histone demethylase JARID1A. *Mol Hum Reprod.* 2014;20(5):442–453.
 131. Ke W, et al. Histone deacetylase 1 regulates the expression of progesterone receptor A during human parturition by occupying the progesterone receptor A promoter. *Reprod Sci.* 2016;23(7):955–964.
 132. Condon JC, et al. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Natl Acad Sci U S A.* 2003;100(16):9518–9523.
 133. Kalkhoven E, et al. Negative interaction between the RelA(p65) subunit of NF-kappaB and the progesterone receptor. *J Biol Chem.* 1996;271(11):6217–6224.
 134. Nadeem L, et al. Molecular evidence of functional progesterone withdrawal in human myometrium. *Nat Commun.* 2016;7:11565.
 135. Amini P, et al. Human parturition involves phosphorylation of progesterone receptor-A at serine-345 in myometrial cells. *Endocrinology.* 2016;157(11):4434–4445.
 136. Romero R, Mazor M. Infection and preterm labor. *Clin Obstet Gynecol.* 1988;31(3):553–584.
 137. Romero R, et al. Infection in the pathogenesis of preterm labor. *Semin Perinatol.* 1988;12(4):262–279.
 138. Gibbs RS, et al. A review of premature birth and subclinical infection. *Am J Obstet Gynecol.* 1992;166(5):1515–1528.
 139. Andrews WW, Hauth JC, Goldenberg RL. Infection and preterm birth. *Am J Perinatol.* 2000;17(7):357–365.
 140. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. *N Engl J Med.* 2000;342(20):1500–1507.
 141. Goldenberg RL, Culhane JF. Infection as a cause of preterm birth. *Clin Perinatol.* 2003;30(4):677–700.
 142. Boggess KA. Pathophysiology of preterm birth: emerging concepts of maternal infection. *Clin Perinatol.* 2005;32(3):561–569.
 143. Romero R, et al. The role of inflammation and infection in preterm birth. *Semin Reprod Med.* 2007;25(1):21–39.
 144. Gravett MG, et al. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *Am J Obstet Gynecol.* 1994;171(6):1660–1667.
 145. Baggia S, et al. Interleukin-1 beta intra-amniotic infusion induces tumor necrosis factor-alpha, prostaglandin production, and preterm contractions in pregnant rhesus monkeys. *J Soc Gynecol Invest.* 1996;3(3):121–126.
 146. Romero R, Mazor M, Tartakovsky B. Systemic administration of interleukin-1 induces preterm parturition in mice. *Am J Obstet Gynecol.* 1991;165(4 Pt 1):969–971.
 147. Hirsch E, Muhle R. Intrauterine bacterial inoculation induces labor in the mouse by mechanisms other than progesterone withdrawal. *Biol Reprod.* 2002;67(4):1337–1341.
 148. Sadowsky DW, et al. Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. *Am J Obstet Gynecol.* 2006;195(6):1578–1589.
 149. Cox SM, Casey ML, MacDonald PC. Accumulation of interleukin-1beta and interleukin-6 in amniotic fluid: a sequela of labour at term and preterm. *Hum Reprod Update.* 1997;3(5):517–527.
 150. Thomson AJ, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. *Hum Reprod.* 1999;14(1):229–236.
 151. Osman I, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. *Mol Hum Reprod.* 2003;9(1):41–45.
 152. Mittal P, et al. Characterization of the myometrial transcriptome and biological pathways of spontaneous human labor at term. *J Perinat Med.* 2010;38(6):617–643.
 153. Trivedi S, et al. Fetal-placental inflammation, but not adrenal activation, is associated with extreme preterm delivery. *Am J Obstet Gynecol.* 2012;206(3):e1–e8. 236.
 154. Norwitz ER, et al. Molecular regulation of parturition: the role of the decidual clock. *Cold Spring Harb Perspect Med.* 2015;5(11).
 155. Tan H, et al. Progesterone receptor-A and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. *J Clin Endocrinol Metab.* 2012;97(5):E719–E730.
 156. Lei K, et al. Progesterone and the repression of myometrial inflammation: the roles of MKP-1 and the AP-1 system. *Mol Endocrinol.* 2015;29(10):1454–1467.
 157. Georgiou EX, et al. The study of progesterone action in human myometrial explants. *Mol Hum Reprod.* 2016;22(8):877–889.
 158. Edey LE, et al. Progesterone, the maternal immune system and the onset of parturition in the mouse. *Biol Reprod.* 2017;98(3):376–395.
 159. Peters GA, et al. Inflammatory stimuli increase progesterone receptor-a stability and transrepressive activity in myometrial cells. *Endocrinology.* 2017;158(1):158–169.
 160. Amini P, et al. Mechanism by which progesterone and cAMP synergize to maintain uterine quiescence during pregnancy. *Mol Cell Endocrinol.* 2019;479:1–11.
 161. Boyd HA, et al. Maternal contributions to preterm delivery. *Am J Epidemiol.* 2009;170(11):1358–1364.
 162. Porter TF, et al. The risk of preterm birth across generations. *Obstet Gynecol.* 1997;90(1):63–67.
 163. Winkvist A, Mogren I, Högberg U. Familial patterns in birth characteristics: impact on individual and population risks. *Int J Epidemiol.* 1998;27(2):248–254.
 164. Zhang G, et al. Genetic associations with gestational duration and spontaneous preterm birth. *N Engl J Med.* 2017;377(12):1156–1167.
 165. Rayman MP, et al. Maternal selenium status during early gestation and risk for preterm birth. *CMAJ.* 2011;183(5):549–555.
 166. Huang B, et al. Interleukin-33-induced expression of PIBF1 by decidual B cells protects against preterm labor. *Nat Med.* 2017;23(1):128–135.
 167. Haataja R, et al. Mapping a new spontaneous preterm birth susceptibility gene, IGF1R, using linkage, haplotype sharing, and association analysis. *PLoS Genet.* 2011;7(2). e1001293.

168. McElroy JJ, et al. Maternal coding variants in complement receptor 1 and spontaneous idiopathic preterm birth. *Hum Genet.* 2013;132(8):935–942.
169. Huusko JM, et al. Whole exome sequencing reveals HSPA1L as a genetic risk factor for spontaneous preterm birth. *PLoS Genet.* 2018;14(7). e1007394.
170. Zhang G, et al. Assessing the causal relationship of maternal height on birth size and gestational age at birth: a mendelian randomization analysis. *PLoS Med.* 2015;12(8). e1001865.
171. Liu X, et al. Variants in the fetal genome near pro-inflammatory cytokine genes on 2q13 are associated with gestational duration. *BioRxiv.* 2018. 423897.

6

Ambiguous Genitalia

Selma Feldman Witchel, Peter A. Lee

CHAPTER OUTLINE

INTRODUCTION	124
TALKING WITH THE PARENTS	124
TERMINOLOGY	125
SEX DETERMINATION	126
DEVELOPMENT OF THE REPRODUCTIVE SYSTEM	126
Urogenital Ridge and Bipotential Gonad Development	126
Human Testicular Development	127
Human Ovary Development	128
Germ Cell Development	128
Human Adrenal Development	129
Development of Internal Genital Structures	129
Development of External Genital Structures	130
Anogenital Distance	130
Sexual Differentiation of the Brain	130
MOUSE MODELS	130
SEX CHROMOSOME DISORDERS	131
45,X/46,XY Mosaicism	131
DISORDERS ASSOCIATED WITH DISORDERS OF SEX DEVELOPMENT AND ADDITIONAL CONGENITAL ANOMALIES	131
ARX (X-Linked Lissencephaly 2)	131
CDKN1C (IMAGe Syndrome)	131
CHD7 (CHARGE Syndrome)	133
DHCR7 (Smith-Lemli-Opitz)	133
EMX2 Gene	133
GLI3 (Pallister-Hall Syndrome)	133
HHAT (Nivelon-Nivelon-Mabille Syndrome)	133
HOXA13 (Hand-Foot-Genital Syndrome)	133
KAT6B (Genitopatellar Syndrome)	134
NR2F2 Gene	134
RSP01	134
SAMD9 (MIRAGE Syndrome)	134
SOX9	134
TBX3 (Ulnar-Mammary syndrome)	135
WT1	135
XH2 (ATRX Syndrome)	135
DISORDERS ASSOCIATED WITH 46,XX DISORDERS OF SEX DEVELOPMENT AND 46,XY DISORDERS OF SEX DEVELOPMENT	136
SF1/NR5A1 Gene	136
SOX8	137
NONSYNDROMIC 46,XY DISORDERS OF SEX DEVELOPMENT (GONADAL DYSGENESIS)	137
Chromobox Homolog 2 (CBX2)	137
Desert Hedgehog	137
Doublesex and Mab-3–Related Transcription Factor 1 (DMRT1) and Doublesex and Mab-3–Related Transcription Factor 2 (DMRT2)	137
GATA4	137
Mastermind-Like Domain Containing 1 (MAMLD1)	137
Mitogen-Activated Kinase Kinase Kinase 1 (MAP3K1)	137
Nuclear Receptor Subfamily 0, Group B, Member 1 (NROB1)	138
PBX1 (Pre-B-Cell-Leukemia Factor 1)	138
Protein Phosphatase Two Regulatory Subunit B”Gamma (PPP2R3C)	138
SRY	138
WW Domain-Containing Oxidoreductase (WWOX)	138
Zinc Finger and Ring Finger Protein 3 (ZNRF3)	139
Zinc Finger Protein, Multitype 2 (ZFPM2)	139
OVOTESTICULAR DISORDER OF SEX DEVELOPMENT	139
NONSYNDROMIC XX DISORDERS OF SEX DEVELOPMENT	139
46,XX Testicular Disorder of Sex Development	139
XX DISORDER OF SEX DEVELOPMENT/PREATURE OVARIAN FAILURE	139
FOXL2	141
NOBOX	141
FIGLA	141
SPIDR	141
NUP107	141
WNT4 Gene	141
VANISHING TESTES	141
DISORDERS OF CHOLESTEROL AND STEROID BIOSYNTHESIS	141
Luteinizing Hormone Choriogonadotropin Receptor Gene	143
Congenital Lipoid Adrenal Hyperplasia	144
SIDE CHAIN CLEAVAGE CYTOCHROME P450 ENZYME	144
VIRILIZING CONGENITAL ADRENAL HYPERPLASIAS	144
21-Hydroxylase Deficiency	144
11 β -Hydroxylase Deficiency	146
3 β -Hydroxysteroid Dehydrogenase Deficiency	146
DEFECTS IN SEX STEROID BIOSYNTHESIS	146
17 α -Hydroxylase/17,20-Lyase Deficiency	146
Cytochrome b ₅ Deficiency	146
3 α -Hydroxysteroid Dehydrogenase Isozyme Deficiencies	146
Cytochrome P450 Oxidoreductase Deficiency	147
17 β -Hydroxysteroid Dehydrogenase Deficiency	147
5 α -Reductase Deficiency	147
Placental Aromatase Deficiency	147
MATERNAL HYPERANDROGENISM	148
DISORDERS OF ANDROGEN ACTION	148

MULLERIAN DUCT ABNORMALITIES 149

- Persistent Mullerian Duct Syndrome 149
- Mullerian Duct Abnormalities in 46,XX Individuals 149

MICROPHALLUS, HYPOSPADIAS, AND CRYPTORCHIDISM 149

- Hypospadias 149
- Cryptorchidism 149
- Hypogonadotropic Hypogonadism 151

ENVIRONMENTAL DISRUPTORS 152**OTHER DISORDERS 152****EXSTROPHY OF BLADDER 152****DIAGNOSIS 152**

- History 153
- Physical Examination 153

Laboratory Studies 154

- Genetic Tests 155
- Hormone Determinations 155
- Newborn Screening 157
- Imaging, Endoscopy, and Laparoscopy 157

TREATMENT 158

- Sex of Rearing 158
- Medical Treatment 159
- Considerations With Regard to Surgery 160
- Risks for Gonadal Tumors 161
- Transition From Pediatrics to Adult Care 161
- Long-Term Outcome Studies 161
- Psychological and Genetic Counseling and Support and Ethical Considerations 161

CONCLUSIONS 162

- References 162

INTRODUCTION

Throughout history, humans have sought to understand the differences between biological sexes and the mechanisms responsible for these biological differences.¹ Normal gonadal differentiation and sex development depend on the meticulous choreography and synchrony of a network of endocrine, paracrine, and autocrine signaling pathways. This network involves the actions and interactions of specific genes, epigenetic influences, transcription factors, and hormones. Perturbations of the intricate network of gene regulation and gene expression governing fetal gonadal development result in disorders of sex development (DSD). Approximately one in 4000 infants are estimated to be born with a DSD.^{2,3} However, this estimate increases if all variations of sex development including cryptorchidism and all forms of hypospadias are included. The prevalence for some subgroups have been merely accurately reported, such as 46,XY females at 6.4/100,000; 4.1/100,000 for androgen insensitivity syndrome (AIS); and 1.5/100,00 for gonadal dysgenesis.⁴

DSDs comprise a spectrum of disorders in which chromosomal, genetic, gonadal, hormonal, or anatomic aspects of sex are atypical. Understanding the developmental biology and embryology of the urogenital system is crucial to categorizing and identifying the molecular basis of the disorder in an individual patient. It has become apparent that multiple genes, epigenetic factors, environmental factors, and protein interactions are involved in the processes of gonadal differentiation and sex development. The pediatric endocrinologists' role in the care of an infant with ambiguous genitalia begins at the time of initial presentation—birth, childhood, adolescence, or before birth for infants identified through prenatal testing. The physician's responsibility for the management of this child starts early and continues until transition to adult care providers is deemed appropriate.

Following the 2006 Consensus Statement regarding the management of intersex disorders,⁵ knowledge regarding the molecular basis of sex differentiation/development has expanded. The number and capability of the genetic tools available to identify the molecular basis have increased. Considerations include a holistic approach to the child with a DSD, inclusion of the parents, recognition of psychological well-being, the role for support groups, and need for comprehensive longitudinal care.^{6,7} Outcome data collection, although still limited, expands medical knowledge and quality improvement. Development and evaluation of multidisciplinary care models focusing on quality of care is ongoing.⁸

TALKING WITH THE PARENTS

For parents, the birth of their child is a long-anticipated and exciting event. Because of the increased frequency of prenatal ultrasound examinations, parents have usually been told the sex of their child, may have selected their infant's name, and often hosted a "gender reveal" celebration. In some instances, abnormal genital development has been identified through ultrasound and the parents have been informed of this situation. In this scenario, parents and healthcare providers can confer regarding the likely diagnostic testing and treatment recommendations. In the absence of prior knowledge, the parents are suddenly confronted with a newborn with a birth defect involving the external genitalia and uncertainty regarding their infant's sex. Anxiety and apprehension regarding the baby's health and sex are particularly traumatic when parents had no prior awareness of the abnormal genital development.

Initially, the parents need to be congratulated on the birth of their child. They need to hear that their child has a condition affecting sex development and that this condition will be carefully and completely addressed. Appropriate discussions include sharing that abnormalities of sex development involve the complex system that directs the development of reproductive system, including external genital development. Explaining that the sex of their child cannot be established simply by examining the external genitalia is essential. It is important to emphasize that the atypical development is not the parents' fault and that they should not feel guilty or ashamed. Physician and healthcare professionals should not speculate or offer presumptive diagnoses. The parents can be reassured that all appropriate studies will be obtained to provide comprehensive information to best establish their child's sex of rearing. Use of genetic tools may ascertain the specific etiology of the DSD, with the caveat that a specific molecular etiology may remain unknown.

The goal is patient-centered care within the context of the family and a multidisciplinary team that includes pediatric endocrinologists, pediatric urologists/surgeons, geneticists, neonatologists, radiologists, behavioral health providers, and pediatric endocrine nurse educators. An initial treatment goal is to ascertain whether an underlying or associated life-threatening condition requires specific urgent treatment. One team member should serve as the principal communicator with the family.

Although contemporary society includes overt references to sex and sexuality, parents may have difficulty thinking of their child as a sexual being and feel embarrassed discussing their

child's sex, gender identity, and future sexuality. Cultural attitudes, preexisting expectations, and family support systems impact the parent's responses and may influence their choices for the infant.⁹ Parents need to be informed that the child's gender identity is a personal experience of one's gender. The medical team needs to promote an open and caring network to provide support for the parents. Importantly, the medical team needs to engage the parents in the medical decision-making process and discuss pertinent information. It is important to be aware that the clinicians' use of clinical uncertainty may misdirect parents and exacerbate their anxiety. For surgical decision-making, clinicians should strive for balance between caution and benefit and consideration of options beyond the dichotomy of "surgery" versus "no surgery."¹⁰

If the child's sex remains unclear, information needs to be obtained to assist the parents and healthcare team to determine the most appropriate sex of rearing. Usually, this can be accomplished within a matter of hours or days. In more complex instances, the diagnostic process may take longer. In situations in which it is impossible to identify the specific etiology, the general DSD category (see later) provides a basis for decision-making.

Factors relevant for the medical decision-making process include the extent of external and internal reproductive system development, evidence of gonadal functionality (potential for pubertal hormone secretion and fertility), and hormone responsiveness. In some instances, these factors are more relevant than the karyotype. Genes and gene products involved in sex development are mapped to autosomes and sex chromosomes. Genetic variants mapped to coding and noncoding regions influence sex differentiation and sex development of the fetus and child.

When consensus has been reached regarding a diagnostic category, available outcome information should be reviewed. Knowledge of a specific etiology, including immediate details and long-term outcome, enables optimal planning of therapeutic interventions and genetic counseling for future pregnancies. Healthcare providers need to be cognizant that available outcome data to assist in the decision-making processes are limited. Currently accessible information in published reports is largely based on retrospective studies obtained using diverse methods and strategies.¹¹ The extent, timing, and duration of prenatal androgen exposure, which can only be estimated, likely influences development of the central nervous system (CNS) and other tissues.¹² Prenatal androgen exposure may influence development of gender identity.

One multicenter cross-sectional study reported short-term outcome data on 92 children found to have genital ambiguity with karyotype distribution of 57%, 46,XX; 34% 46,XY; and 9% sex chromosome anomalies. Over 90% of 46,XX individuals had congenital adrenal hyperplasia (CAH); 65% of the 46,XY individuals lacked a molecular diagnosis; and most 46,XY individuals were being raised as males.¹³ Changing concepts regarding medical and surgical interventions urge caution regarding decision-making, while additional outcome studies are underway.¹⁴ Studies may address specific aspects, such as compliance with hormone therapy and patient satisfaction or psychological health, quality of life, and self-perceptions among males with CAH.

The first conversation with the parents should reinforce parental bonding with their infant. As much as possible, a positive and optimistic tone is helpful. Indeed, the emotional tone of this initial conversation is more meaningful than the factual information provided and is recalled by parents for many years. Respect for the family and individual viewpoints, together with a willingness to repeat or defer detailed explanations, are crucial. In the midst of the emotional distress associated with the uncertainty of their infant's sex, parents may be unable to

assimilate the vast amount of information that eventually needs to be shared. Repeated discussions with the parents will enable them to acknowledge their emotional and intellectual concerns regarding their infant. Familiarity and understanding will enable the parents to bond with their infant and interact with family members, friends, and colleagues.

Unless the natal sex is clear at this point, the healthcare team should recommend that the parents delay naming the infant, announcing the baby's birth, and registering the birth, until more information becomes available. The message should be clear that they will be actively involved in the process to establish the sex of rearing for their child. Until sex of rearing is established, it is best to refer to the infant as "your baby" or "your child." Terms such as *he*, *she*, and *it* should be avoided. The multidisciplinary team is responsible for educating ancillary staff regarding how to refer to the infant as "your baby."

Factual explanations regarding the process of sexual differentiation with a focus on their infant's situation should be initially outlined. The primary goal at this point is to provide the parents with a basic understanding that the internal and external genital structures for both boys and girls develop from the same primordial tissues. It is also helpful to explain that there are no exclusively male and female hormones. Rather, the environments in which male and female fetuses develop are characterized by differing relative amounts of these hormones. Using simple sketches, pictures, and diagrams can be helpful to explain the embryology of genital development to the parents. Some parents may benefit from practicing the words they will use to discuss the infant's health with other family members. Detailed explanations can be reviewed multiple times as the child ages, especially because the child is unable to actively participate in the initial discussions.

During the early conversations, examining the infant with the parents to identify the specific physical findings of their infant is often beneficial. Viewing their child's external genitalia can reduce their apprehension and reinforce the perception that their infant's needs are similar to all infants. Information can be presented to minimize anxiety and better equip parents to participate in the decision-making process. Discussion of the many concerns (particularly those related to gender identity, pubertal development, sexual orientation, sexual function, and fertility) may be helpful. Honestly addressing parents' concerns will ultimately create trust and positive feelings to help the parents promote their child's self-esteem. Ideally, each parent achieves a personal resolution with a commitment to a positive perspective for the child's future.

TERMINOLOGY

Under the auspices of the Pediatric Endocrine Society (NA) and the European Society for Pediatric Endocrinology, an international consensus statement was formulated that recommended a revised classification of the medical terminology used for disorders of sex development to avoid confusing and derogatory terms. This descriptive classification attempts to be sensitive to concerns of parents and flexible enough to incorporate novel molecular genetic information. The updated classification system integrates molecular genetic considerations into the nomenclature for "disorders of sexual differentiation (DSD)" and provides an approach to the diagnostic evaluation. There are objections to use of the word "disorders" because this implies pathology, with "differences" sometimes being used.

Terms, such as *pseudohermaphrodite*, *true hermaphrodite*, and *gender labeling* in the diagnosis should be eliminated.³ However, some patients still prefer to use the term *intersex*. To accommodate all types of DSD, the classification system is broad and includes some conditions that do not present with

TABLE 6.1 Sex Chromosomal Disorder of Sex Development

Syndrome	Karyotype	Chromosomal Defects
Turner syndrome	45,X mosaic 45,X/46,XX	X monosomy Mosaic monosomy X
Turner syndrome with structural X chromosome rearrangements	46,X,i(Xq) 46,X,del(Xp) 46,X,+mar	isochromosome Xq deletion Xp marker chromosome
Gonadal dysgenesis	45,X/46,XY XX/XY	Mosaic loss of the Y chromosome in XY, chimerism
XX male SRY gene positive Gonadal dysgenesis	46,XX or 46,X,der(X)t(X;Y)	Yp (SRY gene) translocation to the X chromosome or autosome
XX male SRY gene negative	46,XX	SRY gene is absent
Klinefelter syndrome and its variants	XXY mosaic 46,XXY/46,XY XXYY XX/XXY	Disomy X, Mosaic disomy X Disomy X and Y Mosaic loss of the Y chromosome in XXY

obvious abnormalities of genital development (Table 6.1). The primary goal of this classification system is to provide a framework for diagnosis, assessment, and care management based largely on sex chromosome status. Currently, microarray, candidate gene analyses, and whole exome/genome sequencing are increasingly used. The DSD categories include sex chromosome DSDs, such as 45,X/46,XY; ovotesticular DSD; 46,XY DSDs, such as disorders of testicular development, disorders of androgen synthesis and action, 46,XY sex reversal, and 46,XX DSDs; and 46,XX sex reversal. Some diagnoses are included in more than one category because of the complexities of chromosomal and gonadal development. The number of genes identified to be involved in sex development continues to increase. Nevertheless, despite many recent advances, the specific molecular etiology of the genital ambiguity in an individual cannot always be identified, especially among those with 46XY DSDs.

SEX DETERMINATION

Through Alfred Jost's experiments with fetal rabbits in the 1940s and 1950s, the critical requirements for a testis and testosterone for male sexual differentiation were established.¹⁵ Chromosomal composition of the human embryo, XX or XY, determines gonadal sex. Investigation of human sex chromosome evolution suggested that the human sex chromosomes evolved from a pair of ancestral autosomes approximately 300 million years ago. The emergence of the SRY locus, several Y chromosome inversions, and loss of X-Y crossing-over led to the current human situation.¹⁶

Studies of patients with disorders of sex development ultimately led to identification of the genetic locus primarily responsible for this binary switch, the sex-determining region on the Y (SRY) gene on the Y chromosome. Studies involving creation of transgenic SRY+ mice confirmed the essential role of SRY and provided further molecular understanding of testicular differentiation.^{17,18}

Genetic sex is established at fertilization followed by sex determination, the binary switch that launches the developmental destiny of the embryonic gonads to become testes or ovaries. Sex determination is largely influenced through transcriptional regulation, whereas secreted hormones and hormone receptors influence phenotypic development. Sexual

differentiation refers to the process through which male or female phenotype develops.¹⁹

The gonads, internal genital ducts, and external genital structures all develop from bipotential tissues. Each cell in the developing gonad has the potential to differentiate into either a testicular or ovarian cell, depending on how the transcriptome of the undifferentiated cell realizes its pathway to develop into an ovary or testis. In this bipotential state, pluripotency with genes poised for either activation or repression exists.²⁰ In other words, sex determination of the bipotential gonads depends on cell fate commitment to one pathway at a precise moment in development, while maintaining active repression of the alternative developmental pathway.

In the usual situation, the karyotype (46,XY or 46,XX) of the primordial gonad determines whether it differentiates into a testis or ovary, respectively. Inherent differences exist between XX and XY cells; approximately 85% of the second X chromosome in an XX cell undergoes X-inactivation and Y chromosome genes related to spermatogenesis are expressed in XY cells.²¹ Local factors (such as hormones secreted by the developing gonads or tissue-specific transcription factors) influence the ensuing differentiation of the internal and external genital structures. This process integrates sex-specific pathway signals that appear to antagonize each other.

Chromatin configuration and its spatial three-dimensional architecture influence gene expression by modulating the ability of transcription factors to bind to deoxyribonucleic acid (DNA).²² Histone modification and DNA methylation influence chromatin organization. The chromatin landscape actively modulates the unending ovary versus testis antagonism. Genes involved with male pathway development lose their repressive marks when the testis pathway is activated and vice versa for genes involved in the ovarian pathway. Cis-regulatory elements, such as silencers and enhancers, coordinate the specific spatiotemporal expression of genes within the transcriptional network; sex-specific regulatory elements are acquired during development.²³ Polycomb proteins are conserved transcriptional regulators that coordinate chromatin structure and chromosome architecture. Chromatin regulators, for example, polycomb proteins, represent critical nodes where biological signals modulate gene expression.

Divergence from the normal sequence of events leads to disorders of sex development that can manifest as abnormal gonadal differentiation, inconsistent internal genital differentiation, or ambiguity of the external genitalia. Although genital ambiguity is usually not considered to be a medical emergency, this type of birth defect is usually extremely distressing to the parents and extended family. When adrenal insufficiency accompanies the genital ambiguity, immediate evaluation and treatment are essential. Regardless, prompt referral and evaluation by a multidisciplinary team with expertise in disorders of sex differentiation is strongly recommended.

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Urogenital Ridge and Bipotential Gonad Development

The urogenital ridge gives rise to the gonads, adrenal cortex, kidney, and reproductive tract. The gonads are derived from intermediate mesoderm and depend on the correct ingression, proliferation, and orientation of the coelomic epithelial cells (Fig. 6.1). In humans, at 4 to 6 weeks of gestation, the urogenital ridges develop as paired outgrowths of coelomic epithelium (mesothelium) on the ventral side of the mesonephros. As the coelomic epithelium proliferates, its basement membrane disintegrates to allow ingression of coelomic cells to form the developing gonad. Notch signaling ensures proper

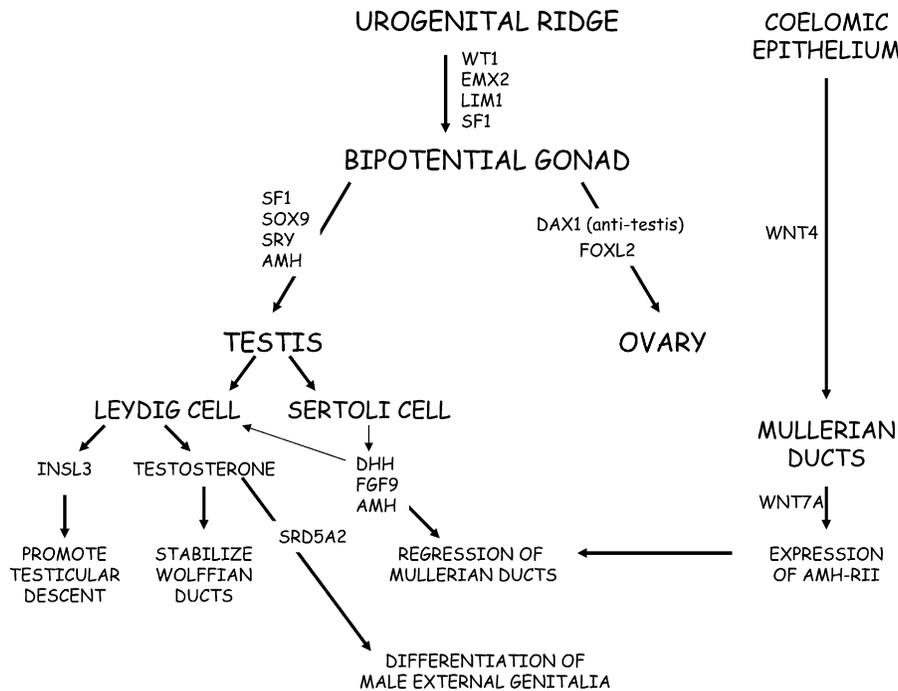


Fig. 6.1 Genes involved in the process of sexual differentiation. Wilms tumor (*WT1*), *EMX2*, *LIM1*, *CBX2*, and steroidogenic factor-1 (*SF1*) play roles in differentiation of gonad from urogenital ridge. Genes involved in testicular differentiation include *SF-1*, *SOX9*, sex-determining region on Y (*SRY*), and anti-Müllerian hormone (*AMH*). The dosage-sensitive sex-adrenal hypoplasia congenital critical region on X (*DAX1*) appears to function as an antitestis factor. *Wnt4* promotes development of the Müllerian ducts, whereas *Wnt7a* promotes expression of the receptor for *AMH* (*AMH-RII*). Sertoli cells secrete *AMH*, which (acting through its cognate receptor [*AMH-RII*]) promotes regression of the Müllerian ducts. Leydig cells secrete testosterone and insulin-like hormone-3 (*INSL3*). Testosterone stabilizes the Wolffian ducts and is converted to dihydrotestosterone (*DHT*) by 5α -reductase in target tissues to promote differentiation of the prostate and development of male external genitalia. *INSL3* is involved in transabdominal testicular descent. *FOXL2*, *RSPO1*, *WNT4*, and β -catenin are involved in ovarian development.

polarization of these cells. In the developing testis, the ingressing cells initially form the Sertoli cells, whereas the later ingressing cells develop into the interstitial cells, including Leydig cells.²⁴ In the ovary, ingressing cells give rise to both theca and granulosa cells.²⁵

The somatic cells of the bipotential gonad require expression of Wilms tumor (*WT1*), GATA binding protein 4 (*GATA4*), and steroidogenic factor 1 (*NR5A1/SF1*) genes. The *WT1* gene codes a zinc transcription factor, which is expressed in embryonic mesodermal tissues and appears to influence mesodermal-epithelial interactions.²⁶ *GATA4* is expressed in the somatic cells of the urogenital ridge and bipotential gonad before showing sex-specific expression. Chromobox homolog 2 (*CBX2*) is expressed in the developing gonad at 7 weeks of gestation and plays a role in early gonadal development.²⁷ *CBX2* functions upstream of *SRY*, promotes transactivation of *NR5A1* and *SOX9*, and represses forkhead transcription factor 2 (*FOXL2*).²⁸ *NR5A1* is expressed in the urogenital ridge and appears to upregulate *SRY* expression, furthering testis development. *FOXL2*, a forkhead transcription factor expressed in the ovary, orchestrates cell fate decisions; *FOXL2* favors ovarian development and represses testicular development. In addition to transcription factors and specific secreted factors (hormones), physical contact with the mesonephros appears to be important for subsequent gonadal differentiation.

Because of their origin within the developing urogenital system, ovaries, and testes are initially located high in the abdomen near the kidneys. Specific signaling molecules activate or repress gonad determination for both testes and ovaries. As noted earlier, ongoing mutual antagonism or competition between specific genes and proteins influences cell fate decisions in gonad development.²⁹ Transcription factors modulate

this “competition” by influencing gene expression. Specific examples include *FOXL2* versus *SRY*-box 9 (*SOX9*) and *SOX9* versus Wingless-type MMTV integration site family member 4 (*WNT4*)/ β -catenin, which are discussed later. Hence, sex determination and differentiation reflect mutual antagonism between the testes determining factors, *SRY*-*SOX9*-*FGF9*, and the ovary determining factors, roof plate-specific spondin-1 (*RSPO1*)-*WNT*- β -catenin-*FOXL2*. Curiously, the antagonism persists postnatally (see later). As would be anticipated, steroidogenic enzyme genes show higher expression in developing human testes compared with ovaries.³⁰

Human Testicular Development

Testicular differentiation begins earlier than ovarian development. The first evidence of testicular differentiation is the appearance of primitive Sertoli cells at 6 to 7 weeks’ gestation in the human fetal testis. Cells, mostly endothelial cells, migrate from the mesonephros and interact with the pre-Sertoli cells to promote development of the testicular cords, which is initiated at approximately 7 to 8 weeks of gestation with identifiable cords by 9 to 10 weeks of gestation.³¹ The testicular cords are precursors of the seminiferous tubules that will contain Sertoli and germ cells. Interactions between endothelial and mesenchymal cells appear to influence development of the testicular cords.³²

The binary switch responsible for testicular development is the *SRY* gene located on the short arm of the Y chromosome. The precise mechanism(s) responsible for triggering *SRY* expression are incompletely defined. In the human testis, *SRY* is expressed in supporting cells at approximately 6 weeks of gestation; low-level expression persists throughout

gestation.^{33,34} The SRY protein contains a high-mobility group (HMG) domain and is encoded by a single exon gene. Two nuclear localization signals are located in the HMG domain. The SRY protein is expressed in pre-Sertoli cells, where it triggers a molecular switch to induce Sertoli cell differentiation, thus, initiating the process of male sexual differentiation. The HMG domain of the SRY protein binds to the minor DNA groove, where it functions as a transcription factor by bending DNA to presumably permit other proteins access to regulatory regions and to promote assembly of nucleoprotein transcription complexes. A threshold SRY level must be achieved at a critical time during gestation to establish male sexual differentiation. Otherwise, the ovarian differentiation pathway is activated.

SRY expression is independent of the presence of germ cells. SRY increases the expression of the SRY-related HMG box-containing-9 (SOX9) gene. Phenotype-genotype studies of humans and mice demonstrate that SOX9 expression is a crucial step, downstream of SRY, in testis development. Upstream from the SOX9 transcription start site, at least three enhancers, eSR-A, eSR-B, and eALDI, synergize to promote SOX9 expression. Available data suggest that SRY and NR5A1 bind at eALDI to promote initial SOX9 expression. Once expression has been initiated, SOX9 positively autoregulates its own expression. Subsequently, SOX9 and NR5A1 bind to regions within all three enhancer loci to amplify testicular SOX9 expression.³⁵ Using both direct and indirect mechanisms, SOX9 interferes with genes promoting ovarian differentiation. These data are consistent with the suggestions that mammalian gene regulation depends on multiple redundant enhancers and that copy number variants in this region may cause sex reversal.³⁶

In addition to SRY, SOX9, and NR5A1, expression of other genes is required for normal male sexual differentiation. Fibroblast growth factor (FGF9) acting through FGF receptors (FGFRs) promotes expression of SOX9 and repression of WNT4 and FOXL2.³⁷ Genes found to have expression pattern similar to SOX9 during testis development include *CITED1*, *ANKRD18A*, *G6PD*, *SLC52A3*, *KEL*, *ZNF280B*, *PRPS2*, and *INHBB*.³³ In addition to activation of the testicular differentiation pathway, the developing testis expresses factors to actively repress ovarian development. Specifically, the developing testis represses the R-spondin-WNT signaling pathway. Transmembrane E3 ubiquitin ligases, ZNRF3 and RNF43, inhibit WNT signaling by targeting the Frizzled receptor for degradation.³⁹

By immunohistochemistry, NR5A1 and SOX9 proteins can be detected in human embryonic gonadal tissue at 6 to 7 weeks of gestation. At this time, SOX9 expression becomes limited to nuclei of Sertoli cells in a 46,XY fetus but remains cytosolic in a 46,XX fetus. Subsequently anti-Mullerian hormone (AMH) is expressed; with increased AMH protein expression Wilms tumor (WT1) and GATA-4 protein expression increase in the fetal testis.⁴⁰ Male-limited expression of doublesex-related and Mab-3-related transcription factor 1 (*DMRT1*) was found in 6- and 7-week-old human fetuses.⁴¹ *DMRT1* appears to be necessary to maintain testicular cords and prevent transdifferentiation to a female phenotype.⁴² The most rapid growth in Sertoli cell number appears to occur during the latter half of the first trimester and the second trimester.⁴³ After Sertoli cells have developed, fetal Leydig cells appear around the seventh to eighth week of gestation and produce androgens to promote internal and external male genital structures.⁴⁴

Leydig cells are comprised of two distinct populations; fetal Leydig cells differentiate in utero and adult Leydig cells emerge after birth. Fetal Leydig cell differentiation depends on paracrine signals, such as platelet-derived growth factor receptor-alpha (PDGFR- α), Desert hedgehog (DHH), PTCH1, and Aristaless-related homeobox (ARX). *NR5A1* is expressed in Leydig cells to promote steroidogenic enzyme gene expression. The number of fetal Leydig cells reflects gonadotropin

stimulation because the number is decreased in anencephalic male fetuses and increased in 46,XY fetuses, with elevated gonadotropin concentrations secondary to complete androgen insensitivity.⁴⁵

Expression of genes associated with testicular steroidogenesis increases around 8 weeks of gestation. Placental human chorionic gonadotropin (hCG) stimulates early testicular androgen secretion. Later in gestation, pituitary LH secretion drives testicular androgen secretion. By 11 weeks of gestation, the testicular compartments, tubular and interstitial components, and the cell types of interest (Leydig, Sertoli, and germ cells) can be visualized. In human fetal testes, *HSD17B3*, *CYP11A1*, and *PTC1* messenger ribonucleic acid (mRNA) levels increased significantly through the second trimester without significant changes in *CYP17A1*, *LHR*, or *INSL3* levels.

Human Ovary Development

Rather than ovarian differentiation being the default pathway occurring in the absence of SRY gene expression, it is clear that specific genes influence ovarian differentiation. Genes involved in ovarian differentiation include *WNT4*, *FOXL2*, follistatin (*FST*), bone morphogenic protein-2 (*BMP2*), *GATA4/FOG2*, and *RSPO1*. *RSPO1* is a secreted factor that activates the β -catenin WNT signaling pathway.³³ *FOXL2* represses male-specific genes, especially SOX9 beginning in the fetus; this active repression continues throughout adulthood.⁴⁶ Hence *FOXL2* and SOX9 expression are mutually exclusive in developing gonads, as well as postnatal ovaries and testes.^{47,48}

Normal ovarian development requires *FOXL2*, *RSPO1*, and canonical WNT/ β -catenin signals. *RSPO1* acting through *LGR4/5* cell-surface receptors sequesters the transmembrane E3 ubiquitin ligases ZNRF3 and RNF43, resulting in increased WNT signaling.⁴⁹ *RSPO1* and *WNT4* stabilize and amplify β -catenin signaling to activate target gene transcription.³⁹ *WNT4* suppresses androgen-secreting interstitial cells, inhibits coelomic vascularization, and supports Mullerian derivatives.

Two genes that factor in germline development, alpha (*FIGLA*) and newborn ovary homeobox (*NOBOX*), are expressed in fetal or neonatal germ cells, where they recruit developing granulosa cells to form primordial and primary follicles. Additional transcription factors involved in ovarian development include *SOHLH1*, *SOHLH2*, *NOBOX*, *LHX8*, *FIGLA*, and *LHX9*.⁵⁰ The second trimester human fetal ovary expresses proteins necessary to synthesize and respond to estrogenic, progestogenic, and androgenic signaling.⁵¹

Germ Cell Development

Despite the prerequisite for germ cell development to perpetuate the species, germ cells are not required for the initial development of ovaries or testes. The primordial germ cells originate in the epiblast. At approximately 6 weeks of gestation in the human, the primordial germ cells proliferate and migrate from the hindgut along nerve fibers to colonize the genital ridges.⁵²⁻⁵⁴ SOX17 is a pluripotency factor that is critical for human primordial germ cell specification through its action to promote germ cell-specific gene expression.⁵⁵ SOX17 appears to promote *BLIMP* expression, which appears to suppress endodermal and mesodermal genes.⁵⁵

Upon reaching the developing gonad, the local environment directs the fate of the primordial germ cells toward male or female development. Hence the terminal differentiation pathways for germ cells are sexually dimorphic. When this migration process goes awry, the gonadal germ cell population may be deficient. Aberrant migration can result in ectopic migration into other organs. Ectopic germ cells are primarily located in the CNS, but can also be found in the mediastinum,

thorax, and pelvic region. Germ cell tumors may develop in ectopic germ cells when the germ cells are not eliminated by apoptosis.

Developmental phases for female germ cells include initiation of meiosis, formation and breakdown of germ cell nests, and assembly of single oocytes into primordial follicles. For male germ cells, the characteristic phases are migration, mitosis, and cell-cycle arrest.⁵⁶ Oocytes undergo meiosis, whereas meiosis is actively inhibited in male germ cells.⁵⁷ The RNA binding protein deleted in azoospermia-like (DAZL) appears to license entry into meiosis; *DAZL* expression increased between 9 and 14 weeks' gestation.⁵⁸ Several RNA-binding proteins, such as LIN28, *DAZL*, and *BOLL*, are expressed during oogenesis.⁵⁹ Another protein, meiosis specific with coiled-coil domain (MEIOC) stabilizes mRNAs that encode proteins relevant for meiosis; its expression appears to be independent of retinoic acid.⁶⁰ *DAZL* inhibits *SOX17* expression limiting germ cell pluripotency. Hence *DAZL* plays dual roles: it initiates meiosis in ovaries and represses pluripotency factors.

The fetal ovary is characterized by a developmental gradient with existence of multiple subpopulations of germ cells at different developmental stages; the more differentiated germ cells are located in the center of the developing ovary. In the developing ovary, the germ cells initially form oogonial clusters connected by intracellular bridges. Selected oogonia enter meiosis and progress through meiotic prophase I (MPI). Somatic cells surround individual oocytes to form follicles. Around 16 to 20 weeks' gestation, the oogonial clusters break down to form primordial follicles.⁶¹ The oocytes within the primordial follicles undergo growth arrest at the diplotene stage, lasting until oocyte growth is reinitiated with the onset of puberty. The greatest number of follicles exists at midgestation. From a peak of 6.8 million oocytes at approximately 5 months of gestation, approximately 2 million are present at birth.⁶² Notch signaling mediates interactions between oocytes and granulosa cells, regulates oocyte survival, and promotes breakdown of the oogonial nests.⁶³ The internal environment throughout fetal ovary development has the potential to directly influence the fertility of the developing female fetus (controlling the size of the ovarian reserve) and the quality of the oocytes that will eventually become her child (by influencing the extent of selection and apoptosis).⁶⁴

The primordial follicles constitute the ovarian reserve. Postnatally, recruitment and maturation of the primordial follicles is largely governed by AMH, androgen, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) secretion. Premature germ cell loss can lead to gonadal dysgenesis or premature ovarian insufficiency (POI). Accelerated follicular atresia contributes to the follicular depletion characteristic of streak gonads in X monosomy. Although not typically associated with genital ambiguity, mutations in genes governing oocyte and ovarian development cause DSDs characterized by delayed puberty or POI. These genes influence germ cell mitosis, germ cell meiosis, and DNA repair.⁶⁵

In contrast, testicular germ cell development is less dynamic. The Sertoli cells envelop the germ cells to form seminiferous cords at approximately 7 to 9 weeks of gestation in the human XY gonad. The germ cells in the developing testis enter a state of mitotic arrest until the onset of puberty.

Early during gestation, primordial germ cells undergo epigenetic reprogramming, which modifies chromatin architecture. In other words, inherited imprints are erased and DNA methylation is decreased at CpG islands, transcription start sites, genes, and intergenic regions.⁶⁶ Demethylation affects almost the entire genome, including the inactive X chromosome by 10 to 11 weeks of gestation.⁶⁷ During meiosis, both X chromosomes need to be active to enable

efficient pairing.⁶⁸ Subsequently, maternal and paternal alleles undergo sexually dimorphic imprinting, such that developing oocytes and sperm are differentially marked reflecting "parent-of-origin"-specific methylation patterns. This process occurs late in fetal development in the male and postnatally in female germ cells.^{69,70} Study of parent-of-origin-dependent disorders, such as Beckwith-Wiedemann, Prader-Willi, and Angelman syndromes and neonatal diabetes mellitus has elucidated the importance of this imprinting process.

Human Adrenal Development

By 33 days postconception, the human fetal adrenal cortex is distinct from the developing gonad. Because of its role as the source of dehydroepiandrosterone sulfate (DHEA-S) for placental estrogen biosynthesis, the fetal adrenal cortex grows rapidly. By 50 to 52 days postconception, expression of several steroidogenic enzymes, steroidogenic acute regulatory protein (*StAR*), 11 β -hydroxylase (*CYP11B1*), 17 α -hydroxylase/17,20-lyase (*CYP17A1*), and 21-hydroxylase (*CYP21A2*) in the fetal adrenal cortex have been demonstrated immunohistochemically.⁷¹ Transitory fetal adrenal cortisol biosynthesis peaks at 8 to 9 weeks' gestation, which coincides with transient adrenal expression of both nerve growth factor IB-like (*NGFI-B*) and 3 β -hydroxysteroid dehydrogenase-2 (*HSD3B2*).⁷¹ At the same time, adrenocorticotropin hormone (ACTH) can be detected in the anterior pituitary, suggesting the presence of negative feedback inhibition during the first trimester. During the time male sexual differentiation begins, this negative feedback inhibition may serve to prevent virilization of female fetuses to ensure normal female sexual differentiation.⁷² Distinct expression profiles for fetal adrenal steroidogenic enzymes have been described with differences between the first and second trimesters.⁷³ Chromaffin cells derived from the sympathetic nervous system migrate into the adrenal cortex to form the adrenal medulla.

Development of Internal Genital Structures

The Wolffian duct originates as the excretory duct of the mesonephros and develops into the epididymis, vas deferens, ejaculatory duct, and seminal vesicle. The Mullerian or paramesonephric duct originates as an invagination of the coelomic epithelium and develops into the Fallopian tubes, uterus, and upper third of the vagina.

The Sertoli cells of the developing testis secrete AMH, also known as *Mullerian inhibitory hormone (MIH)*. In human 46, XY fetuses, AMH expression can be detected by 7 weeks of gestation, is not dependent on the presence of germ cells within the testis, and promotes regression of the Mullerian ducts. AMH, a member of the transforming growth factor- β (TGF- β) family, undergoes proteolytic cleavage to become biologically active. AMH binds to its receptor, AMH-RII, on the surface of the Mullerian duct mesenchymal cells to induce increased matrix metalloproteinase 2 expression. The net result is degeneration and loss of basement membrane integrity of the epithelial and mesenchymal Mullerian cells, leading to regression of the Mullerian ducts.

AMH expression is highly regulated; inappropriate expression in a 46,XX fetus would lead to uterine agenesis. In the 46,XX fetus with absence of both AMH and testosterone, the Mullerian duct derivatives persist and the Wolffian ducts regress. When a female fetus is inappropriately exposed to AMH (as in freemartin cattle), Mullerian duct regression and ovarian masculinization occur. The paired Mullerian ducts arise as coelomic epithelial invaginations. Around the eighth week of gestation, the Mullerian ducts fuse, followed by

degeneration of a midline epithelial septum. Variable persistence of this septum can lead to uterine malformations. Multiple factors influence the development of the uterus, cervix, and vagina.⁷⁴ *HOX* genes specify the development program for distinct uterine regions; *HOXA9* specifies the uterine tube, *HOXA10* and *HOXA11* specify the uterus, and *HOXA13* specifies the vagina.⁷⁵ The vaginal plate appears during the 11th week of gestation through occlusion of the Mullerian-derived uterovaginal canal and ultimately disappears as the vaginal plate lengthens and canalizes.⁷⁶

The fetal hypothalamic-pituitary-gonadal (HPG) axis is active by midgestation, with peak fetal testosterone concentrations occurring at approximately 15 to 16 weeks of gestation. Before this time, placental hCG stimulates testosterone production by the fetal Leydig cells. Secretion of testosterone by the fetal Leydig cells stabilizes the Wolffian ducts in 46,XY fetuses. Region-specific signaling molecules, such as BMPs, homeobox (*HOXA10* and *HOXA11*), growth differentiation factor 7 (*GDF7*), relaxin, an orphan G-protein-coupled receptor (*LGR4*), platelet-derived growth factor A (*PDGFA*), and its cognate receptor (*PDGFRA*) influence the development of the epididymis and seminal vesicle.

The prostate, a male accessory sex gland, contributes to seminal fluid plasma and develops from the urogenital sinus. After the initial testosterone-dependent induction of prostate differentiation, subsequent development involves epithelial-mesenchymal interactions that lead to cell differentiation and branching morphogenesis. The requisite signaling molecules, FGFs, sonic hedgehog (*SHH*), BMPs, *HOXA13*, and *HOXD13*, are similar to those required for external genital differentiation.^{77,78}

Development of External Genital Structures

The genital tubercle, urethral folds, and labioscrotal swellings give rise to the external genitalia. Androgens play a time-dependent role in formation, differentiation, and growth of the fetal male external genitalia at approximately 8 to 14 weeks of gestation, during the masculinization programming window (MPW).^{79,80}

During this period, a cylindrical 2-mm phallus with genital swellings develops by 9 weeks of gestation. Within the developing penis, the urethra forms by canalization of the urethral plate with subsequent fusion to form the penile urethra by 12 to 14 weeks of gestation.⁸¹ The genital tubercle develops into the corpora cavernosa of the penis, and the labioscrotal folds fuse to form the scrotum. By 14 weeks, the external genitalia are clearly masculine, apart from testicular location. Thereafter, the penis grows at a rate of 0.7 mm/week from 14 weeks to term.⁸² Deficiency of androgen or androgen action during the MPW reduces penis length, an effect that cannot be fully rescued by postnatal T therapy.⁸³ The MPW predetermines potential phallic size, whereas postnatal androgen action is required to realize normal potential.

In the 46,XX fetus, in the absence of androgens, the urethral folds and labioscrotal swellings do not fuse and develop into the labia minora and labia majora, respectively. The genital tubercle forms the clitoris, and canalization of the vaginal plate creates the lower portion of the vagina. By 11 weeks of gestation, the clitoris is prominent and the lateral boundaries of the urogenital sulcus have separated. Minimal clitoral growth, well-defined labia majora, hypoplastic labia minora, and separate vaginal and urethral perineal openings are present by 20 weeks of gestation.

Anogenital Distance

The anogenital distance (AGD), the distance from anus to the genital tubercle, indicates prenatal androgen action and, as

anticipated, is sexually dimorphic. Investigators have used different anatomic landmarks to assess AGD in humans. Salazar-Martinez and colleagues defined AGD as being from the anus to the perineoscrotal junction (anoscrotal distance) in males and from the anus to the anterior fourchette (anofourchettal distance) in females; this definition appears to improve interobserver reliability.⁸⁴ Salazar-Martinez and colleagues reported normative ranges for anoscrotal and anofourchettal distances to be 21 ± 3.0 mm in males and 11 ± 2 mm in females (Salazar). In both sexes, AGD increased up to 12 months and maintained the sex dimorphic pattern. AGD also showed positive correlation with penile length at birth and with the increase in AGD from birth to 3 months. AGD provides a biomarker of early fetal androgen exposure and masculinization. Measurement of AGD can be used as a diagnostic tool for in utero androgen exposure and assessing impact of potential environmental endocrine disruptors on external genital development.^{85–88} AGD at birth predicts adult anogenital distance, but correlations with adult male reproductive parameters are inconsistent.^{89,90}

Sexual Differentiation of the Brain

Clinical investigations suggest that the brain is sexually dimorphic and that testosterone is a masculinizing hormone in human. Males with aromatase deficiency manifest male psychosexual behavior and gender identity. Alternatively, 46,XY individuals with complete androgen insensitivity syndrome (CAIS) develop female gender identity. However, preliminary data implicate genetic differences, independent of sex steroid exposure, as the molecular basis for some aspects of sexual dimorphism of the brain and other nongonadal tissues.^{91,92} Androgens have organizational and activation effects on CNS function. Complex interactions between genetic factors and hormone contribute to human brain sex differences.⁹³

MOUSE MODELS

For many patients with DSDs, the specific molecular etiology is unknown. Hence investigation of animal models, especially transgenic mouse models, has been helpful to delve into the processes involved in sex development. Investigation of normal and transgenic mice confirmed the crucial role of the sex-determining region on Y (*SRY*) gene in male differentiation when XX mice carrying only a 14-kb fragment of the Y chromosome showed a male phenotype.⁹⁴ However, the molecular mechanisms responsible for sex differentiation/sex development and postimplantation development differ among rodents and humans. Despite these limitations, information gleaned from preclinical models provides useful information regarding molecular mechanisms of sex development.

Understanding gene regulation, epigenetic influences, and protein-protein interactions are essential to clarify regulation of the sex development pathways; this knowledge may identify factors relevant for patients with DSDs. The outcome of cell fate decisions involves antagonism between the male and female developmental programs. During the bipotential phase, both male- and female-specific genes are expressed. Subsequently, precise spatiotemporal expression of specific genes ensues. Abnormal gonadal development has been described in mice homozygous for targeted deletions of genes involved in urogenital differentiation, that is, *Wt1*, *Sf1*, *Emx2*, *Cbx2/M33*, *Six1/Six4*, and *Lim1/Lhx9*. The phenotype of *Wt1* knockout mice includes embryonic lethality, failure of gonadal and kidney development, and abnormal development of the mesothelium, heart, and lungs.⁹⁵ Homozygous deletion of *Emx2*, a homeodomain transcription factor, results in an embryonic lethal phenotype associated with absence of kidneys, ureters,

gonads, and genital tracts.^{96,97} As *Wt1* expression is initially normal in the metanephric blastema of *Emx2* knockout mice, *Emx2* is likely downstream of *Wt1*. Interestingly, adrenal gland and bladder development are normal in *Emx2* knockout mice. Although the phenotype of specific knockout mice have been reported, details regarding the regulatory mechanisms governing gene expression are often lacking.

Epigenetic mechanisms modify chromatin architecture to reversibly regulate gene expression in a developmental manner. These mechanisms include histone modifications, that is, methylation/demethylation, acetylation/deacetylation, ubiquitination, and DNA methylation.⁹⁸ The polycomb group proteins, consisting of multiple protein subunits, function to modify chromatin structure. One polycomb protein is *Cbx2/M33*. The phenotype of *Cbx2* knockout mice includes male-to-female sex reversal and hypoplastic gonads for both sexes. Although the testes were small, overexpression of *Sry* or *Sox9* in *Cbx2* knockout mice rescued sex reversal in XY mice.⁹⁹ Based on available animal studies, *Cbx2* modulates expression of *Sfl/Nr5a1*, *Sry*, *Sox9*, *Dax1*, *Gata4*, *Arx*, and *Dmrt1* in both sexes, and influences gonad proliferation and size.

Although the temporal expression patterns for human *SRY* and mouse *Sry* differ, understanding the regulatory mechanisms responsible for mouse *Sry* expression has been germane to human *SRY*. Epigenetic factors influence expression of the mouse *Sry* gene. Both demethylation and histone modification are essential for *Sry* expression. The histone demethylase JMJD1A positively influences *Sry* expression by regulating H3K9me2 marks; *Jmjd1a*-deficient mice show partial male to female sex reversal.¹⁰⁰ Another mechanism involves the GADD4G protein, which mediates DNA demethylation and mitogen-activated protein kinase (MAPK) signaling. Loss-of-function *Gadd45g* mutant mice manifest male-to-female sex reversal; GADD45G appears to activate the MAPK pathway by phosphorylating GATA4 to activate *Sry* expression.^{101,102} Mice with loss-of-function *Gata4* mutation show phenotype similar to *Gadd4g* mice with decreased *Sry* expression and XY sex reversal.¹⁰³

Another epigenetic mechanism involved in *Sry* regulation involves CREB-binding protein (CBP, CREBBP, KAT3A) and p300 (EP300, KAT3B). These proteins are histone/lysine acetyl-transferases that modify chromatin-associated proteins to regulate gene expression. Mice with loss-of-function mutations manifest male-to-female sex reversal.¹⁰⁴ These proteins can act as network hubs interacting with other proteins to influence gene transcription.¹⁰⁵

Investigation of mouse models has been used to learn more about Wolffian and Mullerian duct development. Development of Wolffian duct epithelium depends on signals from Wolffian duct mesenchyme. Testicular testosterone activates mesenchymal androgen receptors to initiate mesenchymal-epithelial cross talk, increase epidermal growth factor production, and antagonize COUP-TFII to promote Wolffian duct stabilization.^{106,107} Factors involved in Mullerian duct regression include AMH, AMHR2, and SMAD; however, the molecular mechanism(s) used by AMH to induce Mullerian duct regression remains to be defined.

SEX CHROMOSOME DISORDERS

45,X/46,XY Mosaicism

Most sex chromosome disorders, such as 45,X and cytogenetic variants and 47,XXY and cytogenetic variants, are not associated with abnormal external genital development and will not be discussed in this chapter (see Table 6.1). In contrast, individuals with 45,X/46,XY and 46,XX/46,XY karyotypes manifest a broad range of phenotypes.¹⁰⁸ Among individuals with

45,X/46,XY karyotype, internal and external genital structures range from normal male to ambiguous to female. Whereas the typical histological features consist of poorly developed seminiferous tubules surrounded by wavy ovarian stroma, gonadal differentiation can range from normal testis to streak gonads. At the time of puberty, virilization can occur.

The majority of individuals identified by prenatal karyotype as being 45,X/46,XY appear to be normally androgenized males; however, individuals diagnosed postnatally tend to have more clinical signs.^{109,110} A normal peripheral blood lymphocyte karyotype in individuals with gonadal dysgenesis suggests the presence of sex chromosome mosaicism within the gonad(s).¹¹¹ Individuals with sex chromosome DSDs because of gonadal dysgenesis have an increased risk of developing gonadal tumors, such as gonadoblastoma or dysgerminoma because a dysgenetic gonad carrying a Y chromosome has an increased risk for neoplastic changes. Although gonadal tumors typically do not develop until the second decade of life, they can occur at younger ages.

Among a series of 63 males with 45,X/46,XY, subjects were classified into two groups; one group presented with genital anomalies and the second group presented with other reasons, such as short stature or infertility. Individuals identified by genital anomalies tended to have lower rates of spontaneous pubertal development, shorter stature, and greater likelihood of germ cell neoplasia.¹¹² Although most gonads were classified as dysgenetic testes, some testes appeared relatively normal with evidence of spermatogenesis. No follicles were observed, suggesting that gonads labeled as ovotestis were mislabeled and consisted of undifferentiated/streak-like tissue.¹¹³ Based on these histological findings, it has been suggested that the etiology of the 45,X/46,XY karyotype is the loss of a Y chromosome in some cells.¹¹⁴ Focal spermatogenesis was identified in approximately 25% of these subjects. Thus when gonadectomy is considered because of risk for neoplasia, fertility preservation should be considered.¹¹²

DISORDERS ASSOCIATED WITH DISORDERS OF SEX DEVELOPMENT AND ADDITIONAL CONGENITAL ANOMALIES (TABLE 6.2)

ARX (X-Linked Lissencephaly 2)

X-linked lissencephaly 2 is characterized by genital ambiguity, lissencephaly, early-onset seizures, absence of the corpus callosum, intellectual disability, and hypothalamic dysfunction, and *Aristaless*-related homeobox (*ARX*) gene mutations. This gene is a member of the paired-type homeodomain transcription factor family and is mapped to Xp21.3. *ARX* contributes to many aspects of brain development including patterning, neuronal proliferation and migration, and cell maturation and differentiation, especially the generation and migration of GABAergic neurons.^{115,116} Findings in *Arx* knockout mice showed no expression in developing ovaries, but Leydig cell differentiation was impaired.¹¹⁷ Available data suggest that *Arx* is a positive regulator for the differentiation of fetal Leydig cells at the progenitor stage.¹¹⁸

CDKN1C (IMAGe Syndrome)

The IMAGe syndrome is characterized by intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia, cryptorchidism, and micropenis.¹¹⁹ Genital anomalies appear to be limited to affected males. Both sporadic and familial cases have been described. Heterozygous missense mutations in the *CDKN1C* gene have been detected in some cases. This gene is located at chromosome 11p15.5, encodes a 316-amino-acid protein, p57^{Kip2}, negatively regulates cellular proliferation, and

TABLE 6.2 Syndromic Disorders Associated With Disorder of Sex Development

Gene (CHR Band)	Syndrome	Karyo-Type	Genetic Defects	Mode of Inheritance	Characteristic Congenital Anomalies
<i>ARX</i> (Xp21.3)	Hydranencephaly with ABNORMAL GENITALIA (OMIM #300215)	XY	point mutations deletion/duplication	X-linked	lissencephaly, absent corpus callosum, early-onset intractable seizures, temperature instability
<i>ATRX</i> (Xq13.1)	Alpha-thalassemia mental retardation syndrome (OMIM # 301040)	XY	point mutations	X-linked	alpha-thalassemia, severe mental retardation, and genital abnormalities
<i>CDKN1C</i> (11p15)	IMAGe syndrome (OMIM # 614732)	XY	point mutations	AD maternal transmission	IUGR, metaphyseal dysplasia, adrenal insufficiency
<i>CHD7</i> (8q12.2)	CHARGE syndrome (OMIM#214800)	XY	point mutations (70%)	AD	coloboma, heart defects, choanal atresia, ear anomalies
<i>DHCR7</i> (11q13.4)	Smith-Lemli-Opitz syndrome (OMIM #270400)	XY	point mutations (96%) deletion/duplication (4%)	AR	characteristic facial features, postaxial polydactyly, 2-3 toes syndactyly, cleft palate
<i>EMX2</i> (10q26.11)		XY	microdeletion		sex reversal, kidney, developmental delay, schizencephaly
<i>GLI3</i> (7p14.1)	Pallister-Hall syndrome (OMIM #146510)	XY XX	point mutations (20%–25%) deletion/duplication (rare)	AD	hypothalamic hamartoma, mesoaxial polydactyly, bifid epiglottis
<i>HHAT</i> (1q32.2)	chondrodysplasia (PMID:24784881)	XY	point mutations	AR	dwarfism with generalized chondrodysplasia
<i>HOXA13</i> (7p15.2)	Hand-foot-genital syndrome (OMIM#140000)	XY XX	polyalanine expansions (60%) point mutations (35%) deletion/duplication (2%–5%)	AD	shortening of the thumbs and great toes, bicornuate uterus
<i>HSD17B4</i> ^a (5q23.1)	Perrault syndrome 1 (OMIM #233400)	XX	point mutations	AR	sensorineural deafness
<i>KAT6B</i> (10q22.2)	Genitopatellar syndrome (OMIM #606170)	XY	point mutations deletion/duplication (rare)	AD	patellar hypoplasia, club feet, microcephaly, flexion contractures, agenesis of the corpus callosum
<i>POR</i> (7q11.2)	Antley-Bixler syndrome (OMIM#201750)	XY XX	point mutations deletion/duplication (rare)	AR	radiohumeral synostosis, midface hypoplasia, choanal stenosis, joint contractures
<i>RSPO1</i> (1p34.3)	Palmoplantar keratosis/sex reversal (OMIM #610644)	XX	point mutations	AD	palmoplantar keratoderma, predisposition to squamous cell carcinoma of the skin
<i>SAMD9</i> (7q21.2)	MIRAGE syndrome (OMIM # 610456)	XY	point mutations	AD	myelodysplastic syndrome, IUGR, adrenal insufficiency, chronic diarrhea, pulmonary infections
<i>SOX9</i> (17q24.3)	Campomelic dysplasia (OMIM #114290)	XY	point mutations (90%) translocation (5%) deletion/duplication (2%)	AD	Pierre Robin sequence, cleft palate, short bowed limbs, 11 pairs of ribs
<i>TBX3</i> (12q24.1)	Ulnar-mammary syndrome (OMIM #181450)	XX	point mutations	AD	ulnar limb deficiencies, mammary gland hypoplasia, dental abnormalities
<i>TP63</i> (3q28)	Limb-mammary syndrome (OMIM #603543)	XX	point mutations	AD	hand/foot anomalies, mammary gland and nipple
<i>TSPYL1</i> (6q22.1)	SIDDT (OMIM #608800)	XY	point mutations	AR	bradycardia, hypothermia, laryngospasm, bronchospasm
<i>WT1</i> (11p13)	Denys-Drash, Frasier, and Meacham syndromes (OMIM #194080)	XY XX	point mutations	AD	Wilms tumor, nephropathy, gonadoblastoma, congenital diaphragmatic hernia
<i>ZEB2</i> (2q22.3)	Mowat-Wilson syndrome (OMIM#235730)	XY	point mutations (80%–85%) deletion/duplications (15%–17%) translocations (1%–2%)	AD	Hirschsprung disease, congenital heart defects, agenesis of the corpus callosum, microphthalmia, Axenfeld anomaly

^aadditional genes

AD, Autosomal dominant; AR, autosomal recessive; IUGR, intrauterine growth restriction; SIDDT, sudden infant death with dysgenesis of the testis syndrome.

inhibits cell cycle progression.¹²⁰ The gene is located within a complex genetic locus containing several genes including, *IGF2* and *H19*, subject to imprinting modulation through cis-acting elements; *CDKN1C* is maternally expressed. Mutations associated with the IMAGE or Silver-Russell syndromes appear to be localized to the proliferating cell nuclear antigen-binding domain, resulting in gain of function with excessive inhibition of growth and differentiation.^{121,122} Curiously, loss-of-function mutations affecting the cyclin-dependent kinase-binding domain of this protein are associated with overgrowth syndromes, such as Beckwith-Wiedemann syndrome.¹²³

CHD7 (CHARGE Syndrome)

The CHARGE syndrome is associated with mutations in the chromodomain helicase DNA binding protein-7 (*CHD7*) gene. Features of this syndrome include eye coloboma, heart malformations, choanal atresia, short stature, genital anomalies, ear abnormalities, and hearing loss. Micropenis and cryptorchidism are found in males. Hypogonadotropic hypogonadism can occur. Although usually sporadic, autosomal dominant cases have been described. The *CHD7* gene, located at chromosome 8q12.1-q12.2, codes for a large protein that participates in chromatin remodeling and transcription.

DHCR7 (Smith-Lemli-Opitz)

Several enzymes catalyze the conversion of lanosterol to cholesterol. Decreased activity of these enzymes leads to cholesterol deficiency. The enzyme, 7-dehydrocholesterol reductase, encoded by the 7-dehydrocholesterol reductase (*DHCR7*) gene, located at chromosome 11q12-q13, catalyzes the last step in cholesterol biosynthesis. Smith-Lemli-Opitz (SLO) is an autosomal recessive disorder caused by *DHCR7* loss-of-function mutations. As would be anticipated, elevated 7-dehydrocholesterol concentrations are required to confirm the diagnosis.

Clinical features include urogenital anomalies, mental retardation, failure to thrive, facial abnormalities, developmental delay, behavioral abnormalities, and autism symptoms.¹²⁴ The most common urogenital abnormalities include male-to-female sex reversal, hypospadias, and cryptorchidism. Facial abnormalities include microcephaly, broad nose, upturned nares, micrognathia, short neck, and cleft palate. Short thumbs, syndactyly of the second and third toes, and postaxial polydactyly are common.¹²⁵ CNS malformations include septum pellucidum anomalies, agenesis of the corpus callosum, or holoprosencephaly in severe cases.¹²⁶

Infants often have hypotonia, feeding problems, and failure to thrive. Tube feedings may be necessary because of the poor weight gain. Impaired cholesterol biosynthesis may be associated with adrenal insufficiency. However, overt adrenal insufficiency is uncommon.¹²⁷ Nevertheless, adrenal insufficiency, especially during stress, can occur.¹²⁸ Thus the hypothalamic-pituitary-adrenal axis function should be assessed and glucocorticoid stress dosing may be beneficial. Although the efficacy remains unclear, dietary cholesterol supplementation is often prescribed.¹²⁹

DHCR7 mutations are associated with decreased cholesterol and accumulation of sterol intermediates proximal to the defective enzyme. Decreased cholesterol concentrations lead to decreased steroid concentrations, with subsequent decreases in glucocorticoid, mineralocorticoid, and sex steroid biosynthesis. In addition to serving as the precursor for vitamin D and bile acids, cholesterol is involved in SHH signaling and cell membranes. Available data indicate that accumulation of sterol intermediates rather than cholesterol deficiency leads to some of the clinical findings. Impaired SHH signaling contributes to malformations, such as agenesis of the corpus callosum,

holoprosencephaly, and postaxial polydactyly. Using induced pluripotent stem cells from subjects with SLOS, data suggest that accumulation of 7-dehydrocholesterol and its isomer, 8-dehydrocholesterol, rather than cholesterol deficiency, likely contributes to the neurological manifestations.¹³⁰

Prenatal diagnosis can be performed by measurement of amniotic fluid 7-dehydrocholesterol concentrations. Women pregnant with affected fetuses have low plasma estriol; elevated blood 16 α -hydroxyestrogens concentrations; and elevated urinary $\Delta 7$ and $\Delta 8$ unsaturated C₁₈, C₁₉, and C₂₁ dehydrosteroid concentrations, presumably because of impaired fetal cholesterol production.¹³¹ The incidence of biochemically confirmed SLO is estimated at 1/20,000 to 1/60,000 live births.¹³² The rate of heterozygosity for *DHCR7* mutations is surprisingly high. Because the prevalence of SLO at 16 weeks of gestation is comparable to the prevalence at birth, early fetal loss and/or reduced fertility of carrier couples is likely occurring.¹³³

EMX2 Gene

One male patient presented with 46,XY DSD, a single kidney, and intellectual disability; he had a small microdeletion involving *EMX2*.¹³⁴

GLI3 (Pallister-Hall Syndrome)

Pallister-Hall syndrome is characterized by genital anomalies, hypothalamic hamartoma, postaxial polydactyly, and imperforate anus.¹³⁵ Hypospadias, micropenis, and bifid or hypoplastic scrotum are described in XY patients whereas hydrometrocolpos and/or vaginal atresia are described in XX patients.¹³⁶ This autosomal dominant disorder is caused by mutations in the Gli-Kruppel Family Member 3 (*GLI3*) gene, which is located at chromosome 7p14.1.¹³⁷ The protein acts as both a transcriptional activator and a transcriptional repressor of downstream targets in the SHH pathway. Pallister-Hall is typically caused by frameshift/nonsense and splicing mutations located in the middle third of the gene, which are predicted to generate a truncated functional repressor protein.¹³⁸ Curiously, mutations outside of this region are associated with Greigs cephalopolysyndactyly syndrome.¹³⁹ Preclinical data using mouse knockout models showed aberrant ureteric budding, hydroureter, hydronephrosis, and renal hypoplasia.¹⁴⁰

HHAT (Nivelon-Nivelon-Mabille Syndrome)

The Nivelon-Nivelon-Mabille syndrome is an extremely rare disorder characterized by primordial short stature, generalized chondrodysplasia, microcephaly, brachydactyly, facial dysmorphism, and XY sex reversal.¹⁴¹ This syndrome is caused by autosomal recessive loss-of-function mutations in the O-acyl-transferase hedgehog acyl-transferase (*HHAT*) gene mapped to chromosome 1q32.2. The HHAT protein palmitoylates the N-terminal domain of the hedgehog proteins. The hedgehog proteins, SHH, DHH, and Indian hedgehog (IHH), play major roles in embryo patterning and differentiation. Based on the phenotype of the knockout mouse, loss of *Hhat* leads to testicular dysgenesis characterized by decreased testicular size, decreased testis cord, and almost complete absence of fetal Leydig cells.¹⁴² Curiously, the phenotype described for XY patients with *DHH* mutations and *Dhh* knockout mice resembles the phenotype reported for *HHAT* patients and *Hhat* knockout mice.¹⁴²

HOXA13 (Hand-Foot-Genital Syndrome)

HOX genes are a group of transcription factors involved in embryonic development and patterning. These genes are organized into clusters characterized by specific spatiotemporal

expression patterns. The hand-foot-genital syndrome (HFGS) (OMIM #140000) is a rare, autosomal dominant disorder associated with mutations or deletions of the *HOXA13* gene on chromosome 7p15.¹⁴³ This syndrome is characterized by congenital genitourinary anomalies and variable skeletal anomalies. The skeletal anomalies mainly affect the hands and feet, such as short thumbs and halluces, shortening of the middle phalanges, fifth finger clinodactyly, and the fusion of distal and middle phalanges of the toes.¹⁴⁴ Although approximately 50% of affected individuals have urogenital anomalies, many experience normal fertility. Males may have hypospadias or cryptorchidism. Females may have incomplete Mullerian fusion or bicornuate uterus associated with increased risks for spontaneous abortion, preterm delivery, or stillbirth. In some instances, the skeletal phenotype can be mild.¹⁴⁵ Additional comorbidities include chronic urinary tract infections, ectopic ureteric orifices, chronic pyelonephritis, and renal failure.¹⁴⁶

KAT6B (Genitopatellar Syndrome)

The genitopatellar syndrome is a rare disorder characterized by skeletal dysplasia, flexion contractures, genital anomalies, craniofacial defects, and developmental delay associated with lysine acetyltransferase 6 (*KAT6B*) gene mutations. The skeletal features include hypoplastic or absent patellae, flat temporal bones, and brachydactyly. Genital anomalies include hypoplastic labia, clitoromegaly, scrotal hypoplasia, and cryptorchidism. Cardiac anomalies, hydronephrosis, and developmental delay have been described. The gene is located at chromosome 10q22.2 and contains 20 exons. The *KAT6B* protein is a component of the histone H3 acetyltransferase complex and functions as a histone acetyltransferase. The *KAT6B* protein functions as a chromatin modifier by catalyzing acetylation of specific lysines. Most mutations are de novo mutations.¹⁴⁷ The Say-Barber-Biececker-Young-Simpson syndrome is also associated with *KAT6B* mutations; specific facial features consisting of blepharophimosis/ptosis and an immobile mask-like face distinguish these syndromes.

NR2F2 Gene

This gene encodes chicken oval albumin upstream promoter transcription factor (COUP-TF2), a nuclear receptor. This protein appears to function as a proovary and antitestis factor. Mutations in this gene are associated with a syndromic 46,XX DSD characterized by genital virilization, congenital heart disease, and varying somatic findings. Frameshift mutations have been reported in three 46,XX patients who were identified by genital ambiguity. All three patients had congenital heart disease. One infant died of hypoplastic left heart; no gonadal histology was available. The other two patients carried de novo c.97_103delCCGCCCG predicted to generate frameshift mutation, p.Pro33Alafs*77; they also had blepharophimosis ptosis epicanthus inversus syndromes (BPES) and uterus. One patient had ovotesticular disorder; gonadal histology was not available for the other patient.

Immunohistochemistry studies performed on human ovaries showed positive COUP-TF2 staining in FOXL2-negative stromal areas consistent with COUP-TF2 functioning as a proovary and antitestis factor in developing female gonads.¹⁴⁸ Preclinical studies in mice showed that female embryos lacking *Coup-tfII* possess both Wolffian and Mullerian ducts; maintenance of the Wolffian ducts is androgen independent and is likely caused by enhanced phosphorylated extracellular signal-regulated kinase signaling in Wolffian duct epithelium.¹⁴⁹ These data suggest that COUP-TFII actively promotes Wolffian duct regression in female embryos.

RSPO1

The R-spondin 1 (*RSPO1*) gene codes for a secreted furin-like domain protein that stabilizes β -catenin in the Wnt-signaling pathway. Mutations in this gene, located at chromosome 1p34, are associated with 46, XX sex reversal. This gene was initially identified by investigating in individuals with palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal in one family.¹⁵⁰ Affected XX, sex-reversed individuals lack Mullerian structures.¹⁵⁰ A 46,XY individual, homozygous for *RSPO1* mutations, fathered two children. An XX individual with ovotesticular disorder and palmoplantar keratoderma was found to be homozygous for a splicing mutation in the *RSPO1* gene.¹⁵¹ In addition to sex reversal and palmoplantar keratosis, reported features include microphthalmia and nail dystrophy.¹⁵¹

SAMD9 (MIRAGE Syndrome)

Mirage syndrome is characterized by myelodysplasia, infections, intrauterine growth restriction (IUGR), adrenal hypoplasia, XY male-to-female sex reversal, and enteropathy.^{152,153} Pulmonary disease, severe infections, and myelodysplasia contribute to deaths during the first 2 years of life. This disorder has been associated with heterozygous mutations in the sterile α motif domain-containing protein 9 (*SAMD9*) gene located at chromosome 7q21.2. The 1589-amino-acid protein is highly expressed in the fetal adrenal; immunohistochemistry showed *SAMD9* localization in the cytoplasm of adrenal definitive and fetal zone cells colocalized with cells positive for *NR5A1* expression. *SAMD9* is considered to be a tumor suppressor gene; mutations are gain-of-function mutations, leading to IUGR and consequences for adrenal gland and testes differentiation. Curiously, second mutations, such as acquired monosomy for chromosome 7, modify the phenotype and prolong survival. Buonocore and colleagues suggest that *SAMD9* is essential to control the balance between cell proliferation and cell differentiation; activating *SAMD9* mutations interfere with cell proliferation resulting in hypoplastic tissues.¹⁵⁴

SOX9

SOX9 is a member of the SRY-related HMG domain gene family located at chromosome 17q24.3-17q25.1 that encodes a 508-amino-acid protein. Heterozygous loss-of-function *SOX9* mutations are associated with autosomal-dominant campomelic dwarfism and male-to-female sex reversal.¹⁵⁵ Features of campomelic dwarfism include congenital bowing of long bones, hypoplastic scapulae, 11 pairs of ribs, narrow chest, congenital dislocated hips, and clubfeet. Facial features include micrognathia, cleft palate, large head, flat nasal bridge, and low-set malformed ears. When bone malformations are severe, postnatal survival may be limited. Affected 46,XY fetuses manifest sex reversal, with external genital differentiation, ranging from ambiguous to female. Gonadal dysgenesis and persistence of Mullerian duct derivatives are typical. Phenotypic heterogeneity with differing phenotypes, including ovotesticular DSD and complete sex reversal, has been described in affected siblings.¹⁵⁶ Acampomelic dysplasia has been described in which the typical limb anomalies are absent.¹⁵⁷ Patients with acampomelic dwarfism and sex reversal have been reported.^{158,159} Familial campomelic dysplasia associated with a deletion upstream of *SOX9* has been described in a mother and 46,XY child with female external genitalia, normal uterus, and streak gonads.¹⁶⁰

SOX9 mutations can affect DNA-binding affinity, DNA bending ability, nuclear import, transactivation, and nuclear export. Haploinsufficiency is the mechanism responsible for

many of the consequences of *SOX9* mutations. Somatic cell mosaicism, de novo germline mutations, and mitotic gene conversion events have been described. Mutations located upstream of *SOX9* in the regulatory regions have been identified in patients with varying degrees of sex reversal. Duplications of the eSR-A locus were associated with 46,XX testicular disorder and 46,XX ovotesticular disorder, whereas deletions have been identified in patients with 46,XY sex reversal.³⁵

***TBX3* (Ulnar-Mammary syndrome)**

The Ulnar-Mammary syndrome is characterized by cryptorchidism, micropenis, breast hypoplasia/aplasia, nipple hypoplasia/aplasia, delayed puberty, absent axillary hair, and skeletal anomalies. Skeletal anomalies include hypoplasia of humerus and ulna, oligodactyly, polydactyly, and finger anomalies. The *TBX3* gene plays a role in development of the dorsoventral limb axis. Additional endocrine features include hypogonadotropic hypogonadism, short stature, growth hormone deficiency, and obesity.¹⁶¹ Neuroimaging studies have identified pituitary and brain malformations.¹⁶²

WT1

The Denys-Drash syndrome is characterized by genitourinary anomalies, Wilms tumor, nephropathy, and Wilms tumor suppressor (*WT1*) gene mutations. Among affected 46,XY individuals, the external genitalia can range from ambiguous to normal female. Internal genital differentiation varies because of inconsistent Wolffian and/or Mullerian structure development and regression. Typically, the nephropathy begins during the first few years of life, manifests with proteinuria, and results in end-stage renal failure because of focal or diffuse mesangial sclerosis.^{163,164} Typically, the gonads are usually dysgenetic in 46,XY individuals. Affected 46,XX individuals typically have normal female external genital development.

However, a novel *WT1* mutation was reported in an XX individual identified at birth because of clitoromegaly and a single perineal opening. She was found to have an immature right uterine tube; gonadal histology revealed bilateral testes with seminiferous tubules containing predominantly Sertoli cells and rare germ cells. The karyotype of both gonads was XX. Genetic analysis revealed a de novo frameshift variant in exon 10, which encodes the fourth DNA finger. This variant is predicted to generate a deleterious mutation p.Arg485Glyfs*14, which appears to be a gain-of-function mutation increasing DNA binding affinity, promoting *NR5A1* overexpression and *SOX9* upregulation, and culminating in testicular development.¹⁶⁵

The Wilms tumor suppressor (*WT1*) gene, located at chromosome 11p13, plays an important role in both kidney and gonadal differentiation. Through alternative splicing, multiple translation start sites, and posttranslational RNA editing, over 30 isoforms are derived from this one gene. The carboxyl terminal domain of the *WT1* protein contains four zinc fingers that serve as the nucleic acid binding domain.

The two major isoforms differ by the inclusion or exclusion of three amino acids, lysine, threonine, and serine (KTS), between the third and fourth zinc-finger domains. Subnuclear localization studies have shown that the -KTS form colocalizes predominantly with transcription factors and preferentially binds to DNA, whereas the +KTS form colocalizes mainly with splicing factors and plays a role in RNA processing.¹⁶⁶ The ratio of the +KTS/-KTS isoforms appears to be tightly regulated. Hence depending on cell context, *WT1* can function as a transcriptional activator, a transcriptional repressor, or tumor suppressor. *WT1* plays a role in the balance between mesenchymal-epithelial transitions.¹⁶⁷

Patients with *WT1* mutations manifest phenotypic heterogeneity. Although Wilms tumor and genitourinary abnormalities can be associated with heterozygous *WT1* deletions, only 6% to 15% of sporadic Wilms tumors are associated with *WT1* mutations. Heterozygous deletions at chromosome 11p13 can be part of a contiguous gene deletion syndrome known as WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, gonadoblastoma, and mental retardation). In general, missense mutations in exons 6–9 are associated with severe gonadal dysgenesis and early-onset nephropathy. The (-) KTS form appears to be protective from development of Wilms tumor, whereas mutations located in the N-terminal repression domain are associated with development of Wilms tumor.

Frasier syndrome is characterized by gonadal dysgenesis, progressive glomerulopathy, and an increased risk for gonadoblastoma. Wilms tumor is extremely rare in Frasier syndrome.¹⁶⁸ The typical renal lesion is focal glomerular sclerosis. The majority of cases are associated with a specific *WT1* point mutation in intron 9, associated with altered splicing and decreased amounts of the +KTS isoform.

Meacham syndrome is characterized by genital anomalies, cyanotic congenital heart defects, and pulmonary hypoplasia secondary to diaphragmatic abnormalities. In 46,XY infants, the spectrum of internal genital anomalies extends from presence of a uterus to a blind ending vaginal pouch. External genitalia have been described as ranging from normal female to ambiguous.¹⁶⁹

***XH2* (ATRX Syndrome)**

ATRX (α -thalassemia, mental retardation, X-linked protein) syndrome is an X-linked disorder characterized by mild alpha-thalassemia, severe mental retardation, and genital abnormalities because of mutations in the *XH2* gene located at chromosome Xq13.3. Urogenital anomalies, including ambiguous genitalia, cryptorchidism, hypoplastic scrotum, hypospadias, shawl scrotum, and small penis occur in approximately 80% of patients.¹⁷⁰ Additional features include short stature, psychomotor retardation, microcephaly, seizures, talipes equinovarus, and gastrointestinal problems. The facies are described as coarse with midface hypoplasia, short nose, and widely spaced incisors. The hemoglobin H inclusions associated with alpha-thalassemia can be demonstrated on brilliant cresyl blue stained peripheral blood smears.¹⁷¹ Typically, Wolffian duct structures are present, whereas Mullerian duct structures and germ cells are absent.¹⁷² Phenotype-genotype correlations are inconsistent. Most cases are inherited from carrier mothers and most, but not all, carrier females show preferential inactivation of the X chromosome carrying *ATRX* mutations.¹⁷³

This disorder, also known as Carpenter-Waziri syndrome, is caused by mutations in the *ATRX* (also known as *XH2* or *XHP*) gene located at Xq13.3.¹⁷⁴ The *ATRX* protein is a member of the SWI/SNF DNA helicase family and consists of three domains: an N-terminal containing a plant-homeodomain-like (PHD) domain, a coiled-coil motif, and C-terminal helicase domain.¹⁷⁵ The urogenital anomalies are typically associated with mutations located in the PHD domain and predicted to generate a truncated protein. During meiosis, *ATRX* maintains genomic integrity by modulating DNA damage response mechanisms.¹⁷⁶ *ATRX* mutations are associated with abnormal methylation of repetitive DNA sequences, failure to establish necessary epigenetic modification for X chromosome inactivation, and impaired silencing of imprinted genes.^{177,178}

Sertoli cell *ATRX* knockout mice had small testes, decreased seminiferous tubule volume, decreased Sertoli cell number, and increased Sertoli cell apoptosis associated with prolonged

G2/M phase during fetal life. This finding is consistent with the concept that ATRX protein expression impacts on G2-M progression with ultimate consequences on cell survival.¹⁷⁹ Tissue-specific consequences of the epigenetic regulation and disrupted cell cycle progression may explain the disparate features associated with *ATRX* mutations.

DISORDERS ASSOCIATED WITH 46,XX DISORDERS OF SEX DEVELOPMENT AND 46,XY DISORDERS OF SEX DEVELOPMENT (TABLE 6.3)

SF1/NR5A1 Gene

The *SF1/NR5A1* gene, located at chromosome 9q33, codes for a 461-amino-acid protein. This protein is a nuclear receptor transcriptional regulator involved in adrenal, gonadal, and hypothalamic development and function. The protein contains an amino terminal DNA binding domain containing two zincfingers, a hinge domain, and ligand-binding domain. The protein modulates expression of *SRY*, *SOX9*, *AMH*, and *AMHR*. In Leydig cells, *NR5A1* promotes expression of *LHCGR*, *STAR*, *CYP11A1*, and *CYP17A1*, which are required for testosterone biosynthesis.

Phenotypic heterogeneity is characteristic for patients with *NR5A1* mutations. Among 46,XY individuals, reported phenotypes range from impaired testicular development to male infertility. Among 46,XX individuals, reported phenotypes range from 46,XX ovotesticular disorder to primary ovarian insufficiency. Adrenal insufficiency may be the primary feature in some patients. Nonsense, frameshift, and missense changes have been identified. Most mutations are heterozygous. Familial cases have been reported.¹⁸⁰ Differing phenotypes have been described in a single family.¹⁸¹

Because of the multiple *NR5A1* actions in gonadal differentiation and function, several potential mechanisms of action are possible with *NR5A1* mutations. Among 46,XY individuals, *NR5A1* mutations cannot adequately activate the male pathway leading to undervirilization. Among XX (SRY-negative) virilized patients, based on in vitro studies, *NR5A1* mutations do not appear to abnormally activate the male pathway. Rather, in vitro studies showed that p.Arg92Trp or p.Ala260Val missense mutations repressed Wnt signaling, thus interfering with ovarian differentiation in virilized 46,XX (SRY-negative) patients.¹⁸² In other words, downregulation of the female “antitestis” genes decreases inhibition of the male developmental pathway, shifting cell fate determination to promote testicular differentiation in XX individuals.¹⁸³ Curiously, 46XX

TABLE 6.3 Nonsyndromic Disorders of Sex Development in XY Individuals

Gene	Location	Phenotype	Genetic Defects	Mode of Inheritance
<i>AMH</i>	19p13.3	Persistent Mullerian duct syndrome, gonadal dysgenesis (OMIM #261550)	Point mutations	AR
<i>AMHR2</i>	12q13.13	Persistent Mullerian duct syndrome (OMIM #261550)	Point mutations	AR
<i>AR</i>	Xq12	Androgen resistance, gonadal dysgenesis (OMIM # 300068)	Deletions, point mutations	X-linked
<i>AXIN1</i>	16p13.3	Cryptorchidism, caudal duplication anomaly (OMIM # 603816)	Point mutations	unknown
<i>CBX2</i>	17q25	Complete gonadal dysgenesis, ovotesticular DSD (OMIM #613080)	Point mutations	AR
<i>DHH</i>	12q13.12	Gonadal dysgenesis, partial or complete (OMIM #233420)	Point mutations	AR
<i>DMRT1</i>	9p24.3	Gonadal dysgenesis, partial or complete Additional anomalies in patients with 9p24.3 deletions (OMIM #154230)	Point mutations, 9p24.3 deletions (contiguous gene deletion syndrome)	AD
<i>ESR2</i>	14q23.2-q23.3	XY sex reversal (OMIM # 601663)	Point mutations	AD, AR
<i>FGFR1</i> , multiple	8p11.23, multiple	Hypogonadotropic hypogonadism with or without anosmia, see (OMIM # 147950) for additional loci	Deletions/duplications, point mutations	AD, AR, oligogenic
<i>GATA4</i>	8p23.1	Testicular anomalies with/without congenital heart disease (OMIM#615542)	Deletions/duplications, point mutations	AD
<i>KAL1</i>	Xp22.31	Hypogonadotropic hypogonadism with anosmia (OMIM #308700)	Deletions/duplications, point mutations	X-linked
<i>LHCGR</i>	2p16.3	Leydig cell hypoplasia (OMIM #238320)	Point mutations	AR
<i>MAMLD1</i>	Xq28	Micropenis, bifid scrotum, penoscrotal hypospadias (OMIM #300758)	Point mutations	X-linked
<i>MAP3K1</i>	5q11.2	Gonadal dysgenesis (OMIM #613762)	Point mutations	AD
<i>NR0B1(DAX1)</i>	Xp21.2	Complete gonadal dysgenesis, sex reversal (OMIM #300018)	Duplications, X-autosome translocations, deletions upstream	X-linked
<i>NR5A1 (SF1)</i>	9q33.3	Underandrogenization, complete gonadal dysgenesis, adrenal insufficiency (OMIM # 612965)	Deletions/duplications, point mutations	AD
<i>SOX8</i>	16p13.3	Ambiguous genitalia, complete gonadal dysgenesis (PMID:21373258) (28)	Point mutations, upstream duplications	AD
<i>SRY</i>	Yp11.2	Gonadal dysgenesis (OMIM #400044)	Deletions/duplications, point mutations	Y-linked
<i>WVVOX</i>	16q23.1-q23.2	Gonadal dysgenesis (PMID:22071891)	Deletions	AD
<i>ZFPM2 (FOG2)</i>	8q23.1	Gonadal dysgenesis with/without congenital heart disease (OMIM #616067)	Point mutations	AR
<i>ZNRF3</i>	22q12.1	Gonadal dysgenesis (OMIM# 612062)	Point mutations	AD

AD, Autosomal dominant; AR, autosomal recessive.

individuals with a different missense mutation, p.Arg92Gln, have been reported as having adrenal insufficiency and with or without ovotesticular DSD.^{184,185}

Incomplete penetrance and variable expression are recognized characteristics of *NR5A1* mutations. The *NR5A1* mutations illustrate how specific variants in a transcription factor act as a cell/organ switch to direct developmental outcomes.¹⁸⁶ With whole exome sequencing (WES), variants in other genes have been demonstrated. Hence, oligogenicity or modifier genes may influence phenotypic expression for *NR5A1* mutations.¹⁸⁷

SOX8

SOX8 is located at chromosome 16p.13.3. *SOX8* mutations are associated with 46,XY sex reversal and infertility in both 46,XX and 46,XY individuals. Heterozygous *SOX8* missense mutations were identified in individuals with 46,XY sex reversal.¹⁸⁸

NONSYNDROMIC 46,XY DISORDERS OF SEX DEVELOPMENT (GONADAL DYSGENESIS)

(SEE TABLE 6.3)

Chromobox Homolog 2 (*CBX2*)

The *CBX2* gene, mapped to chromosome 17q25, is a subunit of the chromatin-associated polycomb repressive complex 1. As noted earlier, the polycomb group proteins regulate chromatin structure, ultimately influencing gene expression. Two isoforms exist, *CBX2.1* and the shorter *CBX2.2*. Loss-of-function missense *CBX2.1* mutations were identified in a 46,XY infant identified by a mismatch between prenatal karyotype and female external genital appearance at birth. Subsequent evaluation identified a normal uterus and ovarian-like tissue on histology.¹²⁰ Two patients with *CBX2.2* mutations have been reported; both patients had 46,XY karyotypes. One patient had dysgenetic testes, whereas no gonadal tissue could be identified in the second patient.¹⁸⁹ Available data suggest that *CBX2.1* is involved in testis determination, whereas *CBX2.2* is involved in early gonad development. The factors that regulate alternative splicing of this gene are unknown.¹⁸⁹

Desert Hedgehog

The Desert hedgehog (*DHH*) gene is located on chromosome 12q12-q13.1 and encodes a protein consisting of 396 amino acids. *DHH* is a member of the Hedgehog family and is also expressed in the Schwann cells of the peripheral nervous system. This is an autosomal recessive disorder associated with XY gonadal dysgenesis and minifascicular neuropathy.^{190,191} However, neuropathy is an inconsistent feature. *DHH* promotes formation of the connective tissue sheath around peripheral nerves and is essential for their survival.¹⁹²

One 46,XY patient had female external genitalia, polyneuropathy, a testis on one side, and a streak gonad on the other side. Genetic analysis showed homozygosity for a single nucleotide substitution at the initiation codon, which is predicted to abolish initiation of translation at the normal start site. Histological analysis of the sural nerve revealed extensive formation of minifascicles within the endoneurium.¹⁹³ The associated minifascicular neuropathy has been attributed to developmental malformation of peripheral nerves and is characterized by the presence of many small fascicles with glove and stocking-type sensory impairment.¹⁹⁴ Gonadal tumors, including seminoma-in-situ, gonadoblastoma, and dysgerminoma have been reported.¹⁹⁵

Doublesex and Mab-3–Related Transcription Factor 1 (*DMRT1*) and Doublesex and Mab-3–Related Transcription Factor 2 (*DMRT2*)

Monosomy for distal chromosome 9p has been reported in male-to-female sex reversal. Most deletions associated with sex reversal involve the chromosome region 9p24.3 where three *DMRT* genes are located.¹⁹⁶ *DMRT1* appears to be involved in Sertoli and cell differentiation. External genitalia have been described as ambiguous or female. The external genitalia may appear symmetric or asymmetric. Differentiation of internal genitalia is highly variable, with the presence of Mullerian and Wolffian remnants being reported. In addition to sex reversal, clinical features include mental retardation, low-set ears, trigonocephaly, wide nasal bridge, single palmar creases, heart defects, epilepsy, and scoliosis. Genital anomalies reported in XY individuals include gonadal dysgenesis, ovotestis, hypospadias, penoscrotal inversion, and cryptorchidism.¹⁹⁷ Gonadoblastoma have been reported.¹⁴⁹ Although phenotype/genotype correlations are not apparent, haploinsufficiency for *DMRT1* appears to be sufficient to cause gonadal dysgenesis.¹⁵⁰ Female external genitalia and short stature were described in a patient with mosaic 46,XY karyotype characterized by ring chromosome 9 and *DMRT1* haploinsufficiency.¹⁹⁸

GATA4

The GATA proteins comprise a family of tissue-specific transcription factors that contain two conserved zinc-finger domains. The C-terminal zinc finger is required for DNA recognition and the N-terminal zinc finger contributes to stability; both zinc fingers are required for protein-protein interactions with other transcription factors.¹⁹⁹ The *GATA4* gene is mapped to chromosome 8p23.1. Heterozygous *GATA4* mutations are associated with congenital heart disease and XY disorders of sex development.²⁰⁰ The observed phenotypic heterogeneity has been attributed to incomplete penetrance and oligogenicity. Three males in one family were reported to manifest congenital heart disease and varying abnormalities in male sex development, whereas the female carrier had congenital heart disease without an ovarian phenotype. This specific mutation, p.Gly221Arg, abrogated the ability of the *GATA4* protein to interact with the ZFPM2/FOG2 protein and disrupted synergistic activation of the AMH promoter by *GATA4* and *NR5A1*.²⁰¹

Mastermind-Like Domain Containing 1 (*MAMLD1*)

MAMLD1 was identified during studies investigating the genetic basis of X-linked myotubular myopathy.²⁰² This gene is also known as chromosome X open reading frame 6 (*CXorf6*).²⁰³ However, some variants have been detected in normal individuals. In vitro, most *MAMLD1* variants acted similarly to the wild type with the exception of the L210X variant.²⁰⁴ The lack of a significant reproductive phenotype in transgenic knockout mice raises doubt whether *MAMLD1* is sufficient to cause a DSD.^{205,206} Hence these findings suggest that *MAMLD1* variations are insufficient to account for a patient's DSD phenotype.

Mitogen-Activated Kinase Kinase Kinase 1 (*MAP3K1*)

The *MAP3K1* gene is located at chromosome 5q11.2. This gene encodes a protein involved in MAPK signaling. Phenotypes of affected individuals include 46,XY complete gonadal dysgenesis, perineoscrotal hypospadias, cryptorchidism, and variable

Mullerian duct development.^{207,208} Gonadoblastoma and dysgerminoma have been reported.²⁰⁹ Inheritance appears to be sex-limited autosomal dominant. Variations associated with gonadal dysgenesis increase phosphorylation of the downstream targets, MAPK11 (p38), MAP3K (ERK1), and MAPK1 (ERK2) and increase binding of the cofactors RHOA and MAP3K4 (AXIN1); the net result is decreased SOX9 expression.²¹⁰ Hence these gain-of-function mutations repress the testis-promoting factors and stabilize beta-catenin, favoring the ovarian differentiation pathway.²¹¹

Nuclear Receptor Subfamily 0, Group B, Member 1 (*NROB1*)

This two-exon gene, *NROB1*, located at Xp21.2 encodes DAX1. The 460-amino-acid protein is an orphan nuclear receptor lacking a typical zinc-finger DNA-binding domain. The N terminus of the 470-amino-acid protein contains a novel DNA-binding domain, whereas the C terminus shows characteristics of a nuclear hormone receptor ligand-binding domain. *NROB1* is expressed throughout the HPG axis. The DAX1 protein functions as a transcriptional repressor of many genes, including NR5A1 and some steroidogenic enzyme genes.

Duplication of the *DAX1/NROB1* locus is associated with male-to-female sex reversal.¹³³ External genital differentiation ranges from female to ambiguous. Descriptions of internal genitalia include the presence of Mullerian and Wolffian structures. Gonads are typically described as streaks. Using high-resolution array comparative genome hybridization (CGH), a submicroscopic interstitial duplication of the *DAX1/NROB1* gene was discovered during thorough evaluation of two sisters. This 637-kb duplication included *NROB1*, four *MAGEB* genes, *CXorf21*, glycerol kinase (*GK*), and part of the *MAP3K7IP3* gene. The older sister had presented with primary amenorrhea, female external genitalia, 46,XY karyotype, and gonadal dysgenesis; the younger phenotypic female sister was prepubertal, but found to have 46,XY karyotype.¹³⁴

Loss-of-function mutations are associated with X-linked adrenal hypoplasia congenita (AHC). In this disorder, development of the fetal adrenal cortex is normal. However, the adult or definitive adrenal cortex fails to develop. Adrenal insufficiency may not be evident in the immediate neonatal period but may become obvious during early infancy. Although adrenal insufficiency generally manifests in infancy or early childhood, phenotypic heterogeneity in severity and age at presentation occurs.¹³⁵ Unilateral or bilateral cryptorchidism can also occur. At the age of expected puberty, hypogonadotropic hypogonadism caused by hypothalamic and pituitary dysfunction may occur among affected males.¹³⁶ Delayed puberty has been recognized in heterozygous females.¹³⁷ One female homozygous for *NROB1* mutations has been reported. Her phenotype was hypogonadotropic hypogonadism.¹³⁸ As part of a contiguous gene deletion syndrome, X-linked AHC can be associated with glycerol kinase deficiency, Duchenne muscular dystrophy, ornithine transcarbamylase deficiency, and mental retardation.¹³⁹

Nonsense mutations have been identified throughout the gene. Missense mutations account for 20% of mutations associated with AHC. These mutations tend to cluster in the carboxyl terminal of the protein corresponding to the putative ligand-binding domain and impair the transcriptional repression activity of the protein. One missense point mutation, located in the hinge region of the protein, was identified in an 8-year-old girl with clinical and laboratory features indicative of adrenal insufficiency. Additional studies showed that this mutation hindered nuclear localization of the protein. Curiously, the hemizygous father and heterozygous younger sister of the proband did not manifest the AHC phenotype.¹⁴⁰

PBX1 (Pre-B-Cell-Leukemia Factor 1)

Pre-B-cell-leukemia factor 1 (*PBX1*), located at chromosome 1q23.3, is a member of the Three Amino Acid Loop Extension (TALE) proteins. *PBX1* dimerizes with other proteins to generate nuclear complexes that promote binding specificity of HOX proteins to DNA.²¹² Mutations in *PBX1* have been reported among patients with congenital anomalies affecting the kidney and urinary tract, in association with developmental delay, ear anomalies, and congenital heart disease.^{213,214} A de novo *PBX1* mutation, p.Arg235Gln, located within the conserved homeo-domain, was identified in a 46,XY child with female external genitalia; the right gonad consisted of sparse seminiferous tubules with fibrous tissue and the left gonad consisted solely of fibrous tissue.²¹⁵ Expression studies showed that this missense mutation failed to localize in the nucleus, implying *PBX1* is necessary for testicular development and that this mutation is a loss-of-function mutation.²¹⁵

Protein Phosphatase Two Regulatory Subunit B"Gamma (*PPP2R3C*)

This gene, mapped to chromosome 14q13.2, has 13 exons encoding a 453 amino acid protein. In conjunction with scaffolding A and catalytic C subunits, the three proteins comprise a heterotrimeric protein phosphatase 2A, which functions in the reversible dephosphorylation of phosphoproteins. Four phenotypic female patients with 46,XY gonadal dysgenesis, flat facies, bilateral epicanthal folds, low-set posteriorly rotated ears, and single palmar crease have been described.²¹⁶ All four were homozygous for missense mutations; parents were heterozygous carriers. Curiously, three of the four heterozygous fathers were found to have impaired spermatogenesis characterized by teratozoospermia with severe head, acrosomal, and nuclear anomalies.

SRY

SRY is a single exon gene encoding a 204-amino-acid protein. The protein contains an HMG DNA-binding domain flanked by nuclear localization signals (NLS). The majority of sex-reversing *SRY* mutations are located in the HMG/NLS domain and affect DNA-binding affinity, DNA bending ability, or nuclear localization. Mutations located in the N-terminal and C-terminal domains have been identified in 46,XY sex reversed individuals. Mutations in the non-HMG domains may influence transcriptional activation, DNA binding, or protein-protein interactions.²¹⁷ Paternal mosaicism for *SRY* mutations in which different cells carry different *SRY* genes has been described.^{218,219} More puzzling are the pedigrees in which fathers and unaffected brothers carry the identical mutant *SRY* allele as the proband.²²⁰⁻²²² These paradoxical findings implicate involvement of other genes, gene-gene interactions, and gene-environment interactions in the process of sexual differentiation. Nevertheless, only 15% to 20% of cases of 46,XY DSD because of gonadal dysgenesis can be attributed to *SRY* mutations. Mutations in the *SRY* gene have also been reported in patients with stigmata typical of Turner syndrome and 45, X/46XY karyotype.^{223,224}

WW Domain-Containing Oxidoreductase (*WWOX*)

The *WWOX* gene is mapped to 16q23.1-16q23.2. This gene encodes a cytoplasmic protein that plays a role in growth, differentiation, and tumor suppression. One patient with 46,XY sex reversal was found to carry a heterozygous deletion of exons 6 to 8 (amino acids 173-352). This patient had unfused labioscrotal folds, bilateral nonpalpable gonads, clitoral

hypertrophy, a single perineal opening, and intraabdominal dysgenetic gonads.²²⁵ *WWOX* mutations have been identified in autosomal recessive cerebellar ataxia with epilepsy and mental retardation.²²⁶

Zinc Finger and Ring Finger Protein 3 (*ZNRF3*)

ZNRF3 mapped to chromosome 22q12.1 encodes a transmembrane E3 ubiquitin ligase that typically interferes with WNT signaling in the gonad to promote testicular differentiation. Heterozygous mutations have been reported in 5 XY patients with sex-reversal; four of five patients presented in late adolescence with delayed puberty.²²⁷

Zinc Finger Protein, Multitype 2 (*ZFPM2*)

The Friend of GATA 2 (*FOG2*) protein, encoded by *ZFPM2* and mapped to chromosome 8q23.1, is a transcriptional cofactor that modulates the activity of GATA4 by binding to its N-terminal zinc finger.²²⁸ One boy with a balanced translocation and breakpoint with intron 4 of the *FOG2* gene had congenital heart disease, retractile testes, and hypergonadotropic hypogonadism.²²⁹

OVOTESTICULAR DISORDER OF SEX DEVELOPMENT

Ovotesticular DSD is defined as the presence of ovarian tissue with follicles and testicular tissue with seminiferous tubules in the same individual. External genital appearance does not predict gonadal histology.²³⁰ The gonads can be an ovotestis, ovary, and/or testis. The most common histology is an ovotestis. An ovotestis is typically located on the right side and is often intraabdominal. An ovotestis can appear ovoid or bilobed on gross appearance. In most ovotestes, ovarian and testicular tissue show distinct separation in an end-to-end arrangement. In this situation, the testicular zone typically shows poor differentiation of the tunica albuginea with atypical interstitial tissue.²³¹ In some instances, oocytes are interspersed among seminiferous cords/tubules. The proportion of ovarian and testicular tissue differs among patients; uterine development also varies.

The most common karyotype is 46,XX. Mosaic karyotypes, such as 46,XX/46,XY, 46,XX/47,XXY, 45,X/46,XY, 47,XY/46,XY/45,X have been described.^{232,233} In some cases, the amount of Y chromosomal material in peripheral blood lymphocytes is limited, such as the *SRY* gene can be detected only by polymerase chain reaction (PCR) amplification. In other instances, *SRY* was apparently absent in peripheral blood lymphocytes and detected in testicular tissue.^{234,235} A deletion of the *SRY* promoter region was identified in the testicular tissue of a 46,XX patient with ovotesticular disorder.²³⁶ However, ovotesticular DSD in the absence of Y chromosomal material occurs.²³⁷ As noted elsewhere in this chapter, specific mutations, for example, *NR5A1*, *NR2F2*, *RSPO1*, and *SOX9*, have been identified among patients with ovotesticular disorder.

Most cases are sporadic. Nevertheless, pedigrees in which both XX males and XX individuals with ovotesticular disorder coexist have been described.^{238–240} Although most patients present in infancy or childhood, phenotypic males can present with bilateral gynecomastia.²⁴¹ One patient with 46,XX/46,XY karyotype presented in infancy with penoscrotal hypospadias, left descended gonad, and right undescended gonad; biopsies revealed normal prepubertal testis on the left and normal ovarian tissue on the right. Additional evaluation and management of this patient led to male sex of rearing accompanied by right salpingo-oophorectomy and hysterectomy. The patient subsequently presented at age 17 years with a painless scrotal mass, which was excised and found to be normal ovarian tissue. This case illustrates the potential for inconsistency in gonadal

development among patients with ovotesticular disorder with the possibility of sampling errors in gonadal biopsies.²⁴²

Limited outcome data regarding fertility are available. Among 33 patients followed longitudinally, germ cells identified in the testicular tissue during infancy degenerated, resulting in azoospermia.²³⁶ Histological findings among adults describe a dysgenetic Sertoli-only appearance with hyalinization of seminiferous tubules.²³⁴ Nevertheless, assisted reproductive techniques have been successfully used to achieve paternity in men with ovotesticular disorder.^{243,244} Several pregnancies have been reported among women with ovotesticular disorder.^{245,246}

NONSYNDROMIC XX DISORDERS OF SEX DEVELOPMENT (TABLE 6.4)

46,XX Testicular Disorder of Sex Development

Testicular DSD is characterized by a male phenotype with a 46,XX karyotype. The frequency of the XX male syndrome is approximately 1 in 25,000 males.²⁴⁷ This form of DSD can be subclassified as *SRY*-positive and *SRY*-negative groups. The *SRY*-positive 46,XX males generally have normal male external genitalia, small azoospermic testes, hypergonadotropic hypogonadism, and often present with infertility.²⁴⁸ In most instances, the *SRY* gene is located on an X chromosome because of recombination between the X and Y chromosomes.²⁴⁹ However, translocation to an autosome can occur. One example was the incidental finding small testes, azoospermia, and translocation of the *SRY* gene onto the terminal end of chromosome 16q in a 61-year-old 46,XX man.²⁵⁰

Approximately 10% of these patients are *SRY*-negative. The phenotypic spectrum ranges from genital ambiguity to normal male external genitalia.²⁵¹ Testes are typically small because of meiotic arrest and germ cell degeneration.²⁵² Molecular etiologies are more diverse for the *SRY*-negative 46,XX male. Duplication of *SOX9* was described in one family; all affected family members had normal male secondary sexual characteristics and azoospermia.²⁵³ Duplications approximately 600 kb upstream of *SOX9* have been identified in several *SRY*-negative XX males.²⁵⁴ Overexpression of the *SOX10* gene at 22q13 was found in one patient with 46,XX sex reversal in association with multiple congenital anomalies.²⁵⁵

SOX3 is a single exon gene mapped to Xq27. It plays a role in brain, pituitary, and craniofacial development.²⁵⁶ An *SRY*-negative 46,XX patient referred for evaluation of hypospadias and bilateral cryptorchidism was found to have bilateral ovotestes and *SOX3* duplication.²⁵⁷ Several other patients have been reported with *SOX3* duplications.^{258,259} Overexpression of *SOX3* in a preclinical transgenic XX mouse model resulted in testicular development and suggested the hypothesis that *SOX3* acts as a surrogate for *SRY* in this situation.²⁶⁰ Hypoplasia of the anterior pituitary and ectopic position of the posterior pituitary has been described in two patients.²⁶¹

XX DISORDER OF SEX DEVELOPMENT/PREATURE OVARIAN FAILURE

46,XX gonadal dysgenesis is a rare disorder associated with delayed puberty and premature menopause associated with hypergonadotropic hypogonadism (see Table 6.4). Affected individuals typically present with lack of spontaneous pubertal development, primary amenorrhea, and uterine hypoplasia.

As noted earlier, ovarian development is an active process involving multiple genes. However, mutations of genes involved in ovarian development are not typically associated with ambiguous genitalia; external genital development is typically female. Rather, these disorders typically present with

TABLE 6.4 Disorder of Sex Development and Gonadal Dysgenesis in XX (SRY-Negative) Individuals

Gene	Location	Phenotype	Genetic Defects	Mode of Inheritance
<i>BMP15</i>	Xp11.22	Ovarian dysgenesis ^a , primary amenorrhea (OMIM #300510)	Point mutations (paternally transmitted), deletions	X-linked
<i>DCAF17</i>	2q31.1	Hypogonadism, ovarian dysgenesis, partial alopecia, Woodhouse-Sakati Syndrome (OMIM #241080)	Point mutations	AR
<i>DIAPH2</i>	Xq21.33	Primary or secondary amenorrhea (OMIM #300511)	Point mutations, deletions	X-linked
<i>EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5</i>	12q24.31, 14q24.3, 1p34.1, 2p23.3, 3q27.1	Leukoencephalopathy with vanishing white matter, gonadal dysgenesis, primary or secondary amenorrhea (OMIM #603896)	Point mutations	AR
<i>FIGLA</i>	2p13.3	Ovarian insufficiency (OMIM #612310)	Point mutations	AD
<i>FMR1</i>	X	Fragile X premutation (OMIM #311360/#300869)	Trinucleotide expansion	X-linked
<i>FOXL2</i>	3q22.3	Blepharophimosis with ovarian dysgenesis (OMIM # 110100)	Point mutations, translocations, deletion/duplications	AD
<i>HFM1</i>	1p22.2	Ovarian dysgenesis, ovarian insufficiency (PMID:27603904) (29)	Point mutations	unknown
<i>LMNA</i>	1q22	Ovarian dysgenesis with cardiomyopathy (OMIM #212112)	Point mutations	AR
<i>MCM8</i>	20p12.3	Ovarian dysgenesis, primary amenorrhea (OMIM #236700)	Point mutations	AR
<i>MCM9</i>	6q22.31	Ovarian dysgenesis, primary amenorrhea (OMIM #616185)	Point mutations	AR
<i>MKKS</i>	20p12.2	McKusick-Kaufman syndrome hypogonadism, hydrometrocolpos (OMIM #236700)	Point mutations	AR
<i>NOBOX</i>	7q35	Ovarian dysgenesis, primary ovarian insufficiency (OMIM #611548)	Point mutations	AD & AR
<i>NR5A1 (SF1)</i>	9q33.3	Gonadal dysgenesis, ovotesticular DSD, secondary ovarian insufficiency, sex reversal (OMIM #612964)	Deletions/duplications Point mutations	AD
<i>NUP107</i>	12q15	Ovarian dysgenesis, primary amenorrhea (OMIM #607617)	Point mutations	AR
<i>PMM2</i>	16p13.2	Ovarian dysgenesis, cerebellar atrophy, mental impairment, pigmentary retinopathy (OMIM #212065)	Point mutations	AR
<i>POF1B</i>		Ovarian insufficiency (OMIM #300604)		
<i>PSMC3IP</i>	17q21.2	Ovarian dysgenesis, primary amenorrhea (OMIM #614324)	Point mutations	AR
<i>REC8</i>	14q12	Ovarian dysgenesis, ovarian insufficiency (PMID:27603904) (29)	Point mutations	unknown
<i>SMC1B</i>	22q12.31	Ovarian dysgenesis, ovarian insufficiency (PMID:27603904) (29)	Point mutations	unknown
<i>SOHLH1</i>	9q34.3	Ovarian dysgenesis, ovarian insufficiency (PMID:27603904) (29)	Point mutations	AR
<i>SOHLH2</i>		Ovarian dysgenesis, ovarian insufficiency (OMIM #616066)		AD
<i>SOX3</i>	Xq26.3	Gonadal dysgenesis, virilizing 46,XX sex reversal (OMIM #300833)	Deletions/duplications	X-linked
<i>SOX8</i>	16p13.3	Ovarian dysgenesis, primary or secondary amenorrhea	Point mutations	AD
<i>SOX9</i>	17q24.3-q25.1	46,XX sex reversal, virilizing chromosome 17q24 duplication syndrome (OMIM #278850)	Duplication 584–516 kb upstream of SOX9	AD
<i>SOX10</i>	22q11.2q13	46,XX sex reversal, Waardenburg syndrome, peripheral demyelinating neuropathy	Duplication	AD
<i>SPDR</i>	8q11.21	Ovarian dysgenesis, primary or secondary amenorrhea (OMIM # 615384)	Point mutations	AR
<i>STAG3</i>	7q22.1	Ovarian dysgenesis, ovarian insufficiency (OMIM #615723)	Point mutations	AR
<i>SYCE1</i>	10q26.3	Ovarian dysgenesis, ovarian insufficiency (PMID:27603904) (29)	Point mutations	unknown
<i>WNT4</i>	1p34.12	Mullerian aplasia, hyperandrogenism, primary amenorrhea (OMIM #158330)	Point mutations	AD

^aAlso associated with large X chromosome deletions/duplications. AD, Autosomal dominant; AR, autosomal recessive.

delayed puberty, primary amenorrhea, and POI.²⁶² In these disorders, follicles may fail to develop or may undergo premature atresia. Mutations in genes involved in ovarian development such as Forkhead box L2 (*FOXL2*), Newborn ovary homeobox (*NOBOX*), bone morphogenetic protein 15 (*BMP15*), and Factor in germline alpha (*FIGLA*) have been described. *BMP15*, a member of the TGF- β superfamily, influences granulosa cell growth and differentiation. Heterozygous missense *BMP15* mutations have been identified among women with POI and

associated with X-linked XX gonadal dysgenesis.²⁶³ Ovarian leukodystrophy is a leukoencephalopathy characterized by vanishing white matter and ovarian dysgenesis associated with mutations in the *EIF2B2*, *EIF2B4*, and *EIF2B5* genes. A mutation in the proteasome 26 subunit, adenosine triphosphatase (ATPase), 3-interacting protein (*PSMC3IP*) gene has been described in autosomal recessive 46,XX gonadal dysgenesis; this loss-of-function mutation reduces estrogen-induced transcriptional activation of the protein encoded by this gene.

Fragile X syndrome is caused by inactivation of the *FMR1* gene caused by DNA hypermethylation of the promoter and expanded 5'-UTR CCG repeat region; the premutation allele containing 55-200 CCG repeats is associated with POI.²⁶⁴ Prevalence of premutation alleles in the general population is estimated at 1 per 150 to 300 females.²⁶⁵

FOXL2

Forkhead box L2 (*FOXL2*) is a member of the winged helix/forkhead transcription family. The protein contains a DNA-binding domain, which is primarily located in the nucleus. The protein contains three α -helices. Mutations in *FOXL2*, located at chromosome 3q23, are associated with the autosomal dominant BPES. BPES is characterized by eyelid dysplasia consisting of small palpebral fissures (blepharophimosis), ptosis, epicanthus inversus, and a broad nasal bridge. The phenotype of BPES type 1 consists of eye findings and premature ovarian failure. Patients with BPES type 2 manifest only the eye features. Patients heterozygous for *FOXL2* mutations generally experience spontaneous pubertal development culminating in menarche, but undergo premature follicle depletion, leading to premature ovarian failure. The histological appearance of ovaries in patients with BPES type 1 is reported to vary from the presence of primordial follicles with some atretic follicles to the complete absence of follicles. Mutations generating nonsense mutations, predicted to generate truncated proteins lacking the transrepression domain, are associated with BPES type 1. Expansion of the poly-alanine tract is associated with BPES type 2.

One major role of *FOXL2* in the ovary appears to be transrepression of genes involved in steroidogenesis, such as *StAR*, *P450sc*, and *aromatase* to prevent premature differentiation and proliferation of granulosa cells.¹⁹⁶ Loss of the transrepressor activity because of nonsense mutations likely contributes to the premature depletion of ovarian follicles and premature ovarian failure. One patient with ovotesticular disorder was found to have expression of both *FOXL2* and *SOX9*. However, in general, expression of these proteins is mutually exclusive.

Curiously in goats mutations in this locus are responsible for the autosomal dominant phenotype characterized by the absence of horns in male and female goats (polledness) and XX female-to-male sex reversal in a recessive manner.

NOBOX

The Newborn Ovary Homeobox (*NOBOX*) gene is expressed in germ cells and primordial oocytes. *NOBOX* encodes a basic helix-loop-helix transcription factor involved in the transition from primordial to primary follicles. The protein is expressed in fetal and adult ovaries.²⁶⁶ The *NOBOX* gene is located at chromosome 7q35. The phenotype of patients with *NOBOX* mutations includes delayed puberty, with primary amenorrhea and secondary amenorrhea.²⁶⁷ POI associated with *NOBOX* mutations shows both autosomal dominant and autosomal recessive inheritance patterns.²⁶⁸

FIGLA

Factor in germline alpha (*FIGLA*) is a germ cell-specific transcription factor located at chromosome 2p13.3. *FIGLA* is expressed in the human fetal ovary. The phenotype of women with *FIGLA* mutations includes secondary amenorrhea and ovaries devoid of follicles.²⁶⁹ Both autosomal dominant and autosomal recessive inheritance have been described.²⁷⁰

SPIDR

Scaffolding protein involved in DNA repair (*SPIDR*), mapped to chromosome 8q11.21, encodes a protein involved in

homologous DNA repair. Sisters who presented with primary amenorrhea were found to have hypergonadotropic hypogonadism associated with homozygous mutation.

NUP107

Nucleoporins comprise nuclear pore complexes that serve as the gatekeepers of nucleocytoplasmic transport. One such protein is nucleoporin-107, encoded by *NUP107*, is mapped to chromosome 12q15. Missense variants in *NUP107* segregated with delayed puberty and hypergonadotropic hypogonadism associated with POI in two ethnically different families. Affected females were otherwise healthy; no male phenotype was evident.^{271,272}

WNT4 Gene

WNT4 is a secreted molecule that binds to members of the frizzled family of receptors, resulting in transcriptional regulation of target genes. *WNT4* increases follistatin expression, which inhibits formation of the coelomic vessel (antitestis action) and supports ovarian germ cell survival (proovarian action).¹¹⁰ Duplication of the *WNT4* gene, located at chromosome 1p31-1p35, has been associated with 46,XY male-to-female sex reversal. One such patient presented with ambiguous external genitalia accompanied by severe hypospadias, fibrous gonads, remnants of both Mullerian and Wolffian structures, cleft lip, microcephaly, and IUGR.¹¹¹ A female-to-male sex reversed patient with kidney, adrenal, and lung dysgenesis was found to have a homozygous loss-of-function *WNT4* mutation.¹¹² Loss-of-function *WNT4* mutations have been detected in 46, XX women with primary amenorrhea, secondary to Mullerian duct abnormalities and androgen excess.¹¹³⁻¹¹⁵ The phenotypes of these patients support the hypothesis that *WNT4* plays a role in ovarian differentiation.

VANISHING TESTES

The terms *testicular regression syndrome* and *vanishing testes* are used to describe testicular absence in boys with undescended testes. In some instances, this situation is associated with ambiguous genitalia and undervirilization, which presumably represents regression of testicular tissue occurring between 8 and 14 weeks of gestation. Physical findings reflect duration of testicular function. At operation, a rudimentary spermatic cord and nubbin of testicular tissue may be identified. Histological examination of the testicular nubbins often reveals hemosiderin-laden macrophages and dystrophic calcification.²⁷³ One potential mechanism involves an antenatal vascular accident, associated with antenatal testicular torsion, leading to testicular regression.²⁷⁴ Another possibility is anomalous vascular development.²⁷⁵ Although usually sporadic, familial testicular regression has been described.¹⁶⁰

DISORDERS OF CHOLESTEROL AND STEROID BIOSYNTHESIS

Abnormalities affecting the biosynthetic pathways involved in cholesterol, cortisol, and sex steroid biosynthesis can lead to genital ambiguity (Table 6.5). Steroidogenesis refers to the multiple enzyme-mediated processes through which cholesterol is converted to biologically active steroid hormones (Fig. 6.2). This process depends on the coordinated regulation of cholesterol uptake into cells, transfer into mitochondria, and actions of tissue-specific enzymes.²⁷⁶ Details regarding the complexities of steroidogenic processes continue to unfold. Three pathways have been characterized: (1) canonical/classical, (2) "alternative backdoor," and (3) 11-oxygenated C-19 androgens.

TABLE 6.5 Steroidogenic Disorders

Gene	Location	Phenotype	Genetic Defects	Mode of Inheritance
<i>AKR1C2</i> and <i>AKR1C4</i>	10p15.1	Complete gonadal dysgenesis, 3 α -Hydroxysteroid Dehydrogenase Isozyme Deficiency (OMIM #614279)	Point mutations	AR
<i>CYP5A</i>	18q22.3	Undervirilized, delayed puberty methemoglobinemia (OMIM # 250790)		AR
<i>CYP11A1</i>	15q24.1	Adrenal insufficiency, sex reversal, partial or complete (OMIM # 613743)	Point mutations	AR/AD?, oligogenic
<i>CYP11B1</i>	8q24.3	Virilizing congenital adrenal 11 β -hydroxylase deficiency, hypertension (OMIM #202010)	Point mutations, deletions	AR
<i>CYP17A1</i>	10q24.32	17 α -hydroxylase/17,20-lyase deficiency, genital ambiguity, delayed puberty (OMIM#202110)		AR
<i>CYP19A1</i>	15q21	Virilizing congenital adrenal placental aromatase deficiency (OMIM # 613546)	Point mutations	AR
<i>CYP21A2</i>	6p21.33	Virilizing congenital adrenal hyperplasia caused by 21-hydroxylase deficiency (OMIM #201910)	Deletions, point mutations	AR
<i>HSD3B2</i>	1p12	3 β -Hydroxysteroid dehydrogenase deficiency, genital ambiguity (OMIM #201810)	Point mutations	AR
<i>HSD17B3</i>	9q22.32	17 β -Hydroxysteroid dehydrogenase deficiency, complete gonadal dysgenesis or hypovirilization and gynecomastia (OMIM# 264300)	Point mutations	AR
<i>POR</i>	7q11.23	virilizing congenital P450 oxidoreductase deficiency, (OMIM #613571)	Point mutations, deletions	AR
<i>SRD5A2</i>	2p23.1	Ambiguous genitalia, 5 α -reductase deficiency, (OMIM# 264600)	Point mutations, intragenic deletions	AR
<i>STAR</i>	8p11.23	Lipoid congenital adrenal hyperplasia (OMIM #201710)	Point mutations	AR

AD, Autosomal dominant; AR, autosomal recessive.

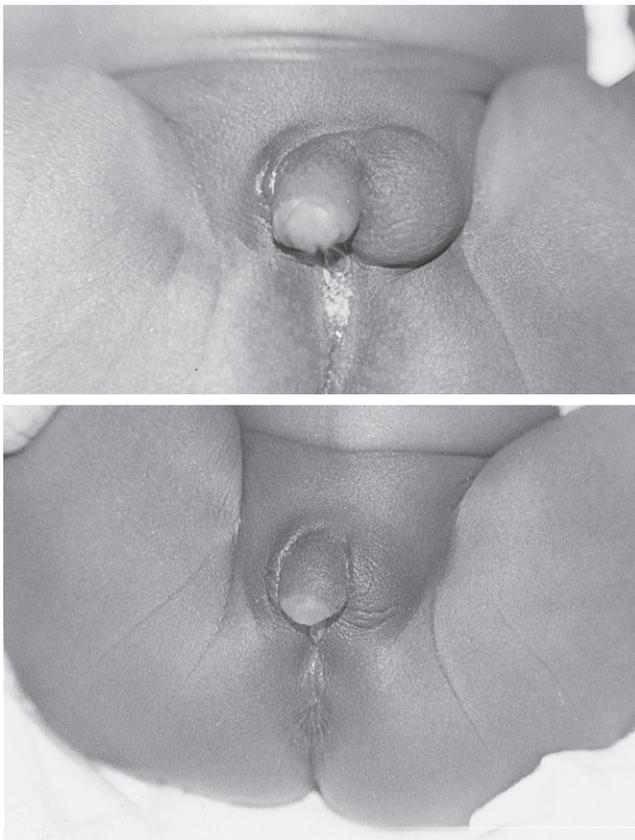


Fig. 6.2 Genital ambiguity with asymmetry in a patient with 46,XY disorder of sexual differentiation (mixed gonadal dysgenesis).

Many of the steroidogenic enzymes are cytochrome P450 heme-containing proteins that absorb light at 450 nm in their reduced state or hydroxysteroid dehydrogenases. The P450 enzymes receive electrons from the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and use

their heme center to mediate catalysis. The type 1 enzymes are located in mitochondria and receive electrons from NADPH from a flavoprotein, ferredoxin reductase, and an iron-sulfur protein, ferredoxin. The type 2 enzymes are located in the endoplasmic reticulum and receive electrons from NADPH from another protein, P450 oxidoreductase.

The hydroxysteroid dehydrogenase enzymes use NAD⁺ and NADP⁺ as cofactors; these enzymes do not contain heme groups. The hydroxysteroid dehydrogenases are classified into two groups. One group is the short-chain dehydrogenase reductase family. The other group is the aldo-keto reductase family.²⁷⁷ These enzymes function as dehydrogenases and reductases. Although these enzymes can perform both dehydrogenase and reductase activities in vitro, most function unidirectionally.

During fetal life, steroidogenic enzymes are expressed in placenta, testis, and adrenal. Inborn errors of testosterone biosynthesis can lead to undervirilization and ambiguous genitalia in 46,XY fetuses. Loss-of-function mutations in adrenal cortisol biosynthesis leads to increased androgen concentrations in XX, fetuses with subsequent virilization of the external genitalia.

In the canonical/classical androgen pathway, the fetal testis secretes testosterone that is converted to dihydrotestosterone (DHT) in target tissues, such as the prostate and external genitalia. In the testis, cholesterol is converted to pregnenolone by cholesterol side chain cleavage enzyme P450_{sc} encoded by *CYP11A1*. Pregnenolone is converted to 17-hydroxypregnenolone by the 17 α -hydroxylase activity of P450_{c17} encoded by *CYP17A1* and then to DHEA by the 17,20-lyase activity of P450_{c17}. DHEA is converted to androstenedione by 3 β -hydroxysteroid dehydrogenase type 2 encoded by *HSD3B2*. Androstenedione is converted to testosterone by 17 β -hydroxysteroid dehydrogenase type 3 encoded by *HSD17B3*. In genital skin, testosterone is converted to DHT by 5 β -reductase type 2 encoded by *SRD5A2*.

Through investigations of the tammar wallaby and patients with disordered steroidogenesis, the presence of another pathway leading to DHT synthesis was uncovered.²⁷⁸ In this "alternate backdoor pathway" steroidogenesis bypasses

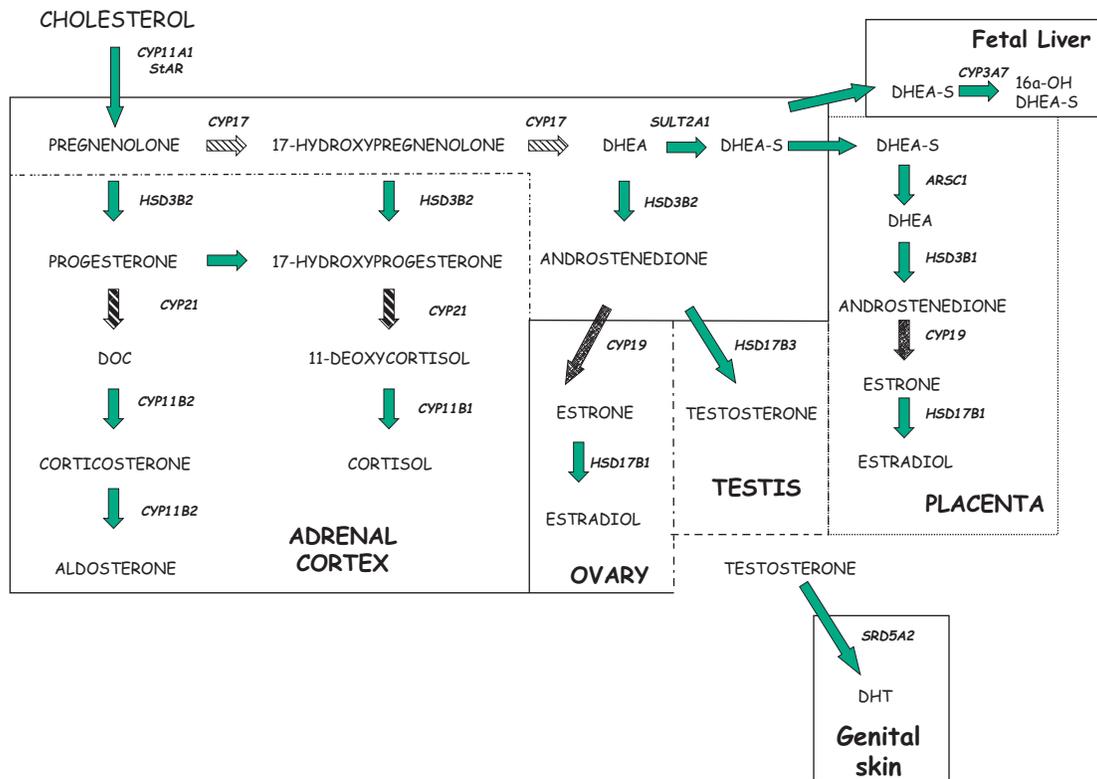


Fig. 6.3 Diagram of classical steroidogenic pathways. Substrates, products, and genes involved in adrenal, ovarian, testicular, and placental steroidogenesis are indicated. Genes are 17 α -hydroxylase/17,20-lyase (*CYP17*), 3 β -hydroxysteroid dehydrogenase (*HSD3B2*), 21-hydroxylase (*CYP21*), 11 β -hydroxylase (*CYP11B1*), aldosterone synthase (*CYP11B2*), aromatase (*CYP19*), 17 β -hydroxysteroid dehydrogenase type 1 (*HSD17B1*), 17 β -hydroxysteroid dehydrogenase type 3 (*HSD17B3*), 5 α -reductase type 2 (*SRD5A2*), sulfotransferase (*SULT2A1*), and steroid sulfatase/arylsulfatase C (*ARSC1*). *CYP3A7* is a cytochrome P450 enzyme expressed in fetal liver, where it catalyzes the 16 α -hydroxylation of estrone (E1) and dehydroepiandrosterone (DHEA). Its expression decreases postnatally. Steroidogenic enzymes that use P450 oxidoreductase (POR), a flavoprotein encoded by *POR*, to transfer electrons are indicated by hatched arrows DHEA-S, Dehydroepiandrosterone sulfate.

DHEA, androstenedione, and testosterone (Fig. 6.3). Rather, 17-hydroxyprogesterone (17-OHP) undergoes 3 α - and 5 α -reduction followed by 17,20-lyase, 17 β -hydroxysteroid dehydrogenase, and 3 α -oxidation steps to generate DHT.²⁷⁹

The third steroidogenic pathway involved the 11-oxygenated C-19 steroids, which have long been recognized as major androgens in teleost fish. The relevance of 11-oxygenated steroids for human health has been explored (see Fig. 6.3). This class of steroids depends on the activity of 11 β -hydroxylase (P450c11B1) encoded by *CYP11B1* and expressed in the adrenal zona fasciculata and zona reticularis. Androstenedione and testosterone are substrates for this enzyme and can be converted to 11 β -hydroxyandrostenedione and 11 β -hydroxytestosterone, respectively.²⁸⁰ Subsequent enzymatic steps can generate 11-ketotestosterone and 11-dihydroketotestosterone; both are potent androgens that activate the androgen receptor.²⁸¹ Whereas the 11-oxygenated C-19 steroids are established to be relevant in postnatal physiology, their contribution, if any, to fetal steroidogenesis is unknown.

Both the canonical androgen biosynthetic pathway and the “alternative backdoor pathway” are critical to promote normal masculinization of the XY fetus.²⁸² Proteins requisite for testosterone biosynthesis include NR5A1, LH receptor, steroidogenic acute regulatory peptide (StAR), P450scc, P450c17, 3 β -hydroxysteroid dehydrogenase type 2, 17 β -hydroxysteroid dehydrogenase type 3, P450-oxidoreductase, 5 β -reductase types 1 and 2, aldo-keto reductase type 1C2 (AKR1C2), and aldo-keto reductase type 1C4 (AKR1C4) (see Fig. 6.3). Enzymes, for example, SRD5A1, associated with the alternative backdoor pathway are primarily expressed in nongonadal

tissues, such as the liver and placenta.²⁸³ Based on a small number of midgestation human male fetuses, androsterone was reported to be the major backdoor androgen in the male fetal circulation; these data also suggested that placental progesterone provided the substrate for androsterone biosynthesis.²⁸³

Inborn errors of glucocorticoid biosynthesis are often associated with the virilizing CAHs. The fetal adrenal cortex is derived from coelomic epithelium and consists of two major zones: the fetal zone and the adult zone. The fetal zone is primarily responsible for DHEA synthesis, which is then sulfated to provide substrate for placental estrogen biosynthesis (see Fig. 6.3). The adult zone, which after birth differentiates into the three zones of the adult adrenal cortex, is primarily responsible for cortisol biosynthesis. By 10 weeks of gestation, the adrenal is secreting DHEA-S and the hypothalamic-pituitary-adrenal axis is functional. The extent of placental *CYP17A1* transcript expression is unresolved, but most recent data indicate minimal expression.^{284,285}

Luteinizing Hormone Choriogonadotropin Receptor Gene

Leydig cell hypoplasia is an autosomal recessive disorder characterized by failure of testicular Leydig cell differentiation, secondary to inactivating *LHCGR* mutations, with ensuing target cell resistance to LH.²⁸⁶ The *LHCGR* gene, mapped to chromosome 2p21, codes for a 674-amino-acid protein. The *LHCGR* is a seven-transmembrane domain G-protein-coupled receptor. Specific mechanisms through which the loss-of-function

mutations induce LH resistance include decreased receptor protein, decreased ligand binding, altered receptor trafficking, and impaired ability to activate G_s .

Inability to respond to hCG or LH decreases Leydig cell testosterone biosynthesis. The phenotype of affected 46,XY infants ranges from undervirilization to female external genitalia. Affected individuals raised as females often seek medical attention for delayed breast development. Mullerian duct derivatives are absent because AMH is secreted by the unaffected Sertoli cells. Testes are typically inguinal or intraabdominal. Laboratory studies show elevated LH, low testosterone, and normal FSH concentrations. There is no significant testosterone response to hCG stimulation. Testicular histology reveals absence of Leydig cells; seminiferous tubules and testicular volume are preserved because of normal FSH secretion and responsiveness. Undervirilization with hypospadias, micropenis, and cryptorchidism can occur with incomplete loss-of-function missense mutations.

Genetic females, sisters of affected 46,XY individuals, who carry the identical mutations show normal female genital differentiation and normal pubertal development but have amenorrhea and infertility.²⁸⁷ The uterus is small to normal in size. Ovarian cysts may develop. Estrogen concentrations generally fail to achieve the threshold required for ovulation. Genetic analysis may be helpful to distinguish LHCGR mutations from other disorders affecting testosterone biosynthesis.^{288,289}

Congenital Lipoid Adrenal Hyperplasia

This autosomal-recessive disorder is characterized by a severe defect in the conversion of cholesterol to pregnenolone, leading to impaired synthesis of all adrenal and gonadal steroid hormones. Impaired testosterone biosynthesis in utero prevents male sexual differentiation. Hence, all affected fetuses (46,XY or 46,XX) have female external genitalia. In the XY fetus, Sertoli cells are intact, AMH secretion is unaffected, and Mullerian duct elements regress. Low or undetectable steroid hormone concentrations, elevated ACTH concentrations, and elevated plasma renin activity (PRA) are consistent with this diagnosis. Elevated 17-hydroxypregnenolone or pregnenolone concentrations distinguish 3β -hydroxysteroid dehydrogenase deficiency from congenital lipoid adrenal hyperplasia.

Congenital lipoid adrenal hyperplasia is caused by mutations in the *StAR* gene mapped to chromosome 8p11.23.²⁹⁰ The *StAR* protein facilitates cholesterol transport across the mitochondria to P450_{scc}. In congenital lipoid adrenal hyperplasia, the impaired mitochondrial cholesterol transport leads to accumulation of cholesterol esters and sterol autooxidation products. Ultimately, the lipid accumulation alters the cell cytostructure provoking cell destruction and complete loss of *StAR*-dependent steroidogenesis.²⁹¹ Hence congenital lipoid adrenal hyperplasia has “two hits”: one is defective steroidogenesis and the other is destruction of the steroidogenic cell.^{292,293} Because the definitive zone of the adrenal cortex is relatively quiescent during gestation, aldosterone deficiency may not be obvious in the immediate newborn period. This two-hit mechanism enables spontaneous pubertal development in affected girls because significant estrogen synthesis does not take place until puberty. Ovarian follicular cells are sequentially recruited and damaged, resulting in hypergonadotropic hypogonadism.^{294,295}

Nonclassic lipoid CAH is a milder form with variable adrenal and gonadal function presenting in childhood and adolescence.²⁹⁶ Nonclassic lipoid CAH may present with isolated glucocorticoid deficiency and resembles familial glucocorticoid deficiency because of mutations in the ACTH receptor (*MC2R*) gene.²⁹⁷

In humans, because 17-OHP is not a favored substrate for the 17,20-lyase reaction, this pathway acquires functional importance in disorders of steroidogenesis associated with increased 17-OHP concentrations.

SIDE CHAIN CLEAVAGE CYTOCHROME P450 ENZYME

Side chain cleavage enzyme (also known as cholesterol desmolase) is a cytochrome P450 enzyme encoded by the *CYP11A1* gene located at chromosome 15q23-q24. This enzyme converts cholesterol to pregnenolone, is essential to steroidogenesis, and plays a crucial role in placental progesterone synthesis. Children affected with this autosomal-recessive disorder have adrenal insufficiency and female external genitalia irrespective of the karyotype. Postnatally, adrenal enlargement is typically not evident on ultrasound or magnetic resonance imaging. The absence of adrenal/gonadal tissue has been speculated to result from lipid accumulation similar to the cell destruction observed in patients with *StAR* mutations.²⁹⁸ The phenotypic spectrum includes adrenal insufficiency with hypospadias, normal external genital development, normal pubertal progression, or gonadal failure.²⁹⁹ *CYP11A1* mutations have been detected in patients with primary adrenal insufficiency.³⁰⁰ Genetic analysis may be necessary to distinguish this disorder from congenital lipoid adrenal hyperplasia.³⁰¹

VIRILIZING CONGENITAL ADRENAL HYPERPLASIAS

The virilizing CAHs are a group of disorders caused by mutations in the steroidogenic enzyme genes involved in cortisol biosynthesis. These genes are 3β -hydroxysteroid dehydrogenase type 2 (*HSD3B2*), 21-hydroxylase (*CYP21A2*), and 11 β -hydroxylase (*CYP11B1*). These three disorders share a common pathophysiology. Insufficient cortisol production leads to decreased negative feedback inhibition, increased ACTH production, accumulation of steroid intermediates proximal to the deficiency enzyme, and increased androgen concentrations.^{302,303} The specific manifestations and laboratory abnormalities vary depending on which enzyme gene is involved. Indeed, the magnitude of glucocorticoid and mineralocorticoid deficiencies varies generally in proportion to the severity of the enzyme deficiency.

Accumulation of steroid intermediates, such as 17-OHP, results in increased androgen concentrations. In the affected female, the increased androgen exposure promotes virilization of the external genitalia. Excessive 17-OHP can be converted through the alternate backdoor pathway to DHT. This alternate route involves 5α and 3α -reduction of 17-OHP to 5α -pregnane- $3\alpha,17\alpha$ -diol-20-one (pdiol), ultimately generating androstane-diol, which is the substrate for 3α -oxidation and conversion to DHT.³⁰⁴ (Fig. 6.4). During fetal life, accumulation of 17-OHP because of mutations in *CYP21A2*, *CYP11B2*, or P450-oxidoreductase (*POR*) may increase flux through this “backdoor pathway,” leading to elevated DHT concentrations.³⁰⁵

21-Hydroxylase Deficiency

The most common type of CAH (accounting for 90%–95% of cases) is 21-hydroxylase deficiency because of mutations in the 21-hydroxylase (*CYP21A2*) gene located at chromosome 6p21.3 in the human leukocyte antigen (HLA) class III region.³⁰⁶ The incidence of the classic forms is reported to range from 1:5000 to 1:15,000 and varies among ethnic/racial backgrounds.³⁰⁷ Decreased 21-hydroxylase activity impairs conversion of 17-OHP to 11-deoxycortisol in the zona fasciculata (the primary site of cortisol biosynthesis) and conversion of progesterone to deoxycorticosterone in the zona glomerulosa, the primary site of aldosterone biosynthesis. An extensive clinical practice guideline has recently been published.³⁰⁸

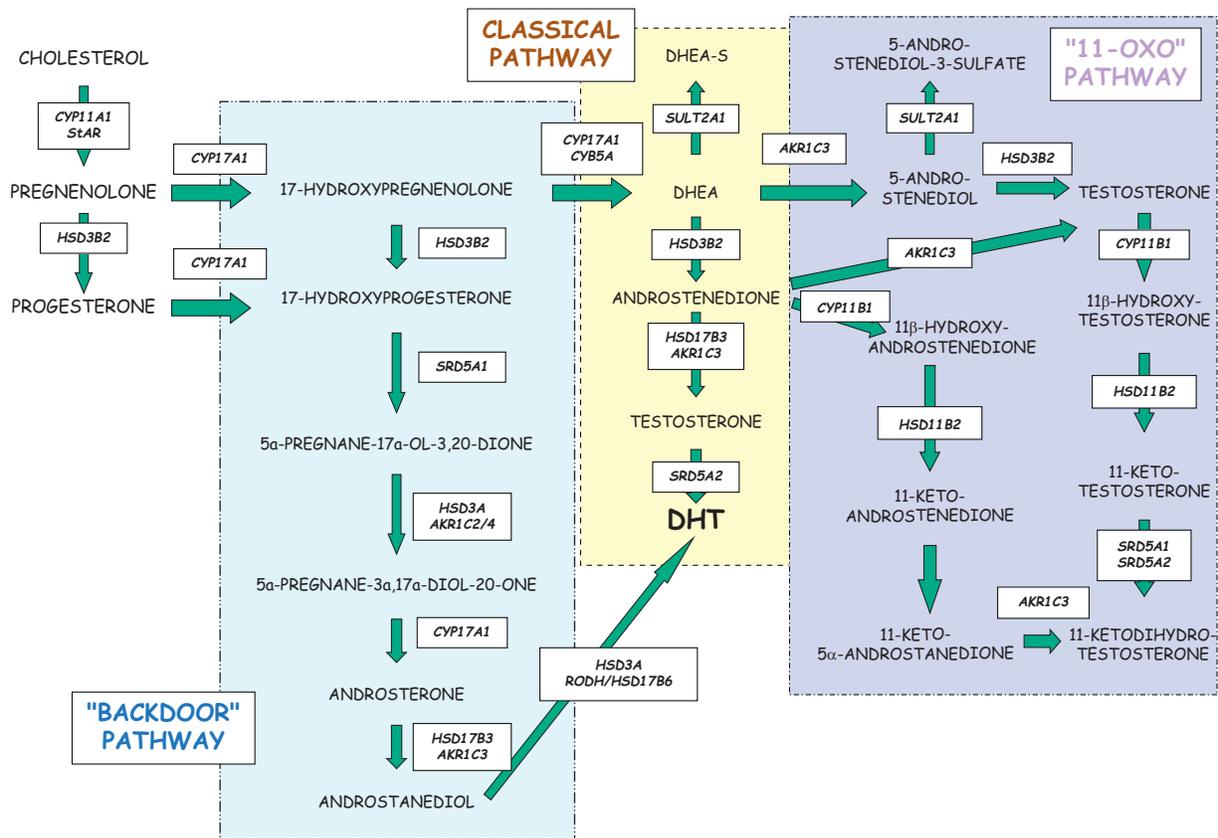


Fig. 6.4 Classical, backdoor, and 11-oxo-C19 steroidogenic pathways. The classic steroidogenic pathway relevant to the testis (*light background*), the "backdoor pathway" (*dark background*), and the 11-oxo-C19 androgens (*purple background*) are indicated. In the presence of elevated ACTH and 17-OHP concentrations because of *CYP21*, *CYP11B1*, or *POR* mutations, the backdoor pathway may contribute to the excessive androgen concentrations responsible for virilization of XX fetuses.

Infant girls with classic salt-losing 21-hydroxylase deficiency usually present in the immediate neonatal period because of genital ambiguity (Fig. 6.5). When the diagnosis is delayed, affected girls develop dehydration, hyponatremia, and hyperkalemia because of glucocorticoid and mineralocorticoid deficiencies. In affected females, the spectrum of genital virilization varies from clitoromegaly to perineal hypospadias to complete

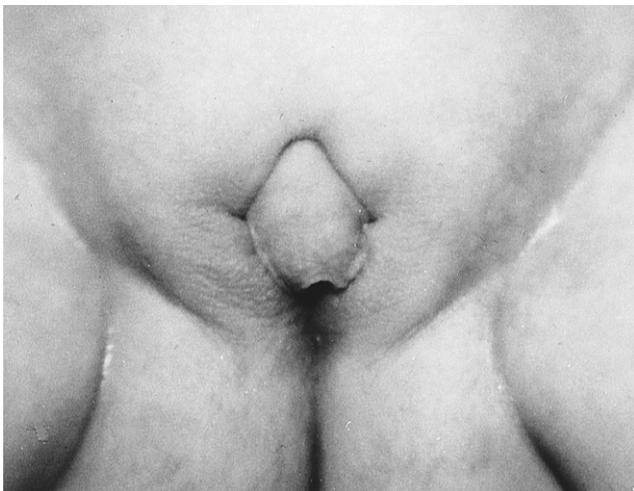


Fig. 6.5 Genital ambiguity in virilized female with 21-hydroxylase deficiency. The labioscrotal folds are fused, and the clitoris is enlarged.

fusion of labiourethral and labioscrotal folds, giving rise to a phallus with chordee and urethral meatus at the tip of phallus. The magnitude of external genital virilization may be so extensive that affected female infants appear to be males with bilateral undescended testes.^{309,310} Unless identified by neonatal screening, infant boys typically present at 2 to 3 weeks of age with failure to thrive, poor feeding, lethargy, dehydration, hypotension, hyponatremia, and hyperkalemia. When the diagnosis is delayed or missed, CAH is potentially fatal. Newborn screening programs decrease the morbidity and mortality associated with acute adrenal insufficiency.

In affected infants, random 17-OHP concentrations are usually elevated. Concentrations are greater than 5000 ng/dL and may be much higher.³¹¹ Androstenedione and progesterone concentrations are also typically elevated. In some instances, PRA can be helpful to assess mineralocorticoid status. Measurement of 21-deoxycortisol is extremely helpful, but availability of this hormone assay is limited. For female infants, a normal uterus is present and can be identified on ultrasound. Ovaries may be too small to be readily identified on ultrasound. Despite excessive antenatal androgen exposure, ovarian position is normal and internal Wolffian structures are not retained.

The spectrum of impaired 21-hydroxylase activity ranges from complete glucocorticoid and mineralocorticoid deficiencies to mild deficiencies manifested principally by compensatory excessive adrenal androgen secretion. Infants capable of adequate aldosterone synthesis do not usually manifest overt salt loss. Female infants capable of adequate aldosterone synthesis may still have sufficient androgen exposure in utero to virilize their external genitalia. In the absence of newborn

screening programs, affected males capable of aldosterone biosynthesis may not be identified until they present with genital overgrowth or premature pubarche. Infants with the milder forms of CAH are generally not identified by most newborn screening programs. Urinary steroid hormone gas chromatography/mass spectrometry analyses showed increased ratios of pdiol to metabolites of the Δ^4 and Δ^5 pathways, which indicates postnatal activity of the backdoor pathway, especially during early infancy.³¹²

CYP21A2 is located approximately 30 kilobases from a highly homologous pseudogene, *CYP21A1P*. The tenascin-XB (*TNXB*) gene encoding an extracellular matrix protein is located on the DNA strand opposite *CYP21A2*.³¹³ At this time, more than 200 *CYP21A2* mutations have been reported.³¹⁴ However, only a few mutations account for the majority of affected alleles. Most of the common mutations represent gene conversion events in which *CYP21A2* has acquired deleterious *CYP21A1P* sequences. The frequency of specific mutations varies among ethnic groups. Molecular genotyping can be a useful adjunct to newborn screening. Caveats to bear in mind are that multiple mutations can occur on a single allele and that different *CYP21A2* mutations can occur in one family.³¹⁵

11 β -Hydroxylase Deficiency

CAH because of 11 β -hydroxylase deficiency is characterized by glucocorticoid deficiency, excessive androgen secretion, and hypertension. This form of CAH is caused by mutations in the 11 β -hydroxylase (*CYP11B1*) gene. The enzyme is expressed in the zona fasciculata, where it converts 11-deoxycortisol to cortisol. CAH, caused by *CYP11B1* mutations, is rare (3%–5% of cases) apart from the high incidence among Moroccan Jews, for whom the incidence approaches one in 6000.³¹⁶ Despite the presence of the identical mutation (R448H), phenotypic heterogeneity for the magnitude of virilization and hypertension occurs even in a single family. Affected females may present with ambiguous genitalia. The typical laboratory finding is an elevated 11-deoxycortisol concentration. Serum concentrations of 17-hydroxyprogesterone, androstenedione, and testosterone may be mildly elevated. PRA concentrations are low or suppressed. Nevertheless, infants may experience salt loss presumably caused by mineralocorticoid resistance. Patients with nonclassic forms have been identified.³¹⁷ Although ACTH-stimulated hormone responses among heterozygotic carriers are usually normal, elevated 11-deoxycortisol and 11-deoxycorticosterone have been reported.

The *CYP11B1* gene is located at chromosome 8q22 in close proximity to a highly homologous gene *CYP11B2*, which codes for aldosterone synthase. Novel mutations associated with classical and nonclassical 11 β -hydroxylase deficiency have been identified. *CYP11B1* is expressed in the zona fasciculata, whereas *CYP11B2* is expressed primarily in the zona glomerulosa.

3 β -Hydroxysteroid Dehydrogenase Deficiency

CAH caused by 3 β -hydroxysteroid dehydrogenase type 2 deficiency, associated with mutations in the 3 β -hydroxysteroid dehydrogenase type 2 (*HSD3B2*) gene, leads to virilization of the external genitalia of 46,XX fetuses because of increased DHEA synthesis. Affected 46,XY fetuses have ambiguous genitalia characterized by undervirilization of the external genitalia secondary to testosterone deficiency. Despite decreased testosterone synthesis, affected 46,XY fetuses usually have intact Wolffian duct structures (including vas deferens). The NAD⁺-dependent enzyme 3 β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 -isomerase catalyzes the conversion of the Δ^5 steroid precursors, pregnenolone, 17-hydroxypregnenolone, and DHEA into the respective Δ^4 -ketosteroids, progesterone, 17-OHP, and androstenedione.²⁵⁰

Two isozymes encoded by two different highly homologous genes have been identified and mapped to chromosome 1p13.1. The type 1 (*HSD3B1*) gene is expressed primarily in skin, placenta, prostate, and other peripheral tissues, whereas *HSD3B2* is primarily expressed in the adrenal cortex and gonads. Mutations in *HSD3B2*, but not *HSD3B1*, have been detected in patients with 3 β -hydroxysteroid dehydrogenase deficiency CAH. Acute adrenal insufficiency occurs in the newborn period when complete loss-of-function mutations impair biosynthesis of mineralocorticoids, glucocorticoids, and sex steroids. Typical presentations for the non-salt-losing forms include premature pubarche and (in 46,XY infants) perineal hypospadias. Confirmatory laboratory findings include elevated pregnenolone, 17-hydroxypregnenolone, and DHEA concentrations with elevated ratios of Δ^5 to Δ^4 steroids. Because enzymatic activity of the type 1 isozyme is unimpaired, elevated 17-OHP and androstenedione concentrations may be found.

DEFECTS IN SEX STEROID BIOSYNTHESIS

17 α -Hydroxylase/17,20-Lyase Deficiency

Synthesis of mineralocorticoids, glucocorticoids, and sex steroids is modulated by the enzyme, 17 α -hydroxylase/17,20-lyase. This enzyme, encoded by a single gene, *CYP17A1* mapped to chromosome 10q24.3, catalyzes two distinct steps in steroidogenesis, pregnenolone is 17 α -hydroxylated to 17 α -hydroxypregnenolone, which then undergoes 17,20-lyase activity to form DHEA. Factors favoring the 17,20-lyase reaction include availability of P450 oxidoreductase and cytochrome b₅ and serine/threonine phosphorylation of the P450c17 protein.³¹⁸

Loss-of-function mutations interfere with glucocorticoid and sex steroid biosynthesis. Affected males present with undervirilization of the external genitalia. Affected females have normal genital development and present with delayed puberty. Patients are often hypertensive because of the increased ACTH-stimulated mineralocorticoid synthesis. Serum levels of progesterone, deoxycortisone, corticosterone, 18-hydroxycorticosterone, and 18-hydroxy deoxycorticosterone (DOC) are elevated and can be suppressed glucocorticoid replacement. Low or absent glucocorticoids and sex steroids with poor ACTH-stimulated responses are consistent with this diagnosis. Mutations solely affecting the 17,20-lyase activity are extremely rare.³¹⁹ The phenotype of apparent isolated 17,20-lyase deficiency can be caused by mutations in other genes, such as cytochrome b₅, P450 oxidoreductase, and several aldo-keto reductase enzymes, as described later.

Cytochrome b₅ Deficiency

Cytochrome b₅ participates in electron transfer for some cytochrome P450 reactions. Although it is not an effective electron donor to P450c17, this protein enables the interactions between P450c17 and oxidoreductase to promote the 17,20-lyase reaction essential for sex steroid synthesis. One 46,XY infant found to be homozygous for a loss-of-function mutation in the cytochrome b₅ (*CYB5*) gene was described to have micropenis, bifid scrotum, scrotal hypospadias, undetectable DHEA-S concentration, low testosterone concentration, and elevated methemoglobin concentration.³²⁰ A mis-sense mutation associated with mild methemoglobinemia and undervirilized 46,XY siblings has been described.³²¹

3 α -Hydroxysteroid Dehydrogenase Isozyme Deficiencies

Reevaluation of the families originally reported to have isolated 17,20-lyase deficiency led to identification of alternative

pathways for androgen synthesis.³²² Investigation of one family revealed no mutations in *CYP17A1*, *NR5A1*, *POR*, or *CYB5*. Rather, mutations were detected in the 3 α -hydroxysteroid dehydrogenase type III (*AKR1C2*) gene, which is mapped to chromosome 10p15.1. The phenotype, undervirilization of affected 46,XY individuals, followed a sex-linked recessive inheritance pattern. A splicing mutation in another closely related 3 α -HSD gene, *AKR1C4*, was found in a second family. Digenic inheritance involving mutations in both *AKR1C2* and *AKR1C4* were found in this second family. Thus both the classic and backdoor pathways appear to be necessary for normal male external genital development.³²² However, details regarding the specific roles of these enzymes remains to be elucidated.

Cytochrome P450 Oxidoreductase Deficiency

In 1985 a disorder with biochemical evidence, suggesting decreased 17 α -hydroxylase and 21-hydroxylase activity, was initially reported.³²³ This disorder was found to be associated with mutations in the cytochrome P450 oxidoreductase (*POR*) gene, mapped to chromosome 7q11-12. *POR* codes for a protein that functions as a mandatory electron donor to microsomal steroidogenic and hepatic P450 enzymes. The *POR* protein plays a major role in glucocorticoid and sex steroid synthesis.

Clinical features included genital ambiguity, craniosynostosis, midface hypoplasia, and radiohumeral synostosis. At birth, genital ambiguity was noted in both male and female infants. However, progressive postnatal virilization does not occur. Insufficient testosterone synthesis likely causes the undervirilization of male infants. Virilization of female fetuses is attributed to shunting of the excessive 17-OHP to the "backdoor pathway," resulting in increased DHT synthesis. During pregnancy, some mothers developed signs associated with androgen excess, such as acne, hirsutism, and clitoromegaly.³²⁴ Typical laboratory findings include elevated 17-OHP, low sex steroid, and normal mineralocorticoid concentrations. Some affected individuals may benefit from daily glucocorticoid replacement therapy. Others may need glucocorticoid treatment only for stress dosing.

The skeletal malformations resemble those found in the Antley-Bixler syndrome, which is an autosomal dominant disorder associated with mutations in the *FGFR2* gene. Patients with Antley-Bixler syndrome caused by *FGFR2* mutations have normal steroidogenesis, whereas patients with *POR* mutations have abnormal steroidogenesis.³²⁵ The molecular basis of the skeletal anomalies is unclear but is suspected to be caused by impaired activity of enzymes involved in sterol biosynthesis, such as 14 α -lanosterol demethylase (*CYP51A1*) and squalene epoxidase or retinoic acid metabolism.³²⁶

17 β -Hydroxysteroid Dehydrogenase Deficiency

This disorder is caused by mutations of the 17 β -hydroxysteroid dehydrogenase type 3 gene (*HSD17B3*) located at chromosome 9q22. The enzyme is expressed almost exclusively in the testis, where it converts androstenedione to testosterone. Loss-of-function mutations result in testosterone deficiency and subsequent undervirilization of 46,XY fetuses. In this autosomal recessive disorder, external genitalia range from female with perineoscrotal hypospadias and a blind-ending vaginal pouch to ambiguous with labioscrotal fusion to hypospadias.³²⁷ Testes are present and may be palpable in the labioscrotal folds or incompletely descended. Despite the presence of female external genitalia, Wolffian structures are typically present.³²⁸

When unrecognized, patients are usually considered to be female at birth. At puberty, progressive virilization occurs. The extent of virilization ranges from hirsutism, deepening of

the voice, and clitoral enlargement. Virilization is attributed to extratesticular conversion of androstenedione to testosterone. Some affected individuals change gender role from female to male. Given the apparently low risk for gonadal neoplasia, there is no contraindication to changing gender role, gender marker, and retention of testes in a scrotal location.³²⁸ Increased conversion of androstenedione to estrogens may cause gynecomastia.

Appropriate male assignment can be made in infancy when the diagnosis is suspected and confirmed. Diagnostic laboratory features include increased basal and hCG-stimulated androstenedione to testosterone ratios. The clinical features are similar to those of 5 α -reductase deficiency and androgen insensitivity; molecular genetic analysis may be beneficial to confirm the diagnosis. Affected 46,XX girls are usually asymptomatic, have normal female internal and external genitalia, and normal pubertal development; infertility has been reported for some.³²⁹

5 α -Reductase Deficiency

This autosomal-recessive disorder is caused by mutations in the 5 α -reductase type 2 gene (*SRD5A2*), which is located at chromosome 2p23. This gene is expressed primarily in androgen target tissues, where it converts testosterone to DHT. Clusters of individuals with *SRD5A2* mutations have been described in regions of the Dominican Republic, Papua New Guinea, Turkey, and the Middle East.

Affected 46,XY individuals have genital ambiguity characterized by differentiation of Wolffian structures, absence of Mullerian-derived structures, small phallus, urogenital sinus with perineoscrotal hypospadias, and blind vaginal pouch. Patients raised as females may present with primary amenorrhea typically associated with genital ambiguity. Deletions, missense mutations, and uniparental disomy have been reported. Because of the extensive heterogeneity in the clinical features, phenotype/genotype correlations are not well established.³³⁰ Random testosterone/DHT ratios may not identify affected individuals; hCG-stimulated testosterone/DHT ratios may be necessary. Caveats regarding this diagnosis include the need for hCG stimulation in prepubertal patients and accurate hormone determinations in which cross-reactivity between testosterone and DHT is minimized.³³¹

At puberty, progressive virilization occurs with muscular development, voice change, and phallic enlargement. These features have been attributed to the actions of 5 α -reductase type 1 (*SRD5A1*). *SRD5A1*, located at chromosome 5p15, is expressed in postpubertal skin and scalp. Phenotypic heterogeneity occurs frequently. Some 46,XY individuals with this disorder who were raised as girls develop a male gender identity and changed gender role in adolescence or adulthood. Despite the virilization, facial hair tends to be scanty, the prostate is hypoplastic, semen tends to be viscous, and the amount of ejaculate is low. Affected men often have azoospermia or oligospermia. Intrauterine insemination with sperm from an affected male has resulted in pregnancy.³³²

Placental Aromatase Deficiency

Placental aromatase deficiency is a rare autosomal recessive disorder arising from mutation in the aromatase gene, *CYP19A1*, which is located at chromosome 15q21.2 and codes for a 503-amino-acid protein. Inactivating *CYP19A1* mutations impair conversion of androgens to estrogens, leading to increased androgens. During pregnancies with affected fetuses, progressive maternal virilization characterized by hirsutism, clitoral hypertrophy, acne, and frontal balding occurs. During pregnancy, testosterone, DHT, and androstenedione concentrations

are elevated and estradiol, estrone, and estriol concentrations are low. In the postpartum period, some clinical features of androgen excess regress and the elevated androgen concentrations return to normal levels.

At birth, 46,XX infants are variably virilized with labioscrotal fusion, clitoromegaly, and perineal scrotal hypospadias. Affected 46,XX individuals generally manifest delayed puberty characterized by minimal or absent breast development, primary amenorrhea, hypergonadotropic hypogonadism, multicystic ovaries, and decreased bone mineral density. At birth, affected 46,XY infants have normal internal and external genital development. Affected males have generally presented after puberty with tall stature, skeletal pain, delayed skeletal maturation, and infertility. Investigation of aromatase-deficient men suggests that estrogen deficiency is associated with abdominal obesity, insulin resistance, dyslipidemia, and relative infertility. Patients with less severe phenotypic features have been described.³³³

Aromatase is a cytochrome P450 enzyme that plays an important role in the biosynthesis of estrogens (C18 steroids) from androgens (C19 steroids). In addition to its role in estrogen biosynthesis in adolescents and adults, aromatase located in the human placenta converts fetal adrenal androgens to estrogens and protects the mother from the potential virilizing effects of the fetal androgens.

MATERNAL HYPERANDROGENISM

During pregnancy, maternal hyperandrogenism can occur secondary to luteomas of pregnancy, androgen secreting tumors, and exposure to exogenous androgen. The excessive maternal androgen concentrations can cause virilization of the external genitalia of 46,XX fetuses. Endocrine disruptors are exogenous chemicals or mixtures of chemicals that interfere with any facet of hormone action. Organochlorine pesticides, polychlorinated biphenyls (PCBs), and alkylpolyethoxylates are considered to be “endocrine disruptors” because of their estrogenic and/or antiandrogenic properties.

DISORDERS OF ANDROGEN ACTION

During the process of sexual development, androgen action is essential to promote retention of Wolffian duct derivatives, development of the prostate, and differentiation of male external genitalia. Androgen action is mediated by the androgen receptor (AR, also known as *NR3C4*), a member of the steroid/thyroid hormone family of hormone receptors. Similar to other members of this receptor family, the androgen receptor is a ligand-dependent transcription factor with a characteristic modular structure. The major modules of the protein include the amino-terminus transactivation (AF1), DNA-binding, and ligand-binding domains. Other features include a nuclear localization signal, another transactivation domain (AF2) in the carboxy terminus ligand-binding domain, and the hinge region.³³⁴ The AR protein has two polymorphic trinucleotide repeat regions located in the amino terminal domain encoding the polyglutamine (CAG) and polyglycine (GCN) repeat regions. In addition to the classical pathway, nongenomic rapid androgen actions occur.³³⁵

Androgen insensitivity is an X-linked recessive disorder because of AR mutations; AR is located near the centromere at Xq11-12.³³⁶ Approximately 30% of cases represent de novo mutations. Somatic cell mosaicism, when the mutation arises in the postzygotic stage, is associated with a lower recurrence risk.³³⁷ Complete androgen insensitivity (CAIS) is characterized by female external genitalia, absence of Mullerian duct derivatives, sparse sexual hair, inguinal masses, and primary amenorrhea in the adolescent girl. Wolffian duct derivatives

(e.g., vas deferens and epididymides) are absent because of deficient androgen action. Although rare exceptions have been described, Mullerian-derived structures are usually absent because Sertoli cell function is normal with in utero AMH secretion. It has been suggested that 1% to 2% of girls with bilateral inguinal herniae may have androgen insensitivity. The finding of a gonad within the hernia sac should prompt cytogenetic studies. The expected LH surge in testosterone concentrations during the first few months of life may be absent in some infants with CAIS.³³⁸ Spontaneous pubertal breast development because of aromatization of androgens to estrogens occurs if the gonads are in situ.

Partial androgen insensitivity (PAIS) is characterized by clinical features, suggestive of a partial biological response to androgens. Typical features include ambiguous genitalia with perineoscrotal hypospadias, microphallus, and bifid scrotum. Testicular position is variable, ranging from undescended to palpable in the scrotum. Infants with PAIS generally manifest the expected neonatal testosterone surge, suggesting that prenatal androgen responsiveness plays a role in imprinting of the HPG axis. Features of mild androgen insensitivity include gynecomastia and infertility in otherwise normal males. Older chronological age at presentation is typical. In all instances, karyotype is 46,XY.

Typical laboratory findings are elevated LH and testosterone concentrations because testicular testosterone synthesis is unimpeded. Yet, there is loss of negative feedback inhibition of gonadotropins. LH concentrations are usually higher than FSH concentrations because testicular inhibin secretion is not impeded. FSH concentrations may be elevated or normal. Infants with PAIS may require dynamic endocrine tests to assess hCG-stimulated Leydig cell testosterone secretion and importantly, end-organ responsiveness to androgens.

In the absence of ligand, the AR protein is located primarily in the cytoplasm, where it is bound to chaperone proteins. Upon ligand binding, the conformation of the androgen receptor changes. The ligand-receptor complexes dimerize and move to the nucleus. A key feature of androgen receptor dimerization is the intramolecular interaction between the N-terminal and C-terminal domains. Binding of ligand stabilizes the androgen receptor and slows its degradation. The increased potency of DHT is attributed to the greater stability of the DHT-receptor complex compared with the testosterone-receptor complex. In the nucleus, the complex binds to androgen response elements and alters target gene transcription.

Over 500 different AR mutations have been described.³³⁹ In general, the phenotype correlates with degree of impaired androgen action. However, clinical features can vary despite the presence of the identical mutation (even within the same family). CAIS or PAIS associated with the same AR mutation can occur in siblings. Different missense mutations at the same position can also be associated with differing phenotypes. Complete loss-of-function mutations and premature termination codons are typically associated with CAIS. Partial loss-of-function mutations are typically missense mutations. Receptors with mutations in the DNA-binding domain bind ligand normally but fail to transactivate target genes. Mutations in the ligand binding domain can be associated with decreased affinity for ligand and/or increased instability of the hormone-receptor complex. In addition to hormone determinations, diagnostic evaluation may include DNA sequence analysis of the AR gene (www.genetests.org and <http://www.androgendb.mcgill.ca>).

Mutations can be located in noncoding regions of the gene; WES identified an intronic mutation resulting in an alternatively spliced transcript in a large family with PAIS associated with neonatal hypospadias and pubertal gynecomastia.³⁴⁰ Two unrelated 46,XY girls diagnosed with CAIS were found

to have the same c.547C>T germline mutation in the *AR*-5'-UTR. This mutation introduced a translated upstream open reading frame in the 5'-UTR, resulting in decreased AR protein.³⁴¹ Two sisters with complete CAIS were found to have an intronic pseudoexon-activating point mutation in the intron between exons 6 and 7 of *AR*; this mutation leads to aberrant mRNA splicing and decreased protein production.³⁴²

AR transcriptional activity depends on other proteins, including coactivators and corepressors. These other proteins presumably modulate physical interactions, linking the basal transcription machinery, the ligand-receptor complex, and chromatin. For example, a missense *AR* mutation altered the interaction of the AR protein with the melanoma antigen A-11 (MAGE-11) protein by interfering with the stimulatory effects of the coregulator.^{343,344}

In addition to androgen insensitivity, Kennedy disease (also known as *spinal and bulbar muscular dystrophy*) is mapped to the *AR* locus. Kennedy disease, a progressive neurodegenerative disorder with onset in the 30s or 40s, is associated with excessive expansion of the CAG polyglutamine trinucleotide repeat region in exon 1 of *AR*.^{304,345} Repeat lengths greater than 35 are associated with spinal and bulbar muscular atrophy. The presumed mechanism for the neurodegeneration is related to the role of AR in the function of the ubiquitin ligase anaphase-promoting complex/cyclosome, which functions with its adaptor protein to influence cell cycle arrest and neuronal architecture. In the presence of the expanded CAG repeat, AR inhibits this complex, leading to abnormal cell cycle reactivation.³⁴⁶ Mild symptoms of androgen insensitivity can be detected with slight decreases in *AR* mRNA and protein concentrations.

MULLERIAN DUCT ABNORMALITIES

Persistent Mullerian Duct Syndrome

Persistent Mullerian duct syndrome (PMDS) is an autosomal recessive disorder because of mutations in the *AMH* gene located at chromosome 19p13.3 or its receptor (*AMH-RII*) gene located at chromosome 12q13.13.³⁴⁷ The phenotypes of patients with *AMH* or *AMH-RII* mutations are comparable. *AMH* is a member of the TGF- β family and signals through two different interacting membrane-bound serine/threonine receptors. The ligand, *AMH*, binds to the type II receptor, which leads to recruitment and phosphorylation of a type I receptor. The type II receptor is specific for *AMH*, whereas there are multiple subtypes of the type I receptors. *AMH* concentrations are low among patients with mutations in the *AMH* gene. Among patients with *AMH-RII* mutations, *AMH* concentrations are normal or elevated. Females who carry mutations on both *AMH* alleles appear to have normal fertility.

The typical clinical features of PMDS include cryptorchidism, testicular ectopia associated with inguinal hernia, and hernia uteri inguinalis. Testicular differentiation is usually normal, but the male excretory ducts may be embedded in the Mullerian duct remnants or incompletely developed. Infertility may ensue secondary to cryptorchidism, intertwining of vas deferens and uterine wall, or lack of proper communication between the testes and excretory ducts. Testicular torsion is not uncommon because the testes may not be anchored properly to the bottom of the processus vaginalis.

Mullerian Duct Abnormalities in 46,XX Individuals

Mayer-Rokitansky-Küster-Hausler (MRKH) syndrome refers to congenital absence of the uterus and upper two-thirds of the vagina; the lower portion of the vagina is usually unaffected. Ovarian development is generally normal. This disorder occurs in approximately 1:4500 girls.³⁴⁸ The typical presentation is an

adolescent girl with primary amenorrhea accompanied by appropriate breast and pubic hair development.

MRKH can be classified as type I or type II. Type I MRKH is characterized by isolated uterine agenesis.

Type II MRKH is characterized by uterine agenesis accompanied by extragenital malformation, commonly renal and skeletal malformations. Renal findings, identified in approximately 40%, include unilateral renal agenesis, horseshoe kidneys, renal ectopia, renal hypoplasia, and hydronephrosis. The MURCS syndrome is defined as Mullerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia; cardiac and nervous system anomalies may also be present.

Hydrometrocolpos, Mullerian hypoplasia, and polydactyly are associated with both the McKusick-Kaufman syndrome and Bardet-Biedel type 6. Both autosomal recessive disorders are associated with mutations in the *MKKS-BBS6* gene located at chromosome 20p12.³⁴⁹ The McKusick-Kaufman syndrome and Bardet-Biedel type 6 are similar; the major differentiating feature is occurrence of retinitis pigmentosa in Bardet Biedel type 6.³⁵⁰

No single gene defect has been identified for MRKH. Recurring copy number variants in specific chromosomal regions, such as 17q12, 16p11.2, 1q21.1, and 22q11.21, have been associated with MRKH.³⁵¹ Mutations in *WNT4*, *LHX1*, *HNF1B*, *TBX6*, *RBM8A*, and *WNT9B* have been associated with Mullerian aplasia.³⁵² Patients with MRKH associated with heterozygous *WNT4* mutations are reported to have clinical or biochemical hyperandrogenism.³⁵³ Mullerian duct hypoplasia has been associated with facioauriculovertebral anomalies, such as Goldenhar syndrome and DiGeorge syndrome.^{354,355} In the absence of a uterine transplant or surrogacy, affected individuals cannot carry pregnancies. Most cases are sporadic. Thus ascertaining genetic transmission has been challenging.

Pelvic ultrasound and magnetic resonance imaging (MRI) demonstrate abnormal development of the uterus, cervix, and upper vagina.^{356,357} Imaging studies should include assessment for renal anomalies and skeletal malformations.³⁵⁸ Ovarian morphology should be appropriate for pubertal stage. Because of the high frequency of associated anomalies, careful physical examination for skeletal malformations should be included in the diagnostic evaluation of women with abnormal development of the Mullerian duct system.

MICROPHALLUS, HYPOSPADIAS, AND CRYPTORCHIDISM

Hypospadias

Hypospadias is a congenital hypoplasia of the penis characterized by ventral displacement of the urethral meatus, chordee, and ventral foreskin deficiency.³⁵⁹ Distal hypospadias is much more frequent than proximal and accounts for approximately 70% of cases. Proximal hypospadias may be associated with chordee, cryptorchidism, and additional congenital anomalies. The reported incidence ranges from 1:100 to 1:1000. Familial hypospadias has been described. Genes involved in early genital tubercle patterning include *BMP4*, *BMP7*, *HOXA4*, *HOXB6*, *FGF8*, *FGF10*, and *FGFR2*. Deletions involving 19q13 are associated with IUGR, microcephaly, postnatal growth retardation, clinodactyly, and hypospadias; the Wilms tumor interacting protein (*WTIP*) gene is located in the deleted region.³⁶⁰

Cryptorchidism

Cryptorchidism (undescended testes) is the most common disorder of sexual differentiation, affecting 3% of male infants.³⁶¹ Cryptorchidism can be categorized as nonsyndromic/isolated or syndromic (Table 6.6).³⁶²

TABLE 6.6 Disorders Associated With Cryptorchidism or Small Penis in Males

Name	Gene	Location	
Aarskog syndrome	<i>FGD1</i> (OMIM # 305400) FGD1 FGD1	Xp11.22	Short stature, hypertelorism, shawl scrotum, brachydactyly
Beckwith-Wiedemann Börjeson-Forsman- Lehman syndrome	<i>CDKN1C</i> (OMIM# 130650) <i>PHF6</i> (OMIM # 301900)	11p15.4 Xq26.2	Beckwith-Wiedemann syndrome Hypogonadism, seizures, developmental delay, obesity
Carnevale syndrome Carpenter syndrome-1	<i>COLEC11</i> (OMIM # 265050) <i>RAB23</i> (OMIM # 201000)	2p25.3 6p12.1-p11.2	Hypospadias, horseshoe kidney, craniosynostosis, hypertelorism Acrocephaly, syndactyly, congenital heart disease, low-set ears, obesity
Carpenter syndrome-2	<i>MEGF8</i> (OMIM # 614976)	19q13.2	Craniosynostosis, polysyndactyly of the hands and feet, situs inversus, congenital heart disease
Cornelia de Lange syndrome	<i>NIPBL</i> (OMIM # 122470)	5p13.2	Hypoplastic male external genitalia, cryptorchidism, mental retardation, synophrys
Costello syndrome	<i>HRAS</i> (OMIM # 218040)	11p15.5	Short stature, coarse facies, hypertelorism, congenital heart disease, skin findings, developmental delay
Hajdu-Cheney syndrome	<i>NOTCH2</i> (OMIM # 102500)	1p12	Short stature, coarse facies, bowing of the long bones, vertebral anomalies, hypertelorism, bushy eyebrows, micrognathia
Juberg-Marsidi syndrome	<i>HUWE1</i> (OMIM # 309590)	Xp11.22	Hypotelorism, small palpebral fissures, dysplastic, large, or low-set ears, long face, high-arched palate, thin upper lip, brachydactyly, developmental delay
Johanson-Blizzard syndrome	<i>UBR1</i> (OMIM # 243800)	15q15.2	Short stature, failure to thrive, hypothyroidism, sensorineural hearing loss, imperforate anus, and pancreatic exocrine insufficiency
Lenz-Majewski hyperostosis syndrome	<i>PTDSS1</i> (OMIM # 151050)	8q22.1	Sclerosing bone dysplasia, loose skin, brachydactyly, progressive generalized hyperostosis, developmental delay
Leopard syndrome	<i>PTPN11</i> (OMIM #151100)	12q24.13	Short stature, ptosis, hypertelorism, low-set ears, webbed neck, congenital heart disease, multiple lentigenes
Lowe syndrome	<i>OCRL</i> (OMIM # 309000)	Xq26.1	Hydrophthalmia, cataract, mental retardation, vitamin D-resistant rickets, amino aciduria
3MC1	<i>MASP1</i> (OMIM # 257920)	3q27.3	Hypertelorism, blepharophimosis, blepharoptosis, and highly arched eyebrows
3MC2	<i>COLEC11</i> (OMIM # 265050)	2p25.2	Short stature, hearing loss, cleft lip/palate
3MC3	<i>COLEC10</i> (OMIM # 265050)	8q24.12	Short stature, ptosis, cleft lip/palate
McKusick-Kaufman syndrome	<i>MKKS</i> (OMIM #236700)	20p12.2	Congenital heart disease, hypoplastic lungs, polydactyly
Meckel-Gruber, type 1	<i>MKS1</i> (OMIM # 249000)	17q22	Polydactyly, polycystic kidneys
Meier-Gorlin syndrome ^a	<i>ORC6</i> (OMIM # 607213)	16q11.1	IUGR, short stature, microtia, microcephaly, cryptorchidism
MEND syndrome	<i>EBP</i> (OMIM # 300960)	Xp11.23	Short stature, cataracts, 2- to 3-toe syndactyly, seizure, development delay
Miller-Dieker syndrome	Contiguous gene deletion (OMIM # 247200)	17p13.3 deletion	Lissencephaly, microcephaly, downward slanting palpebral fissures, small nose and chin, cardiac malformations, short stature, developmental delay
Mowat-Wilson Najjar cardiogenital syndrome	<i>ZEB2</i> (OMIM # 235730) <i>LMNA</i> (OMIM #212112)	2q22.3 1q22	Microcephaly, developmental delay Cardiomyopathy, hypergonadotropic hypogonadism
Noonan syndrome	<i>PTPN11</i> (OMIM # 163950)	12q24.13	Short stature, ptosis, hypertelorism, webbed neck, congenital heart disease, thrombocytopenia, von Willebrand disease
Persistent Mullerian duct syndrome	<i>AMH</i> (OMIM # 600957)	19p.13.3	Persistent Mullerian duct syndrome
Persistent Mullerian duct syndrome	<i>AMHR</i> (OMIM #600956)	12q13.13	Persistent Mullerian duct syndrome
Pfeiffer	<i>FGFR1</i> (OMIM #101600)	8p11.23	Craniosynostosis, broad thumbs, brachydactyly, craniosynostosis, midface deficiency, broad thumbs, broad great toes, brachydactyly
Pfeiffer	<i>FGFR2</i> (OMIM #101600)	10q26.13	Craniosynostosis, broad thumbs, brachydactyly, midface deficiency, broad thumbs, broad great toes
Prader-Willi syndrome	<i>NDN/SNRPN</i> (OMIM # 17620)	15q11.2	Prader-Willi syndrome
Robinow syndrome-RRS1	<i>ROR2</i> (OMIM # 268310)	9q22.31	Skeletal dysplasia, frontal bossing, hypertelorism, short-limbed dwarfism, vertebral segmentation, developmental delay
Robinow syndrome-RRS2	<i>NXN</i> (OMIM # 618529)	17p13.3	Skeletal dysplasia, postnatal mesomelic short stature, relative macrocephaly
Robinow syndrome DRS1	<i>WNT5A</i> (OMIM # 180700)	3p14.3	Macrocephaly, postnatal mesomelic short stature
Rubinstein-Taybi syndrome, type 1	<i>CREBBP</i> (OMIM #180849)	16p13.3	Microcephaly, mental retardation, high arched eyebrows, long eyelashes
Rubinstein-Taybi syndrome, type 2	<i>EP300</i> (OMIM #602700)	22q13.2	Microcephaly, mental retardation, high arched eyebrows, long eyelashes
Sotos syndrome	<i>NSD1</i> (OMIM # 117550)	5q35.3	Cerebral gigantism, conductive hearing loss, developmental delay, advanced bone age
Seckel syndrome, type 1	<i>ATR</i> (OMIM #210600)	3q23	Bird-headed facial appearance
Seckel syndrome, type 2	<i>RBBP8</i> (OMIM #606744)	18q11.2	Bird-headed facial appearance
Seckel syndrome, type 4	<i>CENPJ</i> (OMIM #613676)	13q12.12-q12.13	Bird-headed facial appearance
Seckel syndrome, type 5	<i>CEP152</i> (OMIM #613823)	15q21.1	Bird-headed facial appearance
Shprintzen-Goldberg	<i>KIAA1279</i> (OMIM # 609460)	10q22.1	Microcephaly, dysmorphic features, developmental delay, Hirschsprung disease intellectual disability, microcephaly, and dysmorphic facial features. Most patients also have gyral abnormalities of the brain

TABLE 6.6 Disorders Associated With Cryptorchidism or Small Penis in Males—cont'd

Name	Gene	Location	
Simpson-Golabi-Behmel, type 1	<i>GPC3</i> (OMIM #312870)	Xq26.2	Tall stature, coarse facies, congenital heart disease
Townes-Brocks syndrome	<i>SALL1</i> (OMIM # 107480)	16q12.1	Hypospadias, cryptorchidism, hypoplastic kidneys, bifid uterus
Varadi-Papp syndrome (oral-facial-digital syndrome, type VI)	<i>CPLANE1</i> (OMIM # 277170)	5p13.2	Renal agenesis/dysplasia, short stature, cryptorchidism, polydactyly
Warburg Micro syndrome-1 (WARBM1)	<i>RAB3GAP1</i> (OMIM # 600118)	2q21.3	Microcephaly, microphthalmia, microcornea, congenital cataracts, optic atrophy, corpus callosum hypoplasia, severe mental retardation, spastic diplegia
Weaver	<i>EZH2</i> (OMIM # 117550)	7q36.1	Macrocephaly, hypertelorism, developmental delay, prominent wide philtrum, micrognathia, deep horizontal chin groove, and deep-set nails

^aThe classic triad for Meier-Gorlin syndrome is microtia, absent/hypoplastic patellae, and short stature. Mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1*, and *CDC6* genes are associated with this phenotype. Cryptorchidism is typically associated with *ORC6* mutations. *IUGR*, Intrauterine growth restriction.

Because of spontaneous descent, the prevalence decreases to 1% by 6 months of age. Cryptorchidism can be associated with decreased number of germ cells, impaired germ cell maturation, and decreased number of Leydig cells.³⁶³ In some instances of unilateral cryptorchidism, abnormal histology may be evident in the contralateral normally descended testis.³⁶³ Usually cryptorchidism is an isolated finding, but it can be associated with hypothalamic hypogonadism, aberrant testicular differentiation, impaired testosterone biosynthesis, androgen insensitivity, holoprosencephaly, abnormal AMH production or action, abnormalities affecting *INSL3/LGR8* function, and possibly environmental factors. Other associations include prune belly syndrome, bladder exstrophy, and renal anomalies. Maternal diabetes mellitus, including gestational diabetes, may be a risk factor.

During sexual differentiation, the gonads are positioned between two structures: the cranial suspensory ligament and the gubernaculum. The gubernaculum is a mesenchymal structure that plays a major role in testicular descent. In females, the cranial suspensory ligament persists as the suspensory ligament of the ovary. Androgen action during the intraabdominal phase promotes regression of the cranial suspensory ligament. Testicular descent is divided into two phases: intraabdominal and inguinoscrotal.³⁶⁴ Factors involved in gubernaculum development during the intraabdominal phase include *INSL3* and its receptor, *LGR8/RXFP2*. *INSL3* is secreted by Leydig cells. Its receptor, *LGR8/RXFP2*, is a leucine-rich G-protein-coupled receptor expressed by the gubernaculum. By 13 or 14 weeks of gestation, the gubernaculum anchors the testis to the internal inguinal ring.³⁶⁵ At approximately 22 to 25 weeks of gestation, the testes and epididymis are located at the internal rings of the inguinal canal. Androgen influenced testicular descent through the inguinal canal is usually accomplished by the end of seventh months of gestation, with completion of the inguinoscrotal phase by the end of week 35.

Outcome concerns include risk for infertility and testicular neoplasia.^{366,367} In a prospective randomized study, orchidopexy at 9 months of age resulted in greater testicular volume and increased germ cell number compared with orchidopexy at 3 years of age.³⁶⁸ Hence surgical correction of cryptorchidism in early infancy is preferred.³⁶⁹

Heterozygous missense *INSL3* mutations have been identified in patients with cryptorchidism.³⁷⁰ Mutations have also been identified in the *LGR8* gene in males with cryptorchidism. Sequence variants have been identified in the *HOXA10* gene in boys with cryptorchidism.³⁷¹ Nevertheless, mutations in *INSL3*, *LGR8*, or *HOXA10* are rare causes of isolated

cryptorchidism.³⁷² Typically, the testes of patients with androgen insensitivity have completed the intraabdominal phase but fail to undergo inguinoscrotal descent because this second phase is androgen dependent. However, the more complete the androgen insensitivity, the greater likelihood of finding abdominal testes. Exposure of XX fetuses to androgen does not promote significant ovarian descent in humans, as evidenced by normal ovarian position in females with CAH.

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism associated with GnRH deficiency is characterized by a spectrum of reproductive, olfactory, and nonreproductive clinical features. Inheritance patterns include X-linked, autosomal dominant, and autosomal recessive. Male infants with hypogonadotropic hypogonadism may be identified in the newborn period with microphallus and/or cryptorchidism. Genital ambiguity generally does not occur because placental hCG secretion is unaffected. Decreased gonadotropin secretion causes decreased testosterone secretion, resulting in microphallus and cryptorchidism. Additional anterior pituitary hormone deficiencies may be present.

The 1000 to 2000 GnRH neurons that reside in the postnatal hypothalamus are born in the olfactory placode and migrate during early fetal development from the nose through the forebrain to the hypothalamus. Investigation of families with inherited hypogonadotropic hypogonadism has led to identification of specific genes involved in this process (Table 6.7). Kallmann syndrome is the eponym used for the X-linked recessive form of hypogonadotropic hypogonadism associated with anosmia because of failed migration of GnRH neurons from the olfactory placode into the forebrain along branches of the vomeronasal nerve.³²⁴ Olfactory tract hypoplasia or aplasia has been found on MRI. The molecular basis of this X-linked form is mutations in the Kallmann (*ANOS1*) gene (located at Xp22.3). This gene escapes X-inactivation, codes for a 680-amino-acid protein, and helps target GnRH neurons to the hypothalamus.

Mutations in the *GNRHR*, *FGFR1*, *FGF8*, *NSMF*, *GNRH1*, *KISS1*, *KISS1R*, *PROK2*, and *PROKR2* genes are also associated with hypogonadotropic hypogonadism. Mutations in *TAC3* and its receptor *TACR3* are associated with hypogonadotropic hypogonadism; the prevalence of microphallus among males carrying *TACR3* mutations is high.^{329,373} Gonadal defects may occur in CHARGE syndrome because of *CHD7* mutations; additional features include ocular coloboma, heart defects, choanal atresia, short stature, and hearing loss.³⁷⁴ Rather than

TABLE 6.7 Monogenic Disorders Associated With Hypothalamic Hypogonadism

Gene	Location	Phenotype	Mode of Inheritance
<i>ANOS1 (KAL1)</i>	Xp22.31	Hypothalamic hypogonadism with anosmia	X-linked
<i>CCDC141</i>	2q31.2	Hypothalamic hypogonadism with/without anosmia	AR, oligogenic
<i>FEZF1</i>	7q31.32	Hypothalamic hypogonadism with anosmia	AR, oligogenic
<i>FGFR1</i>	8p11.23	Hypothalamic hypogonadism with/without anosmia; holoprosencephaly; cleft lip/palate	AD,AR, oligogenic
<i>FGF8</i>	10q24.32	Hypothalamic hypogonadism with/without anosmia	AD, oligogenic
<i>FGF17</i>	8p21.3	Hypothalamic hypogonadism with/without anosmia	AD, oligogenic, de novo
<i>FSHβ</i>	11p14.1	Ovarian insufficiency/azoospermia	AR
<i>FSHR</i>	2p16.3	Primary amenorrhea	AR
<i>GNRH1</i>	8p21.2	Hypothalamic hypogonadism without anosmia	AR, oligogenic
<i>GNRHR</i>	4q13.2	Hypothalamic hypogonadism without anosmia	AR, oligogenic
<i>HESX1</i>	3p14.3	Hypothalamic hypogonadism with anosmia	AD
<i>HS6ST1</i>	2q14.3	Hypothalamic hypogonadism with/without anosmia	AD, oligogenic
<i>IGSF10</i>	3q25.1	Delayed puberty	AD
<i>IL17RD</i>	3p14.3	Hypothalamic hypogonadism with anosmia	AR, AD, oligogenic
<i>KISS1</i>	1q32.1	Hypothalamic hypogonadism without anosmia	AR
<i>KISSR1</i>	19p13.3	Hypothalamic hypogonadism without anosmia	AD, AR, oligogenic
<i>LHB</i>	19q13.33	Hypothalamic hypogonadism without anosmia	AR
<i>LHX4</i>	1q25.2	Hypothalamic hypogonadism without anosmia	AR
<i>NSMF</i>	9q34.3	Hypothalamic hypogonadism with/without anosmia	Oligogenic
<i>PROK2</i>	3p13	Hypothalamic hypogonadism with/without anosmia	AD, oligogenic
<i>PROKR2</i>	20p12.3	Hypothalamic hypogonadism with/without anosmia	AD, AR, oligogenic
<i>PROP1</i>	5q35.3	Hypothalamic hypogonadism without anosmia	AR
<i>RNF216</i>	7p22.1	Hypothalamic hypogonadism without anosmia	AR, oligogenic
<i>SEMA3A</i>	7q21.11	Hypothalamic hypogonadism with anosmia	AD, oligogenic
<i>SEMA3E</i>	7q21.11	Hypothalamic hypogonadism with anosmia	AD
<i>SEMA7A</i>	15q24.1	Hypothalamic hypogonadism with/without anosmia	Oligogenic
<i>SOX2</i>	3q26.33	Hypothalamic hypogonadism without anosmia	AR
<i>SOX10</i>	22q13.1	Hypothalamic hypogonadism with anosmia	AD
<i>TAC3</i>	12q13.3	Hypothalamic hypogonadism without anosmia	AR, oligogenic
<i>TACR3</i>	4q24	Hypothalamic hypogonadism without anosmia	AR, oligogenic
<i>WDR11</i>	10q26.12	Hypothalamic hypogonadism with/without anosmia	AD

AD, Autosomal dominant; AR, autosomal recessive.

being a single gene disorder, hypogonadotropic hypogonadism appears to be an oligogenic disorder.^{375,376}

ENVIRONMENTAL DISRUPTORS

Prenatal treatment with diethylstilbesterol (DES), a nonsteroidal synthetic estrogen, has been associated with urogenital abnormalities of both male and female fetuses with cryptorchidism occurring in 46,XY fetuses.^{377,378} The genital ambiguity, described in three 46,XY infants born in heavily agricultural areas, was attributed to fetal exposure to endocrine disruptors especially because no mutations were detected in the *SRY* or *SRD5A2* genes.³⁷⁹ Although no definite causation has been found, various environmental substances, such as herbicides, fungicides, pesticides, and plasticizers, have been considered to be potential endocrine disruptors. Potential mechanisms include binding to nuclear hormone receptors modulating gene expression or epigenetic changes. Reliable evidence confirming detrimental environmental effects on genital development is lacking.³⁸⁰

OTHER DISORDERS

The VACTERL association (VA) is characterized by several anomalies. These abnormalities include vertebral anomalies, anal atresia, cardiovascular malformations, tracheoesophageal fistula and/or esophageal atresia, renal anomalies, limb anomalies, and/or genital ambiguity. Mullerian hypoplasia/aplasia, renal agenesis, and cervicothoracic somite dysplasia (MURCS association) is characterized by primary amenorrhoea. Features of this disorder can include absence of uterus and fallopian tubes, cervical spine abnormalities, and renal anomalies.

Complete absence of penis also known as *aphallia* is rare and may be associated with additional congenital anomalies of the genitourinary and gastrointestinal systems.

EXSTROPHY OF BLADDER

The bladder-exstrophy-epispadias complex (BEEC) describes a primary field defect involving the pelvis, urinary tract, and external genitalia. This spectrum of malformations includes epispadias, bladder exstrophy, and persistent cloaca. Another variant is the OEIS complex (omphalocele, exstrophy, imperforate anus, and spinal defects). Additional findings can include ectopic kidney and renal agenesis as well as anomalies affecting the skeletal, genitourinary, and gastrointestinal systems.³⁸¹ Familial occurrence has rarely been described for this presumably multifactorial disorder.³⁸² Exstrophy of the cloaca is characterized by persistence of a common cloaca associated with failure of fusion of the genital tubercles. The underlying etiology remains to be identified. However, the 22q11.2 microduplication region has been repeatedly detected on array comparative genomic hybridization in several patients; a candidate gene is the Leucine zipper-like transcriptional regulator (*LZTR1*) gene that maps to this region.³⁸³

DIAGNOSIS

A comprehensive individualized approach is appropriate for a child found to have genital ambiguity.^{384,385} In some instances, the diagnosis is first considered when the child presents with delayed puberty. In addition to a thorough medical history and complete physical examination, endocrine assessment, and genetic evaluation provide valuable approaches.³⁸⁶ The list

of potential etiologies especially for 46,XY individuals continues to expand. Yet, despite identification of novel genes and innovative genetic technology, a definitive molecular diagnosis may not be established for all, especially for 46,XY individuals. Ahmed and colleagues suggest recommendations for boys with DSDs.³⁸⁷

History

A detailed family history should be obtained. The family history should include ascertainment of unexplained infant deaths, consanguinity, and infertility. Infants with CAH may have died before diagnosis. Family history may be negative in the case of autosomal recessive disorders. Infertility, POI, and gynecomastia may represent milder phenotypes for some DSDs. For X-linked disorders, such as androgen insensitivity, there may be affected maternal family members (i.e., either amenorrheic or infertile aunts or partially virilized uncles). Pertinent questions include prenatal exposure to exogenous or endogenous androgens, estrogens, or potential endocrine disruptors. Maternal virilization during pregnancy should be queried.

Physical Examination

DSDs encompass a spectrum of physical findings. Atypical genital development may occur as a component of a syndrome. Hence, careful examination of the facies, heart, limbs, and digits assessing for dysmorphic features should be performed. The specific physical findings range from micropenis, hypospadias, undescended testes, minimal clitoromegaly, and scrotalized labia to more extensive forms of genital ambiguity. Severe clitoromegaly with posterior labial fusion in a 46,XX patient may be distinguishable from perineal hypospadias, undescended testes, and a bifid scrotum in a 46,XY individual.

During the physical examination, attention should be focused on phallic size and shape, symmetry of the external genitalia, and presence and location of palpable gonads. The position of the urethra, and whether one or two perineal openings are present should be noted. The extent of virilization should be carefully documented, recording the configuration, stretched dorsal length, and diameter of the phallus (including the glans penis). The location of the urethral opening, degree of fusion of labiourethral folds, and extent of labioscrotal fold fusion and anogenital distance should also be noted. Labioscrotal folds fuse from posterior to anterior; the appearance can range from posterior labial fusion, to a partially fused hemiscrota, or to completely fused scrotum with labiourethral fusion, extending to a midline urethral opening.

Gonadal or adnexal structures may be identified upon careful palpation for content of the labioscrotal structures, scrotum or labia majora, inguinal region, and the lower abdomen. The groin area may be “milked” from anterior to posterior to maneuver the testis into the scrotum. The absence of palpable testes may indicate a virilized XX infant with adrenal hyperplasia or an XY infant with undescended or absent testes. Structures palpated within the labioscrotal folds are usually testes that typically have a characteristic ovoid structure. Rarely, ovaries, ovotestes, or even the uterine cervix can be found within the labioscrotal folds.

Symmetry or asymmetry of external genital differentiation provides clues to the etiology of the genital ambiguity (Figs. 6.6 and 6.7). Unilateral structures with asymmetry of other genital structures suggests ovotesticular or 45X/46,XY DSDs and is often associated with unilateral gonadal maldescent. Asymmetry implies differing local influences, which often reflect abnormalities in gonadal differentiation (see Figs. 6.6 and 6.7). Repeated examinations may be valuable.

Penile length measurements extend from the tip of stretched penis from the pubic ramus. As would be anticipated, penile

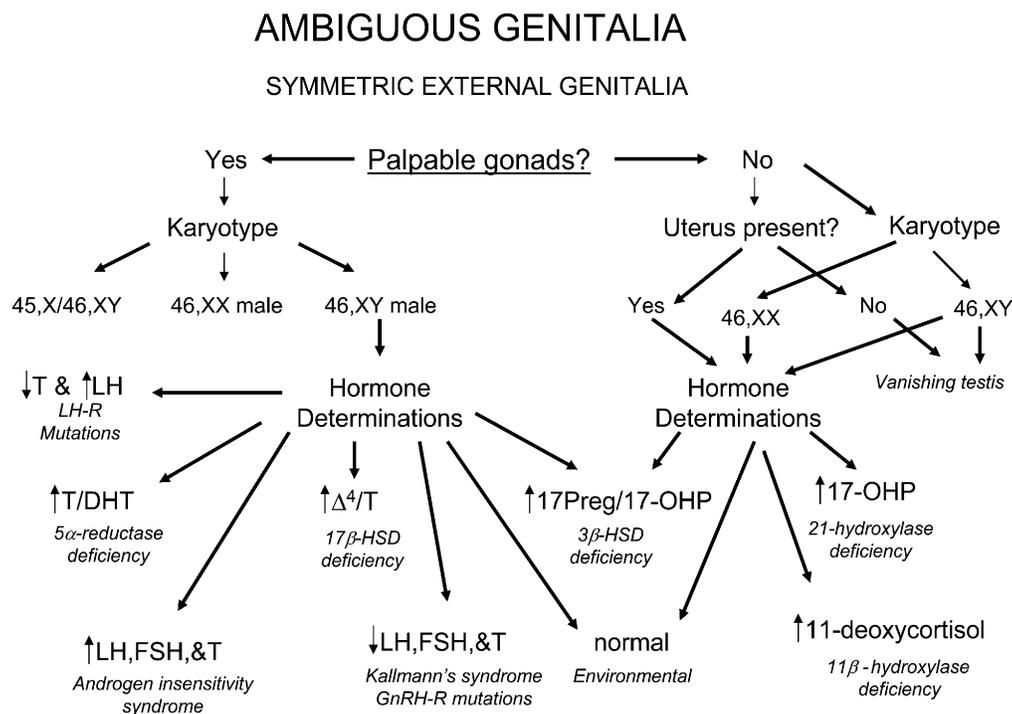


Fig. 6.6 Algorithm for the approach to the child with symmetric genital ambiguity. The configuration of the labioscrotal folds and presence/absence of palpable gonads is comparable on both sides. The presence or absence of palpable gonads directs the initial laboratory evaluation. Ultrasound examination to determine whether a uterus is present is helpful. For example, symmetric fusion of the labioscrotal folds, nonpalpable gonads, and presence of a uterus provide strong circumstantial evidence for the diagnosis of a virilized female with congenital adrenal hyperplasia.

AMBIGUOUS GENITALIA

ASYMMETRIC EXTERNAL GENITALIA

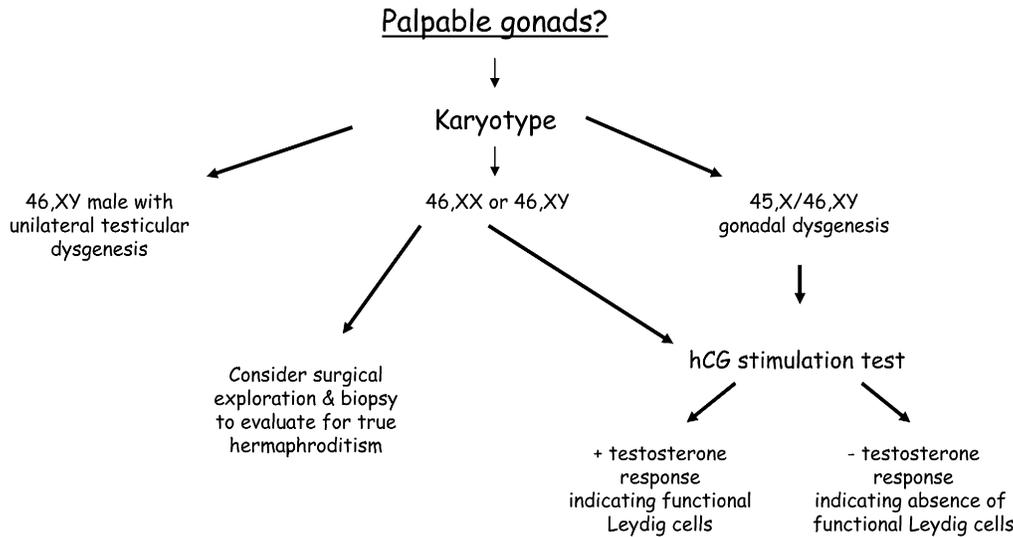


Fig. 6.7 Algorithm for the approach to the child with asymmetric genital ambiguity. In this instance, the labioscrotal folds may appear different or a gonad is palpable only on one side.

length depends on gestational age. For full-term male infants, the average stretched penile length is 3.5 ± 0.7 cm and average diameter is 1.1 ± 0.2 cm³⁸⁸; the lower limit (approximately – 2.5 standard deviation [SD]) at term being 2.0 cm. An isolated micropenis can be a consequence of decreased testosterone exposure in the second half of gestation because of Leydig cell failure, LH deficiency, androgen insensitivity syndrome, *LHCGR* mutations, or GH deficiency. The finding of both a micropenis and hypospadias can be classified as a DSD; additional evaluation is generally warranted.

Measurement of the clitoris requires a careful estimate of the proximal end with exclusion of overlying skin. Clitoral length is usually less than 1.0 cm, although rare variations exist. The length and width of the clitoris can be measured. For term female infants, mean clitoral length is 4.0 ± 1.24 mm and mean clitoral width is 3.32 ± 0.78 mm.³⁸⁹ The clitoral index is the product of length times width; mean clitoral index is 13.3 mm.³⁹⁰ The location of the urethral opening should be ascertained by visualization, witnessing the urinary stream, or with careful insertion of a firm catheter. If urination is observed, force, diameter, and direction of the urinary stream should be noted. The position of the inserted catheter may also provide crucial initial information. If directed toward the anal opening and palpable under the perineal skin, the catheter is likely in a urogenital sinus, as occurs often with virilization of a 46,XX fetus secondary to 21-hydroxylase deficiency. However, a penile urethra is anticipated if the catheter is directed anteriorly and is nonpalpable.

The anogenital ratio is measured as the distance between the anus and the posterior fourchette, divided by the distance between the anus and the base of the phallus. Because pelvic ultrasound is part of the initial laboratory assessment, a rectal examination may not be necessary. If present, a midline uterine cervix can often be palpated upon rectal examination.

The Prader scale is often used to classify the appearance of the external genitalia: (1) normal female genitalia with clitoromegaly; (2) partial labial fusion and clitoromegaly; (3) labioscrotal fusion, so that there is a single opening from the

urogenital sinus and clitoromegaly; (4) fusion of labioscrotal folds so that the single opening is at the base of the phallic structure; and (5) complete male virilization with penis-size phallus, complete labial fusion, and meatus on the glands. An external masculinization score is useful to assess virilization in male infants; this scoring system evaluates scrotal fusion, phallic size, location of urethral meatus, and position of the gonads.³⁹¹

In addition to the genital examination, the examination should include weight, length, and other features to ascertain whether findings are consistent with gestational age, particularly in the apparent female because the clitoris is more prominent in preterm infants. This appearance is caused by the scant subcutaneous fat and completion of clitoral growth before the last trimester of fetal life. A careful examination includes inspection for additional dysmorphic features because genital ambiguity may occur in association with other anomalies. These include midline facial defects, head size, and ear and digital anomalies. Bladder exstrophy and epispadias represent a nonendocrine malformation of the urological system. Infants with CAH, both male and female, may manifest hyperpigmentation of the genitalia and nipples because of adrenal insufficiency and ACTH hypersecretion.

In some instances, an older child or adolescent is referred for evaluation. The genital examination in children may be done subtly and quickly to avoid traumatizing the child. Palpation should be performed only if absolutely necessary. Before examining the child, especially a young child, it is important to remind the child that this is a private area, explain the reason for the examination, and obtain permission from both the child and the parent.³⁹²

Laboratory Studies

Initial laboratory studies to assess genital ambiguity should include karyotype, hormone determinations, and imaging studies. Ultrasound is the typical imaging modality because it does not require sedation, is noninvasive, and does not

require radiation or contrast material. Ultrasound can assess for the presence and location of the gonads, kidneys, adrenal, and uterus. Gonadal structures may be too small to definitively identify by ultrasound. Hence lack of visualization of gonads on ultrasound examination does not substantiate absence of gonads. In some instances, MRI can help evaluate Mullerian structures and abnormalities in the urinary tract. Limitations of MRI include inaccuracy in detecting intraabdominal gonads, streak gonads, or prepubertal uterus. Upon completion of other assessments, laparoscopy may be necessary to accurately evaluate intraabdominal structures and gonads, whereas cystoscopy may be needed to assess the urethra and vagina.³⁹³ When the diagnosis remains uncertain, laparoscopy to visualize the internal genital structures and obtain a biopsy of the gonadal tissue can assist with management recommendations. The extent of gonadal differentiation and germ cell status may predict gonadal function and clarify risk for germ cell tumors.

When gonads are not palpable, the external genitalia are symmetrically ambiguous, and a uterus and possibly ovaries are present, the most likely diagnosis is a virilized 46,XX fetus with CAH. However, the possibility of markedly dysgenetic testes cannot be excluded. If the differential diagnosis based on the presentation includes CAH, the initial laboratory studies should include electrolytes, PRA, serum 17-OHP, AMH, and cortisol concentrations.

Genetic Tests

The karyotype confirms chromosomal sex, even if prenatal chromosome testing was performed. Cytogenetic testing with G-banded karyotyping can detect the presence of two or more cell lines. Because of faster turn-around time, fluorescent in-situ hybridization (FISH) or PCR with markers specific for X and Y chromosomes is recommended.³⁹⁴ In general, peripheral blood karyotypes are sufficient to ascertain chromosomal karyotype. However, the patient may have a mosaic karyotype with one or more additional cell lines restricted to gonadal tissue. CGH microarray analyses are increasingly available and may identify submicroscopic genomic DNA imbalances or copy number variants.³⁹⁴ However, CGH and single nucleotide polymorphism (SNP) assays may fail to detect balanced chromosomal translocations and low-level mosaicism.

Molecular genetic analyses have become increasingly available to determine and confirm the molecular basis of the genital ambiguity and can be considered as part of the diagnostic process to be conducted in parallel to clinical phenotyping and hormone analyses.³⁹⁵ Identifying the specific mutation improves the accuracy of genetic counseling and the prediction regarding recurrence risk. The genetics laboratory can often perform FISH for the SRY gene, or other detailed chromosomal tests, such as CGH microarrays (Fig. 6.8). For some DSDs, molecular genetic analyses of specific genes are available through commercial laboratories. Information regarding the particular details can be obtained from a National Institute of Health-funded web-based resource, Genetests (<http://www.genetests.org>). In some instances, genetic testing is available through research laboratories.

Next generation sequencing (NGS) can benefit patient management by identifying specific disorders and inheritance patterns.³⁹⁶ WES and whole genome sequencing (WGS) are techniques for simultaneous analysis of the whole exome and whole genome, respectively. Whole exome genetic analysis is increasingly available. WES fails to identify variants in non-coding regions. WGS can detect mutations in noncoding regions, including introns, untranslated regions, and proximal and distal *cis*-regulatory regions; such variations can modify chromatin conformation changing structural positioning

between regulatory regions with subsequent misexpression of specific genes.³⁹⁷ Neither technique adequately detects larger Copy Number Variation (CNVs), repetitive sequences, aneuploidy, or epigenetic modifications (us). Subsequently, confirmation of the identified genetic variants by PCR, followed by Sanger sequencing or restriction fragment length polymorphism is necessary.

These NGS approaches generate much information; WES typically detects 15,000 to 20,000 variants and WGS detects 3 to 4 million variants, differing from the human reference genome.³⁹⁸ These tools generate vast amounts of genetic data, necessitating informed bioinformatics support. With identity of multiple variants, parsing the significant variants from benign variants is crucial. Variants can be identified as clearly pathogenic by the specific mutation, inheritance pattern, results of multiple in silico modeling software predictors, functional assays, and conservation between species.³⁹⁹ The American College of Medical Genetics and Genomics provides guidelines for interpretation of variants in the context of standard terminology (Table 6.8).⁴⁰⁰ Given the ongoing rate of gene discovery, reanalysis of variants of uncertain significance may be warranted.⁴⁰¹ Phenotypic heterogeneity and incomplete penetrance complicate interpretation of the genetic analyses. Clinicians also need to be aware of potential ethical issues, such as detecting a pathogenic mutation in a gene unrelated to DSD (incidental finding). Pretest counseling and informed consent are requisite before performing genetic studies.⁴⁰²

In some instances, infants with DSDs are detected through noninvasive prenatal testing (NIPT) using cell-free fetal DNA in maternal serum.⁴⁰³ This technique involves massive parallel sequencing or SNP analyses of the 150 to 180 base pair fragments of fetal DNA and avoids the risks posed by chorionic villus sampling and amniocentesis. At this time, false positive and false negative reports limit the usefulness of this technique. Traditional invasive diagnostic genetic testing is essential if NIPT results indicate a genetic abnormality or differ from ultrasound findings.^{404,405}

Hormone Determinations

Other initial studies depend on the physical findings. If the external genitalia are symmetrically virilized to any degree in the absence of palpable gonads, particularly if a normal uterus is present, additional studies should be directed toward causes of virilization of a female infant. Because 21-hydroxylase deficiency is the most common cause of virilization and genital ambiguity in 46,XX infants, initial laboratory studies should include determination of 17-hydroxyprogesterone (17-OHP) concentrations. In general, 17-OHP concentrations should be measured no earlier than 48 to 72 hours of age because of the potential for false positive results that may be caused by cross-reacting steroid sulfates.³⁵⁰ Nevertheless, extremely elevated 17-OHP concentrations may be detected at earlier timepoints in infants with classical CAH. If one or both gonads can be palpated, the intent of screening studies is to determine the adequacy of androgen synthesis and androgen action in a male infant. Determination of LH, FSH, and testosterone concentrations in infancy provides information regarding the function of the testes and the HPG axis (see Table 6.4).

The pattern of steroid hormone concentrations provides evidence for specific defects in steroidogenesis (see Table 6.4). The diagnosis of CAH arising from 21-hydroxylase deficiency is confirmed by finding elevated 17-OHP concentrations. Typically, 17-OHP concentrations are greater than 10,000 ng/dL (300 nmol/L) in the affected neonate. For 11 β -hydroxylase deficiency, 11-deoxycortisol and 17-OHP concentrations are elevated. For 3 β -hydroxysteroid dehydrogenase deficiency, pregnenolone,

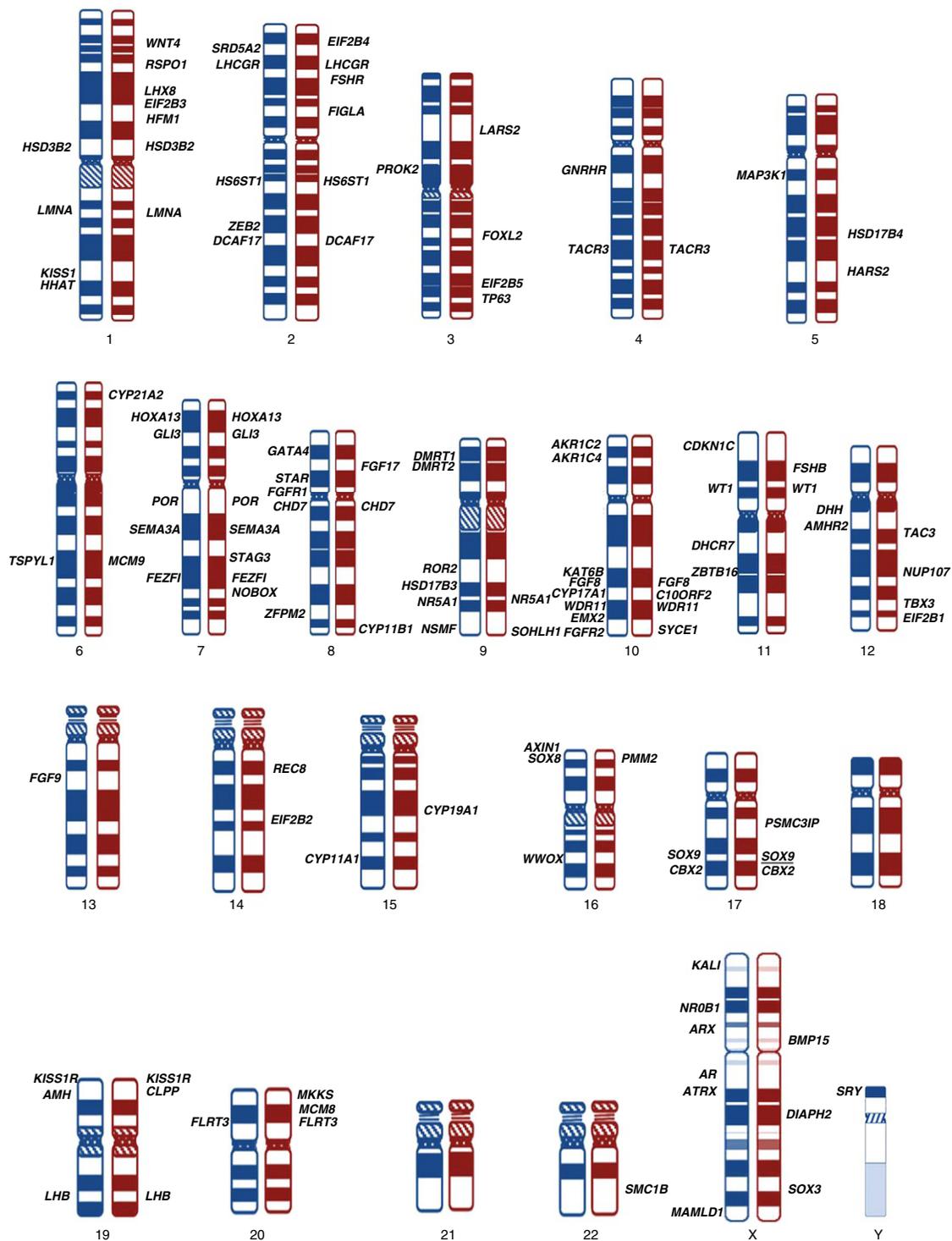


Fig. 6.8 Map of genes associated with disorders of sex development (DSDs). Genes known to cause gonadal dysgenesis, hypogonadotropic hypogonadism, disordered steroidogenesis, isolated urogenital anomalies, syndromic conditions associated with ambiguous genitalia, and primary and secondary gonadal insufficiency are listed along each chromosome. On the left, genes implicated in XY individuals, on the right, genes known to cause ovarian dysgenesis and DSD in XX individuals. (From Yatsenko, S.A., Witchel, S.F. (2017). Genetic approach to ambiguous genitalia and disorders of sex development: What clinicians need to know. *Semin Perinatol*, 41(4), 232–243.)

17-hydroxypregnenolone, and DHEA concentrations are typically elevated. Steroid concentrations are low for patients with congenital lipoid adrenal hyperplasia and CYP11A1 mutations. When salt-losing forms of CAH are included in the differential diagnosis, serum electrolytes and PRA should be monitored. Typically, despite mineralocorticoid deficiency, hyponatremia and

hyperkalemia are not present at birth and develop during the first week of life.

For the milder forms of CAH or other disorders affecting adrenal steroidogenesis, ACTH stimulation testing may be necessary to confirm the diagnosis. After a basal blood sample has been drawn, synthetic ACTH (0.25 mg) can be administered by

TABLE 6.8 Terms Used to Characterize Sequence Variants

Interpretation	Significance
Pathogenic	Contributes to disease
Likely pathogenic/ implicated	Data consistent with pathogenic role with a defined level of confidence
Benign	Available data indicate that variant does not contribute to disease
Likely benign	Data consistent with nonpathogenic role with a defined level of confidence
Variant of uncertain significance (VUS)	Data inadequate to classify variant
Associated	Variant is significantly enriched in affected individuals compared with matched controls
Damaging	Variant affects the normal levels or biochemical function of a gene product
Deleterious	Variant reduces reproductive fitness of carriers

intravenous bolus or intramuscular injection. A second blood sample to measure ACTH-stimulated hormone response can be obtained 30 or 60 minutes later. The milder forms of CAH generally do not affect external genital differentiation and are therefore not usually associated with genital ambiguity. Infants with late-onset CAH are generally not detected through newborn screening programs, presumably because the whole blood 17-OHP concentrations are determined from the newborn screening filter paper are lower than the values used as cutoffs.

In addition to the diagnostic evaluation for disorders of steroidogenesis, hormone measurements in the immediate neonatal period provide an index to the function of the HPG axis. Low testosterone and elevated gonadotropin concentrations in a 46, XY infant with ambiguous genitalia suggest inadequate testosterone biosynthesis. Elevated testosterone and gonadotropin concentrations in an infant with female external genitalia, bilateral labial masses, and 46,XY karyotype are consistent with the diagnosis of androgen insensitivity (see Table 6.5).

AMH concentrations reflect Sertoli cell function; concentrations are sexually dimorphic, with high values in boys (20–80 ng/mL) during the first 6 years of life and low values in girls.^{406,407} AMH concentrations may help distinguish between anorchia and cryptorchidism.⁴⁰⁸ In addition, AMH concentrations can be helpful in disorders of testicular dysgenesis or in virilized females to determine the presence of testicular tissue. Inhibin B concentrations, lower in females than in males, provide another marker of Sertoli cell function.³⁵⁷

Assessing testosterone secretion may be helpful, especially for patients with evidence of testicular tissue by palpation or ultrasound and AMH levels indicating testicular tissue. One approach involves administering hCG and measuring hormone responses. Compared with LH, the additional 24 amino acids on the carboxy-terminal of the protein increase the biological activity of hCG and the presence of a terminal sialic acid on the carbohydrate chain prolongs its half-life.⁴⁰⁹ Specific hCG preparations include hCG extracted from urine of pregnant women and recombinant hCG. Although recombinant hCG is usually more expensive, it shows good batch-to-batch consistency, high specific activity, and greater purity. Multiple protocols have been used with variation in dose and dosing frequency⁴⁰⁹ to assess for gonadal dysgenesis, Leydig cell hypoplasia, defects in testosterone biosynthesis, and androgen responsiveness. One protocol involves administering 1000 to 1500 units subcutaneously, either daily or every other day, for 1 to 5 days, with blood sampling on the day after the last injection. Intermittent injections can be given for up to 3 weeks to stimulate penile growth to demonstrate both testosterone

secretion and target tissue responsiveness. Total dosage should not exceed 15,000 units of hCG. Hormone determinations should include androstenedione, testosterone, and DHT. Testosterone concentrations should more than double.³⁸⁴ Post-stimulation hormone ratios are used to assess for defects in testosterone biosynthesis. Poststimulation, testosterone/androstenedione ratios less than 0.8 among infants and less than 1.0 among prepubertal and adolescent patients are suggestive for 17 β -hydroxysteroid dehydrogenase-3 deficiency.⁴¹⁰ Similarly, basal testosterone/DHT ratios greater than 10 and post-hCG stimulation greater than 20 are suggestive for 5 α -reductase deficiency. However, mutation testing may be needed to accurately differentiate between these disorders.

Newborn Screening

Newborn screening programs (NBS) have been established in all 50 states and many countries to identify infants with classic CAH.⁴¹¹ Typically, screening programs measure whole-blood 17-OHP concentrations eluted from a dried filter paper blood spot using a commercially available immunoassay, such as dissociation-enhanced lanthanide fluoroimmunoassay (DEL-FIA, Perkin-Elmer, Waltham, MA). Slightly increased whole-blood 17-OHP concentrations are detected often enough (especially in preterm infants) to complicate clinical decision-making regarding affected status and need to initiate glucocorticoid therapy. Etiologies of these false positive results include prematurity, cross-reacting steroids, sampling before 48 to 72 hours of age, heterozygosity for 21-hydroxylase deficiency, stressed infant, and late-onset CAH.^{412,413} False negative results can occur; with false negative results being more common in girls than boys.⁴¹⁴ Maternal treatment with glucocorticoids can also lead to false negative results. If the differential diagnosis includes CAH, specific laboratory testing is warranted even if the newborn screen results are reported as negative for 21-hydroxylase deficiency.

To avoid excessive false positive screening results, cutoff levels are typically selected to identify all infants with classic salt-losing or simple virilizing forms. Infants with late-onset CAH are generally not detected through NBS. Improved specificity can be achieved by use of additional procedures, such as organic extraction, chromatography, or liquid chromatography-tandem mass spectrometry (LC/MS/MS) analyses. This technique, LC/MS/MS, may be beneficial to either confirm or eliminate the diagnosis of CAH and can also be used to diagnose other disorders of steroidogenesis.^{351,415,416}

A challenge for NBS has been to ensure maximum sensitivity and specificity. Strategies to improve identification of affected children include a repeat blood sample or molecular genetic analysis. Currently, approximately 22% of American infants are routinely screened a second time.⁴¹⁷ Other approaches include use of gestational-age based cut-points, a second sample for 17-OHP immunoassay, 21-deoxycortisol concentrations, gas chromatography-mass spectrometry analysis, or hormone ratios, such as 17-OHP/11-deoxycortisol ratio.^{418–421}

Imaging, Endoscopy, and Laparoscopy

Endoscopic studies can be used to locate the vaginal-urethral confluence in relation to the bladder neck and the single opening of the urogenital sinus. Using a cystoscope and catheter placement, these distances can be determined before or in conjunction with retrograde contrast studies performed to outline the urethra and to demonstrate (if present) the vagina, uterus, cervix, and uterine cavity. Such information is needed to plan for reconstructive surgery, to assess the risk of medical complications, and in certain instances to provide information to determine the sex of rearing. If such procedures are unhelpful,

laparoscopy may be necessary to visualize and biopsy gonadal and internal genital structures. MRI may be helpful to define anatomic relationships.

TREATMENT

While awaiting results of the initial laboratory studies, and as needed, attention is focused on the main decision: the sex of rearing. In the ideal situation, a specific DSD team meets with the family.³⁸⁴ The DSD team usually includes a pediatric endocrinologist, a pediatric surgeon or pediatric urologist with expertise in urogenital reconstructive surgery, a behavioral health professional (psychologist, social worker, or psychiatrist), and a neonatologist. Input from an ethics consultant may be helpful. Using all available data, the DSD team shares the child's information with the family and educates them. At diagnosis and subsequent interactions, the family benefits from having opportunities to express their fears and concerns in nonthreatening environments in preparation for relevant decision-making.

Families struggle to understand complex medical information and deal with their child's anatomic differences. Additional consideration includes the potential for stigmatization by their community, anxiety about their child's gender identity, distress about their child's fertility potential, uncertainty regarding gonadal tumor risk, and the inconveniences inherent with multiple medical visits.⁴²² The initial participation of parents in discussions and decision-making regarding the options for sex of rearing, medical treatment, and possible surgical interventions should define the approach for subsequent discussion. Honest, sensitive, and candid discussions with the parents only help the child. The overriding goal is a positive strategy to promote the child's positive self-esteem.

Often, the neonatologist is the first physician to communicate with the parents about the genital ambiguity. In addition to talking with the parents, the neonatologist facilitates coordination of care and initiates laboratory studies. In some instances, the neonatologist coordinates transfer of the infant to a tertiary care facility. Long-term management needs to be delineated.

In many instances, the sex of rearing is apparent based on the initial physical examination and laboratory findings. However, for some infants, a decision regarding sex of rearing is necessary. Discussion regarding future gender identity and possibility for fertility are valuable. Consideration of family traditions and customs during the decision-making process reassures the child and family.⁴²³ Following an informed discussion, parents and healthcare providers choose sex of rearing.

Mental/behavioral/psychological health needs to be considered from the initial interactions with the family.⁴²⁴ Psychological impact can be assessed and addressed by relationships involving the members of the DSD team. The behavioral health professional should have thorough training and knowledge of resources available to patients and their families. In addition to the appointments necessary to the patient's medical, surgical, and psychological needs, additional sessions may benefit the parents to address their questions and concerns about their child. These evaluations should be performed promptly.

As the child matures, honest explanations regarding the medical condition are essential. All involved should be alert regarding the overall impact of the condition, as well as need for psychological counseling. The child should progress from a silent partner to a full participating member in this decision-making process, with the child's wishes taking precedence where appropriate.

When a specific diagnosis has been confirmed, decision-making and therapy can be guided accordingly. Considerations for medical care include decisions regarding sex of rearing,

possible need for surgery and a timeline for planned surgery, plan for medical treatment, and timely and appropriate psychological counseling and support.

Sex of Rearing

Decisions regarding the appropriate sex of rearing are based on the specific pathophysiology, external genital features, internal reproductive system anatomy, prognosis for spontaneous pubertal development, capacity for sexual activity and orgasm, and potential for fertility.⁴²⁵ With the use of fertility preservation and assisted reproductive techniques, the potential for future fertility is greater than in the past.^{426,427}

In instances where a decision regarding sex of rearing is necessary, each child warrants careful consideration of the physical findings, laboratory information, and available outcome data. Parents are often apprehensive because of fears regarding social stigmatization of their child and anxiety that the child will reject the assigned gender when older.⁴²⁸ Indeed, uncertainty regarding their infant can be so distressful and traumatic for some parents that their decision-making ability for the child breaks down.⁴²⁹ In most instances, children are raised male or female consistent with the karyotype acknowledging rare exceptions favoring sex reversal (when self-initiated gender reassignment is appropriate). Although the karyotype is informative, the patient's phenotype reflects the interactions of multiple factors. Longitudinal outcome studies are increasingly available regarding adult function, quality of life, and gender identity.^{430,431}

Gender identity refers to a person's self-experience. In most instances, gender identity is congruent with the sex of rearing (natal sex). The primary factors, critical steps, and sequence of psychosexual development that contribute to gender identity remain uncertain. Psychosexual development has three components: gender identity, gender role, and sexual orientation. Gender identity is established in infancy. Gender role, influenced by prenatal hormonal exposure, generally develops during childhood but may shift through life as a result of each individual's response to society's views and expectations. Sexual orientation is typically unknown before puberty and cannot be considered when contemplating sex of rearing.

The best predictor of gender identity among patients with DSDs appears to be sex of rearing.⁴³² Yet, as would be anticipated, an individual with a DSD may have complex feelings, questions, and thoughts about his or her own gender identity. In addition, society increasingly recognizes that gender exists on a nonbinary spectrum. Based on the large DSD-LIFE study, the number of individuals who change gender is higher among certain diagnoses. However, overall the number is low.⁴³³

Gender identity among most 46,XY patients with CAIS is female, whereas gender identity for those with PAIS can be female or male. Decisions regarding sex of rearing for individuals with PAIS may be challenging because androgen impact on CNS development and tissue responsiveness vary. The clinical response to exogenous testosterone can help with the decision-making process. Testosterone responsiveness can be ascertained by assessing penile growth, following one or more intramuscular injections of testosterone. Nevertheless, approximately a quarter of PAIS patients are dissatisfied with the sex assignment, regardless of whether they are raised as male or female.⁴³⁴

The majority of patients with 5 α -reductase deficiency identify as male, and about half of those diagnosed with 17 β -hydroxysteroid dehydrogenase self-reassign from female to male.⁴³⁵⁻⁴³⁷ If questions regarding sex of rearing arise for virilized 46,XX infants, female sex of rearing is usually recommended because most virilized 46,XX individuals with CAH identify as female. Typically, one major issue for virilized

46,XX females is that it relates to questions regarding genital surgery. Yet, among those completely virilized at birth and raised male with delayed diagnosis, male gender development with good long-term outcome has been reported.⁴³⁸ One report contemplates the option of male sex of rearing for extremely virilized 46,XX patients with CAH.⁴³⁹

Prenatal androgen exposure is believed to one factor that influences gender identity.^{440,441} Girls with CAH tend to choose male playmates and preferred male-typical play.⁴⁴² Among virilized 46,XX individuals with CAH, an increased rate of homosexual orientation has been reported primarily based on self-reported sexual imagery and sexual attraction, with reports of actual homosexual involvement being less well documented.⁴⁴³ In general, psychological adjustment has been found to be comparable for females with CAH and their unaffected siblings.⁴⁴⁴

Despite evidence that androgen exposure to the CNS alters general and cognitive behavior, much remains to be learned about the influences of nature and nurture on gender identity.^{445,446} In general, with the exception of CAIS, 46,XY individuals with fetal androgen exposure manifested as partial genital masculinization should be raised as males, unless there are extenuating individual circumstances. Ideally, 46,XY infants with 17 β -HSD3 or 5 α -reductase deficiency will be diagnosed in the newborn period and raised as males. Caution, reflection, and dialog are essential before those with a 46,XY, karyotype are assigned female sex of rearing because fetal androgen exposure appears to impact strongly on self-concept as male despite compromised external genital development.

Among individuals with DSD associated with gonadal dysgenesis, hCG-stimulated testosterone secretion and clinical response facilitates decisions regarding gender of rearing. The genital phenotype, ability of the Leydig cell to secrete testosterone, and extent of genital virilization in response to androgen stimulation are indicators regarding the potential for spontaneous pubertal development and need for hormone replacement therapy. Nevertheless, in gonadal dysgenesis, the impact of prenatal androgen exposure on gender development processes is often indeterminate and cannot be reliably predicted from routine diagnostic studies in the newborn period.

Within the past decade, the number of individuals with gender dysphoria (discordance between experienced gender and the sex assigned based upon biological sex) has increased among persons with typical binary sex development.⁴⁴⁷ Aspects of the care of transgender individuals may be applicable to those born with DSD who develop gender dysphoria.

Medical Treatment

A multidisciplinary team approach is recommended for the care of patients.⁴⁴⁸ With the exception of disorders affecting glucocorticoid and mineralocorticoid biosynthesis, most DSD conditions do not require specific medical therapy in infancy. At the time of expected puberty, patients with hypogonadism will need appropriate hormone replacement therapy. In general, hormone replacement therapy is initiated using low doses of the appropriate sex steroid hormone with incremental increases designed to mirror spontaneous pubertal development. It is helpful to review with families the anticipated frequency of outpatient visits and how the adequacy of replacement therapy will be assessed.

For females, induction of puberty involves low-dose estrogen therapy—usually initiated between 10.5 to 12 years of age to avoid excessive acceleration of skeletal maturation. The initial estrogen dosage is usually the lowest available, such as 0.3 mg of conjugated estrogens every other day or 5 mcg of ethinyl estradiol daily. Transdermal estrogen preparations that provide 0.007 to 0.025 mg daily can be used and are

recommended based upon early outcome data.⁴⁴⁹ Transdermal patches may be used only at night in an effort to mimic spontaneous puberty.⁴⁵⁰ Matrix transdermal patches can also be cut into smaller pieces to provide a lower estrogen dosage. Based on clinical response and the patient's perception, the dose of estrogen can be increased in 6- to 12-month intervals, such that complete replacement doses and development are achieved within 3 years.

Therapy involves the addition of a progestational agent after 12 months of estrogen therapy or when withdrawal bleeding occurs, whichever occurs sooner. Thereafter, cyclic estrogen-progesterone therapy should be used. Once full pubertal development has been reached, the estrogen dosage should be the minimum that will maintain normal menstrual flow and prevent calcium bone loss.⁴⁵¹ Options for cyclic estrogen-progesterone therapy include low-dosage estrogen birth control pills, transdermal estrogen with oral progestin, or estrogen-progestin transdermal patches.

For example, a daily oral estrogen regimen or the transdermal form for 21 days with the addition of progesterone, 5 to 10 mg of medroxyprogesterone acetate, or 200 mg of micronized progesterone daily added for 12 days (day 10–day 21) can be used. This is followed by a week of no hormones. At this point, the replacement regimen may be extended so that less frequent withdrawal bleeding occurs. In the absence of a uterus, progesterone therapy becomes optional. Among patients with low circulating androgen levels, sexual hair growth and libido may be improved by administering small doses of DHEA or methyltestosterone. Gonadotropins, hCG, and/or human menopausal gonadotropin/recombinant FSH are only used to stimulate ovulation or during assisted fertility attempts.

For males, testosterone hormone replacement typically begins at 12.5 to 14 years. In instances where treatment is indicated for psychological reasons, therapy may be initiated 1 to 2 years earlier. If therapy is begun earlier to reassure the patient concerning physical changes, the rate of skeletal maturation should be carefully monitored. Conversely, treatment may be delayed to allow for psychological or emotional maturity or catch-up growth. Testosterone therapy may be given by depot injections intramuscularly or subcutaneously or topically by a patch or gel. Depot testosterone (such as enanthate or cypionate) is begun at a dosage 50 mg every 4 weeks, followed by increased dosing and frequency over about 3 years, to a full replacement dosage of approximately 200 mg every 2 weeks. Availability of the gel in metered-dose pumps allows gradual increases of dosage from 1.25 g daily upward. Among those with differentiated testes and gonadotropin deficiency, assisted fertility techniques may involve intratesticular germ cell retrieval or hFSH/hLH stimulation.

For patients with CAH, carefully monitored hormone replacement therapy is essential.⁴⁵² The goal of glucocorticoid therapy is suppression of excessive adrenal androgen secretion, while maintaining normal growth and development. Typically, oral cortisol doses range from 8 to 20 mg/m²/d. This range is based on provision of 1.5 to 2 times the daily cortisol production rate, 7 to 12 mg/m²/d. Oral fludrocortisone (e.g., Florinef) is commonly used for mineralocorticoid replacement for patients with salt-losing CAH. Patients with simple virilizing CAH may benefit from mineralocorticoid replacement. The typical dose is 0.1 mg administered as a single daily dose. Neonates and infants may require higher fludrocortisone doses, as well as salt supplementation because of relative mineralocorticoid resistance, higher aldosterone production rates, and relatively lower sodium intakes. Topics of discussion with parents include how to crush and administer tablets, what to do if doses are accidentally missed, use of medical alert identification IDs, and when to administer "stress" dosages.

For children with CAH, adequacy of replacement therapy is monitored by periodic reassessment of growth velocity, extent

of virilization, and salt craving. Laboratory monitoring may include serum androgen and 17-OHP concentrations, skeletal maturation, and 24-hour urinary 17-ketosteroid excretion. Androstenedione concentrations are useful to assess adequacy of glucocorticoid replacement, whereas 17-OHP concentrations are useful to assess for overtreatment. Determination of testosterone concentrations is helpful in girls and prepubertal boys. In pubertal and postpubertal girls, menstrual cyclicity is a sensitive indicator of hormone replacement therapy. Adequacy of mineralocorticoid replacement can be judged using PRA. Boys with CAH need to be regularly assessed by ultrasound for the development of testicular adrenal rest tumors; these rest tumors may be too small to palpate on examination.⁴⁵³

At times of physiological stress (such as fever >101° F, persistent vomiting, significant trauma, and surgical procedures), the glucocorticoid dose should be increased. In general, 2 to 3 times the usual dose is sufficient to prevent adrenal insufficiency. Higher doses may be necessary for surgical procedures. All families should have at home and be able to administer injectable hydrocortisone (e.g., Solu-Cortef) intramuscularly in case of medical emergencies. Recommended intramuscular doses are 25 mg for infants, 50 mg for children less than 4 years of age, and 100 mg for all others.

During surgical procedures, additional hydrocortisone can be administered either as continuous intravenous infusion or intramuscular injection. Intravenous normal saline (0.9% NaCl) can be used if oral mineralocorticoid replacement is not tolerated by the patient. While receiving glucocorticoid replacement therapy (and occasionally in the newborn period), physiologically stressed individuals with 11 β -hydroxylase deficiency may develop hyponatremia and hyperkalemia and benefit from mineralocorticoid therapy. At times of physiological stress, individuals with SLO syndrome or P450 oxidoreductase deficiency may benefit from glucocorticoid treatment.

Considerations With Regard to Surgery

For the virilized female patient with female sex of rearing, the extent of ambiguity in combination with the magnitude of clitoromegaly and posterior fusion must be carefully evaluated to determine whether genitoplasty, including clitoral reduction or clitoroplasty, should even be considered. Although current surgical techniques spare more of the neurovascular bundle of the phallus, surgery should be done only after thoughtful consideration. If the urethral outlet is located high in the urogenital sinus, early surgery may be indicated to decrease the risk of recurrent urinary tract infections by providing a direct urinary outflow path. Girls with mild to moderate degrees of clitoromegaly generally do not need surgery because of the potential for compromising genital sensitivity. Despite surgery, lower urinary tract symptoms, such as stress incontinence and incomplete bladder emptying may persist among female DSD patients and among males with hypospadias.

One important point to share with parents of a virilized girl with CAH is that the “stimulated genital tissues” will regress after glucocorticoid therapy is begun. On one hand, the parents need to be informed that it may be appropriate to discourage genital surgery, until the child is old enough to make her own decision.⁴⁵⁴ On the other hand, parents must be empowered to make the choice with which they will be comfortable. This must be done with the understanding that their daughter may criticize them for this decision when she is older. Many parents of daughters with severe ambiguity still choose surgery.

A shared decision-making process may be helpful to encourage thorough discussion between the parents, as well as the healthcare providers.⁴⁵⁵ If surgery is anticipated, the operation should be described in a manner understandable to the parents. An experienced surgeon should discuss the options, risks,

and benefits of surgery, including the innervation of the clitoris or penis and the surgical approach to be used to attempt to spare the neurovascular supply.⁴⁵⁶ Although opinions vary as to whether surgery should be done in one or more stages and whether vaginal reconstruction should be attempted during infancy, the parents need to be informed that subsequent vaginoplasty or vaginal dilatation may be required after puberty.⁴⁵⁷ Among patients who underwent vaginal reconstructive surgery during infancy, the frequency of postoperative vaginal stenosis has been reported to range from 0% to 77%.⁴⁵⁸ Therapeutic goals for vaginal reconstruction surgery include adequate sexual function with minimal need for continual dilatation or lubrication. After the onset of puberty, those with MRKH syndrome and individuals with CAIS can usually achieve increased vaginal size with dilator treatment.⁴⁵⁹

When discussing surgery for boys with hypospadias, pertinent topics include common male concerns about the importance of being able to stand to urinate, adequacy of genital development, and capability of sexual activities. With hypospadias, the location of the meatus does not necessarily indicate that fusion is complete to that point because there may be more proximal inadequate development of the corpus spongiosum and surrounding tissues. Thus the extent of surgery may not be known until labiourethral fusion is better visualized at surgery. Because correction of hypospadias and chordee is generally performed in stages, this discussion also needs to cover the details of the surgical approach, the proposed schedule for follow-up visits, the likely number of surgical procedures, and the optimal age for each surgical stage. The surgeon should review the options, risks, and anticipated outcomes. If the precise location and differentiation status of the testes are unknown, the parents should be aware that exploration and biopsy may be performed. As would be anticipated, individuals with 46,XY DSD have sexual concerns, regardless of gender of rearing. In addition to reconstructive genital surgery, potential confounding factors include self-esteem, genital appearance, infertility, and need for hormone replacement therapy. Adequacy of counseling and support likely influence adult outcome.⁴⁶⁰

Outcome studies reflect the results of the type of surgery and timing of surgery. Although older surgical techniques resulted in impaired genital sensitivity, it is as yet unclear whether current surgical techniques sparing the neurovascular supply to the clitoris will result in better outcomes. It is anticipated (and hoped) that current surgical techniques will have better outcome; however, it will be years before this information is available. In the meantime, available outcome studies can be informative.^{461,462}

The goals of genital reconstruction surgery have been and continue to be a good cosmetic appearance and the potential for functionality involving sensitivity for sexual responsiveness. Function after surgery is related to severity of genital ambiguity at birth, extent of surgery, and complications of surgery. For example, among females diminished vaginal diameter correlated with greater prenatal virilization. Among 21-hydroxylase-deficient CAH females, the outcome of surgery appears to be related to the specific mutations. Those with the null genotype have more surgical complications and poorer scores regarding sexual function.⁴⁶³ Available outcome data indicate that most postsurgical patients have a female gender identity. Yet, studies show that a greater portion of women with CAH, even those with non-classical CAH (NCAH), are sexually attracted to other women compared with the general population. However, long-term outcomes are not well documented. Among 46,XX DSD patients, those living as females indicated that they preferred early surgery and felt that a satisfactory sex life was possible.⁴⁶⁴ Nonetheless, decreased sexual desire, difficulty achieving orgasm, and later sexual debut were common concerns. Fertility is

possible for women with CAH; reduction of progesterone concentrations is one important factor to ensure fecundity for women with classical forms of CAH.⁴⁶⁵

Although most males with 46,XY DSDs are satisfied with long-term results of genitoplasty, consequences of chordee and severe hypospadias can include penis size too short for vaginal penetration. Hence, outcome may be poor. Recurrent complaints not only include penile size and sexual activity, but also urinary symptoms. Despite improved understanding of DSDs and openness with patients and families, quality of life issues need to have further attention.⁴⁶⁶

Risks for Gonadal Tumors

The aberrant fetal gonadal environment and subsequent anomalous germ cell differentiation is associated with an increased risk for germ cell tumors. The presence of Y chromosome material, specifically the testis-specific protein Y-encoded (*TSPY*) gene, and a dysgenetic gonad increase the propensity risk for gonadal tumors. The relative risk for gonadal tumors directs the need for and timing of surgery. Germ cell neoplasia-in-situ (GCNIS) or gonadoblastoma are precursors for germ cell tumors. The prevalence reported for gonadoblastoma ranges from 15% to 30%, depending on age of the patient, gonadal histology, and diagnostic criteria used for GCNIS/gonadoblastoma. Gonadoblastomas are mixed germ cell sex cord-stromal tumors arising in dysgenetic gonads, are composed of immature germ cells and sex cord-stromal cells of indeterminate differentiation, and precede the development of the more invasive neoplasms, such as dysgerminoma, seminoma, and nonseminoma. Assessment of *FOXL2* and *SOX9* expression in the tumor tissue can help to discriminate between those with a Sertoli cell component from those with a granulosa cell component.⁴⁶⁷

Increased and prolonged expression of immunohistochemical markers, *POU5F1* (also known as *OCT3/4*) and *TSPY*, is common in GCNIS and gonadoblastoma. In one series, *TSPY* was abundantly expressed in germ cells within dysgenetic testes and undifferentiated gonadal tissue suggesting upregulation when germ cells are located in an unfavorable environment. It is hypothesized that the normal germ cell maturation process is interrupted, resulting in prolonged expression of *OCT3/4*, erased genomic imprinting, and subsequent immortalization of the cell.^{468,469}

In one series of patients with Turner syndrome, 14/171 (8%) were positive for Y-chromosomal material. Among these 14 patients, the prevalence of gonadoblastoma was 33%.⁴⁷⁰ In some instances, Y-chromosomal material is only detected using PCR methodology.⁴⁷¹ Frasier syndrome, associated with a specific *WT1* mutation, is characterized by steroid-resistant nephrotic syndrome, XY-sex reversal, and increased risk for gonadal tumors.⁴⁷²

Because of a hypothesized increased risk for gonadal tumors, gonadectomy has been typically performed in patients with CAIS. Despite the dearth of long-term outcome studies, available data indicate that the risk for gonadal tumors is extremely low in patients with CAIS during the adolescent years.^{473,474} An international survey highlighted the inconsistent approaches to treatment, low risk for germ cell tumors, and emphasized the need for individualized management recommendations for patients with CAIS.^{475,476}

Limited sample size, ascertainment bias, inconsistent diagnostic criteria for malignant cells, and confusing terminology leave many questions to be answered. For example, which patients with DSD benefit from gonadal biopsy to assess risk for neoplasia? Another consideration is how many biopsies are necessary to be representative of the histology of the gonad. Laparoscopy and video-assisted gonadectomy are invaluable tools when the risk for malignancy is high. Ultimately, the decision

regarding gonadectomy involves consideration of the patient's phenotype (internal and external genital anatomy), karyotype, gender of rearing, psychosocial factors, and gonadal histology.⁴⁰⁹

Transition From Pediatrics to Adult Care

Parents and pediatric healthcare providers envision that children with DSD will become productive adults who will find fulfillment and gratification in their lives. In view of the report from England regarding loss of follow-up of patients with CAH, a planned transition program from the pediatric healthcare team to the adult healthcare team is essential.⁴⁷⁷ This planned transition process needs to consider the medical, psychosocial, educational, emotional, cognitive, and vocational needs of the young adult. Differences in expectations between pediatric and adult healthcare situations should be reviewed. Explanation of the specific details of his/her diagnosis with the young adult is essential.⁴⁷⁸ For many of these patients, the specific details of their disorder were reviewed with the parents at the time of diagnosis when the individual was too young to participate in these discussions. The young adult needs to know the etiology, basic genetics, pathophysiology of his/her disorder, recurrence risk, and fertility status. The young adult, especially those requiring glucocorticoid replacement therapy, needs to understand the rationale for treatment.⁴⁷⁹

Long-Term Outcome Studies

Among 46,XX females with CAH, outcomes differ depending on the severity of the condition or the specific *CYP21A2* mutations. More recent surgical outcome has improved but is not always satisfactory, psychological effects vary, and hence fertility and sexual satisfaction varies. The key approach involves individualizing each patient.⁴⁸⁰ A study of outcome among phenotypic females with 46,XY DSD with an age-matched control group found: (1) overall increased morbidity but not after exclusion of those with specific diagnoses, pregnancy, and birth; (2) mortality similar to controls; and (3) education. Cohabitation and motherhood were reduced. Thus, such individuals appear to perform well in the work environment but family life is negatively impacted.⁴⁸¹ General health status is good, better related to early diagnosis and healthy lifestyle.⁴⁸² Fertility, as would be anticipated, is rare, related to initial genital phenotype, and partnerships (which occur among a minority). Assisted reproductive techniques (ART) are often used. Fertility is extremely rare among those with pure or mixed gonadal dysgenesis, ovotesticular DSD, and XX males.^{483,484} Better surgical techniques, improved socialization, focus on long-term treatment, and ART techniques may improve the potential for fertility in the future. Sexual satisfaction, including early reports of sexual function for females, because the sparing of the neurovascular hood of the clitoris, suggest similar results with the general population, with problems with socialization, interpersonal and intimate relationships continuing to be a factor. Severe forms of hypospadias, whether or not identified as a DSD continue to be a persistent surgical, functional, and psychosocial challenge.^{485,486} Clinical outcomes among boys with PAIS are poorer than with most other XY DSDs, hence the decision-making regarding sex assignment must be particularly cautious.⁴⁸⁷

Psychological and Genetic Counseling and Support and Ethical Considerations

Longitudinal continuity of care and provision for support systems are essential because of the medical and psychological aspects of DSDs. If an experienced social worker, psychologist, or psychiatrist is available, careful assessment and counseling are invaluable throughout childhood and adolescence. Although most such cases of 46,XY DSD may not require

medical therapy during early childhood, intermittent visits with the pediatric endocrinologist are helpful to address the patient's and parents' concerns, update parents concerning new therapy and outcome data, and ensure that appropriate psychological support needs are being met. These visits can foster a positive relationship between the child and the physician. Some parents may find disorder-specific support groups (e.g., AIS, www.aissg.org; CAH, www.caresfoundation.org; Turner syndrome, www.turnersyndrome.org) to be helpful.

Many DSD conditions are inherited. The most common inheritance patterns are autosomal recessive or X-linked traits. Genetic counseling is indicated because parents are often interested to learn about recurrence risks. Because phenotypic heterogeneity occurs in some DSD disorders, hormone determinations and genetic analyses (when available) for other family members may be recommended.

Ethical principles and recommendations have been published in an effort to provide a comprehensive view of the perspective of clinicians, patients, and families, based on the principles of fostering the wellbeing of the child and future adult, uphold their rights to participate in decisions that impact them now and later, and to respect family and parent-child relationships.⁴¹² The general media has noted that intersex babies currently may be caught in the "surgery battle over medical risks and ethical rights."⁴⁸⁸ This controversy highlights the ethical dilemma in which parents serve as surrogates for the best care of their minor children. Parents need to be educated and informed regarding all available information. Numerous critiques discussing various topics have been published; topics have included the struggle against medicalization, politicizing and democratizing standard of care, and legal/medical activism.⁴⁸⁹

CONCLUSIONS

Identification of genes involved in sexual differentiation has elucidated some of the molecular events responsible for normal and abnormal sexual differentiation. Knowledge of the genetic, hormonal, and environmental factors that influence sexual differentiation benefits the affected children, their parents, and their healthcare providers. This information enables better parental education regarding the etiology, natural history, and prognosis for their child. With understanding of factors that affect sexual differentiation, recurrence risks can be estimated.

The evaluation and subsequent management of a child with ambiguous genitalia is based on history, physical examination, and laboratory data. Understanding the family's background and situation enable the medical team to discuss the child's sex development, using terminology that they can understand at the time of diagnosis, as well as during the following years. Initial diagnosis and management are the beginning of a long-term relationship among the patient, family, and healthcare providers.

Despite the advances in characterizing the molecular basis of ambiguous genitalia, sensitivity and compassion demonstrated by the team of healthcare professionals in their interactions with the patient and family remain an essential aspect in the management of an infant with genital ambiguity. The initial evaluation and diagnostic processes are merely the beginning of an extended relationship with the child and family, ultimately culminating in successful transition to adult care providers. Respectful and empathetic interactions foster development of the infant with genital ambiguity to become an adult able to live a contented life. The healthcare providers need to remain cognizant that knowledge regarding all aspects of sex determination, sex development, and gender identity determinants remains to be elucidated, such that management of

individuals with DSDs will still involve uncertainties and likelihoods.

REFERENCES

1. Stévant I, Papaioannou MD, Nef S. A brief history of sex determination. *Mol Cell Endocrinol*. 2018;468:3–10.
2. Lee PA, Houk CP, Ahmed SF. Consensus statement on management of intersex disorders. *Pediatrics*. 2006;118:e488–e500.
3. Thyen U, Lanz K, Holterhus PM, Hiort O. Epidemiology and initial management of ambiguous genitalia at birth in Germany. *Horm Res*. 2006;66(4):195–203.
4. Berglund A, Johannsen TH, Stochholm K, Viuff MH, Fedder J, Main KM, Gravholt CH. Incidence, prevalence, diagnostic delay, and clinical presentation of female 46,XY disorders of sex development. *J Clin Endocrinol Metab*. 2016;101(12):4532–4540.
5. Lee PA, Houk CP, Ahmed SF, Hughes IA, International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. *Pediatrics*. 2006;118(2):e488–e500.
6. Lee PA, Nordenström A, Houk CP, Ahmed SF, Auchus R, Baratz A, et al. Global DSD Update Consortium. Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. *Horm Res Paediatr*. 2016;85(3):158–180.
7. Cools M, Nordenström A, Robeva R, Hall J, Westerveld P, Flück C, et al. COST Action BM1303 working group 1. Caring for individuals with a difference of sex development (DSD): a Consensus Statement. *Nat Rev Endocrinol*. 2018;14(7):415–429.
8. Kyriakou A, Dessens A, Bryce J, Iotova V, Juul A, Krawczynski M, et al. Current models of care for disorders of sex development - results from an International survey of specialist centres. *Orphanet J Rare Dis*. 2016;11(1):155.
9. Zainuddin AA, Mahdy ZA. The Islamic perspectives of gender-related issues in the management of patients with disorders of sex development. *Arch Sex Behav*. 2017;46(2):353–360.
10. Timmermans S, Yang A, Gardner M, Keegan CE, Yashar BM, Fechner PY, et al. Does patient-centered care change genital surgery decisions? the strategic use of clinical uncertainty in disorders of sex development clinics. *J Health Soc Behav*. 2018;59(4):520–535.
11. Schober J, Nordenström A, Hoebcke P, Lee P, Houk C, Looijenga L, et al. Disorders of sex development: summaries of long-term outcome studies. *J Pediatr Urol*. 2012;8(6):616–623.
12. Filippou P, Homburg R. Is foetal hyperexposure to androgens a cause of PCOS? *Hum Reprod Update*. 2017;23(4):421–432.
13. Finlayson C, Rosoklija I, et al. Baseline characteristics of infants with atypical genital development: phenotypes, diagnostics and sex of rearing. *J Endoc Soc*. 2019;3(1):264–272.
14. Röhle R, Gehrman K, Szarras-Czapnik M, Claahsen-van der Grinten H, Pienkowski C, Bouvattier C, et al. dsd-LIFE group. Participation of adults with disorders/differences of sex development (DSD) in the clinical study dsd-LIFE: design, methodology, recruitment, data quality and study population. *BMC Endocr Disord*. 2017;17(1):52.
15. Jost A. Recherches sur la différenciation sexuelle de l'embryon de lapin. *Arch Anat Microsc Morph Exp*. 1947;36.
16. Lahn BT, Page DC. Four evolutionary strata on the human X chromosome. *Science*. 1999;286(5441):964–967.
17. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*. 1990;346(6281):240–244.
18. Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, et al. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature*. 1990;346(6281):245–250.
19. Bashamboo A, Eozenou C, Rojo S, McElreavey K. Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development. *Clin Genet*. 2017;91(2):143–156.

20. Atlasi Y, Stunnenberg HG. The interplay of epigenetic marks during stem cell differentiation and development. *Nat Rev Genet.* 2017;18(11):643–658.
21. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature.* 2005;434(7031):400–404.
22. Garcia-Moreno SA, Plebanek MP, Capel B. Epigenetic regulation of male fate commitment from an initially bipotential system. *Mol Cell Endocrinol.* 2018;468:19–30.
23. Garcia-Moreno SA, Futtner CR, Salamone IM, Gonen N, Lovell-Badge R, Maatouk DM. Gonadal supporting cells acquire sex-specific chromatin landscapes during mammalian sex determination. *Dev Biol.* 2019;446(2):168–179.
24. Lin YT, Barske L, DeFalco T, Capel B. Numb regulates somatic cell lineage commitment during early gonadogenesis in mice. *Development.* 2017;144(9):1607–1618.
25. Mork L, Maatouk DM, McMahon JA, Guo JJ, Zhang P, McMahon AP, Capel B. Temporal differences in granulosa cell specification in the ovary reflect distinct follicle fates in mice. *Biol Reprod.* 2012;86(2):37.
26. Miller-Hodges E, Hohenstein P. WT1 in disease: shifting the epithelial-mesenchymal balance. *J Pathol.* 2012;226(2):229–240.
27. Ostrer H, Huang HY, Masch RJ, Shapiro E. A cellular study of human testis development. *Sex Dev.* 2007;1(5):286–292.
28. Eid W, Opitz L, Bignon-Laubert A. Genome-wide identification of CBX2 targets: insights in the human sex development network. *Mol Endocrinol.* 2015;29(2):247–257.
29. Capel B. Sex in the 90s: SRY and the switch to the male pathway. *Annu Rev Physiol.* 1998;60:497.
30. Mamsen LS, Ernst EH, Borup R, Larsen A, Olesen RH, Ernst E, et al. Temporal expression pattern of genes during the period of sex differentiation in human embryonic gonads. *Sci Rep.* 2017;7(1):15961.
31. Capel B, Albrecht KH, Washburn LL, Eicher EM. Migration of mesonephric cells into the mammalian gonad depends on Sry. *Mech Dev.* 1999;84:127.
32. Cool J, DeFalco TJ, Capel B. Vascular-mesenchymal cross-talk through Vegf and Pdgf drives organ patterning. *Proc Natl Acad Sci U S A.* 2011;108(1):167–172.
33. Del Valle I, Buonocore F, Duncan AJ, Lin L, Barenco M, Parnaik R, et al. A genomic atlas of human adrenal and gonad development. *Wellcome Open Res.* 2019;2:25.
34. Hanley NA, Hagan DM, Clement-Jones M, Ball SG, Strachan T, Salas-Cortes L, et al. SRY, SOX9, and DAX1 expression patterns during human sex determination and gonadal development. *Mech Dev.* 2000; 91(1-2), 403–407.
35. Croft B, Ohnesorg T, Hewitt J, Bowles J, Quinn A, Tan J, et al. Human sex reversal is caused by duplication or deletion of core enhancers upstream of SOX9. *Nat Commun.* 2018;9(1):5319.
36. Osterwalder M, Barozzi I, Tissières V, Fukuda-Yuzawa Y, Mannion BJ, Afzal SY, et al. Enhancer redundancy provides phenotypic robustness in mammalian development. *Nature.* 2018;554(7691):239–243.
37. Bagheri-Fam S, Bird AD, Zhao L, Ryan JM, Yong M, Wilhelm D, et al. Testis determination requires a specific FGFR2 isoform to repress FOXL2. *Endocrinology.* 2017;158(11):3832–3843.
38. deleted at review.
39. Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, Avello M, et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature.* 2012;485(7397):195–200.
40. De Santa Barbara P, Moniot B, Poulat F, et al. Expression and subcellular localization of SF-1, SOX9, WT1, and AMH proteins during early human testicular development. *Dev Dyn.* 2000;217:293.
41. Moniot B, Berta P, Scherer G, et al. Male specific expression suggests role of DMRT1 in human sex determination. *Mech Dev.* 2000;91:323.
42. Macdonald J, Kilcoyne KR, Sharpe RM, Kavanagh Á, Anderson RA, Brown P, et al. DMRT1 repression using a novel approach to genetic manipulation induces testicular dysgenesis in human fetal gonads. *Hum Reprod.* 2018;33(11):2107–2121.
43. O'Shaughnessy PJ, Baker PJ, Monteiro A, Cassie S, Bhattacharya S, Fowler PA. Developmental changes in human fetal testicular cell numbers and messenger ribonucleic acid levels during the second trimester. *J Clin Endocrinol Metab.* 92(12), 4792–4801.
44. Svechnikov K, Landreh L, Weisser J, Izzo G, Colón E, Svechnikova I, Söder O. Origin, development and regulation of human Leydig cells. *Horm Res Paediatr.* 2010;73(2):93–101.
45. Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev.* 2007;87:1.
46. Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, et al. Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell.* 2009;139(6):1130–1142.
47. Hersmus R, Kalfa N, de Leeuw B, Stoop H, Oosterhuis JW, de Krijger R, et al. FOXL2 and SOX9 as parameters of female and male gonadal differentiation in patients with various forms of disorders of sex development (DSD). *J Pathol.* 2008;215(1):31–38.
48. Veitia RA. FOXL2 versus SOX9: a lifelong “battle of the sexes”. *Bioessays.* 2010;32(5):375–380.
49. Zebisch M, Xu Y, Krastev C, MacDonald BT, Chen M, Gilbert RJ, et al. Structural and molecular basis of ZNRF3/RNF43 transmembrane ubiquitin ligase inhibition by the Wnt agonist R-spondin. *Nat Commun.* 2013;4:2787.
50. Yatsenko SA, Rajkovic A. Genetics of human female infertility. *Biol Reprod.* 2019. pii: ioz084.
51. Fowler PA, Anderson RA, Saunders PT, Kinnell H, Mason JI, Evans DB, et al. Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary. *J Clin Endocrinol Metab.* 96(6), 1754–1762.
52. Richardson BE, Lehmann R. Mechanisms guiding primordial germ cell migration: strategies from different organisms. *Nat Rev Mol Cell Biol.* 2010;11(1):37–49.
53. Barton LJ, LeBlanc MG, Lehmann R. Finding their way: themes in germ cell migration. *Curr Opin Cell Biol.* 2016;42:128–137.
54. Mamsen LS, Bröchner CB, Byskov AG, Møllgard K. The migration and loss of human primordial germ stem cells from the hind gut epithelium towards the gonadal ridge. *Int J Dev Biol.* 2012;56(10-12):771–778.
55. Irie N, Weinberger L, Tang WW, Kobayashi T, Viukov S, Manor YS, et al. SOX17 is a critical specifier of human primordial germ cell fate. *Cell.* 2015;160(1-2):253–268.
56. Li L, Dong J, Yan L, et al. Single-cell RNA-Seq analysis maps development of human germline cells and gonadal niche interactions. *Cell Stem Cell.* 2017;20(6):858–873. e4.
57. Baltus AE, Menke DB, Hu YC, Goodheart ML, Carpenter AE, de Rooij DG, et al. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. *Nat Genet.* 2006;38:1430.
58. Gill ME, Hu YC, Lin Y, Page DC. Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of fetal germ cells. *Proc Natl Acad Sci U S A.* 2011;108(18):7443–7748.
59. Rosario R, Childs AJ, Anderson RA. RNA-binding proteins in human oogenesis: Balancing differentiation and self-renewal in the female fetal germline. *Stem Cell Res.* 2017;21:193–201.
60. Abby E, Tourpin S, Ribeiro J, Daniel K, Messiaen S, Moison D, et al. Implementation of meiosis prophase I programme requires a conserved retinoid-independent stabilizer of meiotic transcripts. *Nat Commun.* 2016;7:10324.
61. Bendsen E, Byskov AG, Andersen CY, Westergaard LG. Number of germ cells and somatic cells in human fetal ovaries during the first weeks after sex differentiation. *Hum Reprod.* 2006; 21(1):30–35.
62. Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Med Lond.* 1963;158:417.
63. Xu J, Gridley T. Notch2 is required in somatic cells for breakdown of ovarian germ-cell nests and formation of primordial follicles. *BMC Biol.* 2013;11:13.
64. Hartshorne GM, Lyrakou S, Hamoda H, Oloto E, Ghafari F. Oogenesis and cell death in human prenatal ovaries: what are the criteria for oocyte selection? *Mol Hum Reprod.* 2009;15(12):805–819.
65. Yatsenko SA, Rajkovic A. Genetics of human female infertility. *Biol Reprod.* 2019;101(3):602–616.
66. von Meyenn F, Reik W. Forget the parents: epigenetic reprogramming in human germ cells. *Cell.* 2015;161(6):1248–1251.

67. Guo F, Yan L, Guo H, Li L, Hu B, Zhao Y, et al. The transcriptome and DNA methylome landscapes of human primordial germ cells. *Cell*. 2015;161(6):1437–1452.
68. Heard E, Turner J. Function of the sex chromosomes in mammalian fertility. *Cold Spring Harb Perspect Biol*. 2011;3(10):a002675.
69. Golbabapour S, Abdulla MA, Hajrezaei M. A concise review on epigenetic regulation: insight into molecular mechanisms. *Int J Mol Sci*. 2011;12(12):8661–8694.
70. Schaefer CB, Ooi SK, Bestor TH, Bourc'his D. Epigenetic decisions in mammalian germ cells. *Science*. 2007;316:398.
71. Goto M, Piper Hanley K, Marcos J, Wood PJ, Wright S, Postle AD, et al. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest*. 2006;116:953.
72. White PC. Ontogeny of adrenal steroid biosynthesis: why girls will be girls. *J Clin Invest*. 2006;116:872.
73. Melau C, Nielsen JE, Frederiksen H, Kilcoyne K, Perlman S, Lundvall L, et al. Characterization of human adrenal steroidogenesis during fetal development. *J Clin Endocrinol Metab*. 2019;104(5):1802–1812.
74. Robboy SJ, Kurita T, Baskin L, Cunha GR. New insights into human female reproductive tract development. *Differentiation*. 2017;97:9–22.
75. Du H, Taylor HS. The role of hox genes in female reproductive tract development, adult function, and fertility. *Cold Spring Harb Perspect Med*. 2015;6(1):a023002.
76. Cunha GR, Robboy SJ, Kurita T, Isaacson D, Shen J, Cao M, Baskin LS. Development of the human female reproductive tract. *Differentiation*. 2018;103:46–65.
77. Marker PC, Donjacour AA, Dahiya R, Cunha GR. Hormonal, cellular, and molecular control of prostatic development. *Dev Biol*. 2003;253:156.
78. Powers GL, Marker PC. Recent advances in prostate development and links to prostatic diseases. *Wiley Interdiscip Rev Syst Biol Med*. 2013;5(2):243–256.
79. MacLeod DJ, Sharpe RM, Welsh M, Fiskens M, Scott HM, Hutchison GR, et al. Androgen action in the masculinization programming window and the development of the male reproductive system. *Andrology*. 2010;33:279–287.
80. Welsh M, Suzuki H, Yamada G. The masculinization programming window. *Endocr Dev*. 2014;27:17–27.
81. Baskin L, Shen J, Sinclair A, Cao M, Liu X, Liu G, et al. Development of the human penis and clitoris. *Differentiation*. 2018;103:74–85.
82. Zalel Y, Pinhas-Hamiel O, Lipitz S, Mashiach S, Achiron R. The development of the fetal penis—an in utero sonographic evaluation. *Ultrasound Obstet Gynecol*. 2001;17(2):129–131.
83. Jain VG, Singal AK. Shorter anogenital distance correlates with undescended testis: a detailed genital anthropometric analysis in human newborns. *Hum Reprod*. 2013;28(9):2343–2349.
84. Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker MP, Hernandez-Avila M. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. *Environ Health*. 2004;3(1):8.
85. Jain VG, Goyal V, Chowdhary V, Swarup N, Singh RJ, Singal A, Shekhawat P. Anogenital distance is determined during early gestation in humans. *Hum Reprod*. 2018;33(9):1619–1627.
86. Thankamony A, Pasternski V, Ong KK, Acerini CL, Hughes IA. Anogenital distance as a marker of androgen exposure in humans. *Andrology*. 2016;4(4):616–625.
87. Singal AK, Jain VG, Gazali Z, Shekhawat P. Shorter anogenital distance correlates with the severity of hypospadias in pre-pubertal boys. *Hum Reprod*. 2016;31(7):1406–1410.
88. Liu C, Xu X, Huo X. Anogenital distance and its application in environmental health research. *Environ Sci Pollut Res Int*. 2014;21(8):5457–5464.
89. Priskorn L, Bang AK, Nordkap L, Krause M, Mendiola J, Jensen TK, et al. Anogenital distance is associated with semen quality but not reproductive hormones in 1106 young men from the general population. *Hum Reprod*. 2019;34(1):12–24.
90. Parra MD, Mendiola J, Jørgensen N, Swan SH, Torres-Cantero AM. Anogenital distance and reproductive parameters in young men. *Andrologia*. 2016;48(1):3–10.
91. McCarthy MM, Arnold AP. Reframing sexual differentiation of the brain. *Nat Neurosci*. 2011;14(6):677–683.
92. Arnold AP. The end of gonad-centric sex determination in mammals. *Trends Genet*. 2012;28(2):55–61.
93. McCarthy MM. Multifaceted origins of sex differences in the brain. *Philos Trans R Soc Lond B Biol Sci*. 2016;371(1688):20150106.
94. Koopman P, Gubbay J, Vivian N, et al. Male development of chromosomally female mice transgenic for Sry. *Nature*. 1991;351:117.
95. Kreidberg JA, Sariola H, Loring JM, et al. WT-1 is required for early kidney development. *Cell*. 1993;74:679.
96. Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S. Defects of urogenital development in mice lacking Emx2. *Development*. 1997;124(9):1653–1664.
97. Kusaka M, Katoh-Fukui Y, Ogawa H, et al. Abnormal epithelial cell polarity and ectopic epidermal growth factor receptor (EGFR) expression induced in Emx2 KO embryonic gonads. *Endocrinology*. 2010;151(12):5893–5904.
98. Kuroki S, Tachibana M. Epigenetic regulation of mammalian sex determination. *Mol Cell Endocrinol*. 2018;468:31–38.
99. Katoh-Fukui Y, Miyabayashi K, Komatsu T, Owaki A, Baba T, Shima Y, et al. Cbx2, a polycomb group gene, is required for Sry gene expression in mice. *Endocrinology*. 153(2), 913–924.
100. Kuroki S, Matoba S, Akiyoshi M, Matsumura Y, Miyachi H, Mise N, et al. Epigenetic regulation of mouse sex determination by the histone demethylase Jmjd1a. *Science*. 2013;341(6150):1106–1109.
101. Gierl MS, Gruhn WH, von Seggern A, Maltry N, Niehrs C. GADD45G functions in male sex determination by promoting p38 signaling and Sry expression. *Dev Cell*. 2012;23(5):1032–1042.
102. Warr N, Carre GA, Siggers P, Faleato JV, Brixey R, Pope M, et al. Gadd45γ and Map3k4 interactions regulate mouse testis determination via p38 MAPK-mediated control of Sry expression. *Dev Cell*. 2012;23(5):1020–1031.
103. Tevosian SG, Albrecht KH, Crispino JD, Fujiwara Y, Eicher EM, Orkin SH. Gonadal differentiation, sex determination and normal Sry expression in mice require direct interaction between transcription partners GATA4 and FOG2. *Development*. 2002;129(19):4627–4634.
104. Carré GA, Siggers P, Xipolita M, Brindle P, Lutz B, Wells S, Greenfield A. Loss of p300 and CBP disrupts histone acetylation at the mouse Sry promoter and causes XY gonadal sex reversal. *Hum Mol Genet*. 2018;27(1):190–198.
105. Bedford DC, Brindle PK. Is histone acetylation the most important physiological function for CBP and p300? *Aging (Albany NY)*. 2012;4(4):247–255.
106. Zhao F, Yao HH. A tale of two tracts: history, current advances and future directions of research on sexual differentiation of reproductive tracts. *Biol Reprod*. 2019. pii: ioz079.
107. Gupta C, Siegel S, Ellis D. The role of EGF in testosterone-induced reproductive tract differentiation. *Dev Biol*. 1991;146(1):106–116.
108. Dumeige L, Chatelais L, Bouvattier C, et al. Should 45,X/46,XY boys with no or mild anomaly of external genitalia be investigated and followed up? *Eur J Endocrinol*. 2018;179(3):181–190.
109. Chang HJ, Clark RD, Bachman H. The phenotype of 45,X/46,XY mosaicism: an analysis of 92 prenatally diagnosed cases. *Am J Hum Genet*. 1990;46(1):156–167.
110. Wheeler M, Peakman D, Robinson A, Henry G. 45,X/46,XY mosaicism: contrast of prenatal and postnatal diagnosis. *Am J Med Genet*. 1988;29(3):565–571.
111. Röpke A, Pelz AF, Volleth M, Schlösser HW, Morlot S, Wieacker PF. Sex chromosomal mosaicism in the gonads of patients with gonadal dysgenesis, but normal female or male karyotypes in lymphocytes. *Am J Obstet Gynecol*. 2004;190(4):1059–1062.
112. Ljubicic ML, Jørgensen A, Acerini C, Andrade J, Balsamo A, Bertelloni S, et al. Clinical but not histological outcomes in males with 45,X/46,XY mosaicism vary depending on reason for diagnosis. *J Clin Endocrinol Metab*. 2019;104(10):4366–4381.
113. Andrade JGR, Andrade LA, Guerra-Junior G, Maciel-Guerra AT. 45,X/46,XY ovotesticular disorder of sex development revisited: undifferentiated gonadal tissue may be mistaken as ovarian tissue. *J Pediatr Endocrinol Metab*. 2017;30(8):899–904.

114. dos Santos AP, Andrade JG, Piveta CS, de Paulo J, Guerra Jr G, de Mello MP, Maciel-Guerra AT. Screening of Y chromosome microdeletions in 46,XY partial gonadal dysgenesis and in patients with a 45,X/46,XY karyotype or its variants. *BMC Med Genet*. 2013;14:115.
115. Friocourt G, Parnavelas JG. Mutations in ARX result in several defects involving GABAergic neurons. *Front Cell Neurosci*. 2010;4:4.
116. Curie, A., Friocourt, G., des Portes, V., Roy, A., Nazir, T., Brun, A., et al. Basal ganglia involvement in ARX patients: The reason for ARX patients very specific grasping? *Neuroimage Clin*, 19, 454–465.
117. Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, et al. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002;32(3):359–369.
118. Miyabayashi K, Katoh-Fukui Y, Ogawa H, Baba T, Shima Y, Sugiyama N, et al. Aristaless related homeobox gene, Arx, is implicated in mouse fetal Leydig cell differentiation possibly through expressing in the progenitor cells. *PLoS One*. 2013;8(6): e68050.
119. Vilain E, Le Merrer M, Lecoindre C, et al. IMAGE, a new clinical association of intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies. *J Clin Endocrinol Metab*. 1999;84:4335.
120. Eggermann T, Binder G, Brioude F, Maher ER, Lapunzina P, Cubellis MV, et al. CDKN1C mutations: two sides of the same coin. *Trends Mol Med*. 20(11), 614–622.
121. Hamajima N, Johmura Y, Suzuki S, Nakanishi M, Saitoh S. Increased protein stability of CDKN1C causes a gain-of-function phenotype in patients with IMAGE syndrome. *PLoS One*. 2013; 8(9). e75137.
122. Stampone E, Caldarelli I, Zullo A, et al. Genetic and epigenetic control of CDKN1C expression: importance in cell commitment and differentiation, tissue homeostasis and human diseases. *Int J Mol Sci*. 2018;19(4). pii: E1055.
123. Arboleda VA, Lee H, Parnaik R, Fleming A, Banerjee A, Ferraz-de-Souza B, et al. Mutations in the PCNA-binding domain of CDKN1C cause IMAGE syndrome. *Nat Genet*. 2012;44(7): 788–792.
124. Thurm A, Tierney E, Farmer C, Albert P, Joseph L, Swedo S, et al. Development, behavior, and biomarker characterization of Smith-Lemli-Opitz syndrome: an update. *J Neurodev Disord*. 2016;8:12.
125. Bianconi SE, Cross JL, Wassif CA, Porter FD. Pathogenesis, epidemiology, diagnosis and clinical aspects of Smith-Lemli-Opitz syndrome. *Expert Opin Orphan Drugs*. 2015;3(3):267–280.
126. Lee RW, Conley SK, Gropman A, Porter FD, Baker EH. Brain magnetic resonance imaging findings in Smith-Lemli-Opitz syndrome. *Am J Med Genet A*. 2013;161A(10):2407–2419.
127. Bianconi SE, Conley SK, Keil MF, Sinaii N, Rother KI, Porter FD, Stratakis G. Adrenal function in Smith-Lemli-Opitz syndrome. *Am J Med Genet A*. 2011;155A(11):2732–2738.
128. Jayamanne C, Sandamal S, Jayasundara K, Saranavabavananthan M, Mettananda S. Smith-Lemli-Opitz syndrome presenting as acute adrenal crisis in a child: a case report. *J Med Case Rep*. 2018;12(1):217.
129. Svoboda MD, Christie JM, Eroglu Y, Freeman KA, Steiner RD. Treatment of Smith-Lemli-Opitz syndrome and other sterol disorders. *Am J Med Genet C Semin Med Genet*. 2012;160C(4):285–294.
130. Francis KR, Ton AN, Xin Y, O'Halloran PE, Wassif CA, Malik N, et al. Modeling Smith-Lemli-Opitz syndrome with induced pluripotent stem cells reveals a causal role for Wnt/ β -catenin defects in neuronal cholesterol synthesis phenotypes. *Nat Med*. 2016; 22 (4), 388–396.
131. Shackleton CH, Marcos J, Palomaki GE, et al. Dehydrosteroid measurements in maternal urine or serum for the prenatal diagnosis of Smith-Lemli-Opitz syndrome (SLOS). *Am J Med Genet A*. 2007;143A(18):2129–2136.
132. Cross JL, Iben J, Simpson CL, Thurm A, Swedo S, Tierney E, et al. Determination of the allelic frequency in Smith-Lemli-Opitz syndrome by analysis of massively parallel sequencing data sets. *Clin Genet*. 2015;87(6):570–575.
133. Nowaczyk MJ, Wayne JS, Douketis JD. DHCR7 mutation carrier rates and prevalence of the RSH/Smith-Lemli-Opitz syndrome: where are the patients? *Am J Med Genet A*. 2006;140:2057.
134. Piard J, Mignot B, Arbez-Gindre F, Aubert D, Morel Y, Roze V, et al. Severe sex differentiation disorder in a boy with a 3.8 Mb 10q25.3-q26.12 microdeletion encompassing EMX2. *Am J Med Genet A*. 2014;164A(10):2618–2622.
135. Hall JG. The early history of Pallister-Hall syndrome—Buried treasure of a sort. *Gene*. 2016;589(2):100–103.
136. Narumi Y, Koshi T, Tsuruta G, Shiohara M, Shimazaki E, Mori T, et al. Genital abnormalities in Pallister-Hall syndrome: report of two patients and review of the literature. *Am J Med Genet A*. 2010;152A(12):3143–3147.
137. Kang S, Graham Jr JM, Olney AH, Biesecker LG. GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. *Nat Genet*. 1997;15(3):266–268.
138. Johnston JJ, Olivos-Glander I, Killoran C, et al. Molecular and clinical analyses of Greig cephalopolysyndactyly and Pallister-Hall syndromes: robust phenotype prediction from the type and position of GLI3 mutations. *Am J Hum Genet*. 2005;76(4): 609–622.
139. Biesecker LG. The Greig cephalopolysyndactyly syndrome. *Orphanet J Rare Dis*. 2008;3:10.
140. Blake J, Hu D, Cain JE, Rosenblum ND. Urogenital development in Pallister-Hall syndrome is disrupted in a cell-lineage-specific manner by constitutive expression of GLI3 repressor. *Hum Mol Genet*. 2016;25(3):437–447.
141. Nivelon A, Nivelon JL, Mabilille JP, et al. New autosomal recessive chondrodysplasia—pseudohermaphroditism syndrome. *Clin Dysmorphol*. 1992;1(4):221–227.
142. Callier P, Calvel P, Matevossian A, Makrythanasis P, Bernard P, Kurosaka H, et al. Loss of function mutation in the palmitoyl-transferase HHAT leads to syndromic 46,XY disorder of sex development by impeding Hedgehog protein palmitoylation and signaling. *PLoS Genet*. 2014;10(5). e1004340.
143. Innis JW, Mortlock D, Chen Z, Ludwig M, Williams ME, Williams TM, et al. Polyalanine expansion in HOXA13: three new affected families and the molecular consequences in a mouse model. *Hum Mol Genet*. 2004;13(22):2841–2851.
144. Halal F. The hand-foot-genital (hand-foot-uterus) syndrome: family report and update. *Am J Med Genet*. 1988;30(3):793–803.
145. Tas E, Sebastian J, Madan-Khetarpal S, Sweet P, Yatsenko AN, Pollock N, et al. Familial deletion of the HOXA gene cluster associated with Hand-Foot-Genital syndrome and phenotypic variability. *Am J Med Genet A*. 2017;173(1):221–224.
146. Yokoyama E, Smith-Pellegrin DL, Sánchez S, Molina B, Rodríguez A, Juárez R, et al. 7p15 deletion as the cause of hand-foot-genital syndrome: a case report, literature review and proposal of a minimum region for this phenotype. *Mol Cytogenet*. 2017;10:42.
147. Simpson MA, Deshpande C, Dafou D, Vissers LE, Woollard WJ, Holder SE, et al. De novo mutations of the gene encoding the histone acetyltransferase KAT6B cause Genitopatellar syndrome. *Am J Hum Genet*. 2012;90(2):290–294.
148. Bashamboo A, Eozenou C, Rojo S, McElreavey K. Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development. *Clin Genet*. 2017;91(2):143–156.
149. Zhao F, Franco HL, Rodríguez KF, et al. Elimination of the male reproductive tract in the female embryo is promoted by COUP-TFII in mice. *Science*. 2017;357(6352):717–720.
150. Radi O, Parma P, Imbeaud S, Nasca MR, Uccellatore F, Maraschio P, Tiepolo L, et al. XX sex reversal, palmoplantar keratoderma, and predisposition to squamous cell carcinoma: genetic analysis in one family. *Am J Med Genet A*. 2005;138A(3):241–246.
151. Tallapaka K, Venugopal V, Dalal A, Aggarwal S. Novel RSPO1 mutation causing 46,XX testicular disorder of sex development with palmoplantar keratoderma: A review of literature and expansion of clinical phenotype. *Am J Med Genet A*. 2018;176(4): 1006–1010.
152. Narumi S, Amano N, Ishii T, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. *Nat Genet*. 2016;48(7): 792–797.
153. Shima H, Hayashi M, Tachibana T, Oshiro M, Amano N, Ishii T, et al. MIRAGE syndrome is a rare cause of 46,XY DSD born SGA without adrenal insufficiency. *PLoS One*. 2018;13(11). e0206184.
154. Buonocore F, Kühnen P, Suntharalingham JP, Del Valle I, Digweed M, Stachelscheid H, et al. Somatic mutations and progressive monosomy modify SAMD9-related phenotypes in humans. *J Clin Invest*. 2017;127(5):1700–1713.

155. Cameron FJ, Hageman RM, Cooke-Yarborough C, et al. A novel germ line mutation in SOX9 causes familial campomelic dysplasia and sex reversal. *Hum Molec Genet.* 1996;5:1625.
156. Chen SY, Lin SJ, Tsai LP, Chou YY. Sex-reversed acampomelic campomelic dysplasia with a homozygous deletion mutation in SOX9 gene. *Urology.* 2012;79(4):908–911.
157. Kim GJ, Sock E, Buchberger A, Just W, Denzer F, Hoepffner W, et al. Copy number variation of two separate regulatory regions upstream of SOX9 causes isolated 46,XY or 46,XX disorder of sex development. *J Med Genet.* 2015;52(4):240–247.
158. Corbani S, Chouery E, Eid B, Jalkh N, Ghoch JA, Mégarbané A. Mild campomelic dysplasia: report on a case and review. *Mol Syndromol.* 2011;1(4):163–168.
159. Bhagavath B, Layman LC, Ullmann R, Shen Y, Ha K, Rehman K, et al. Familial 46,XY sex reversal without campomelic dysplasia caused by a deletion upstream of the SOX9 gene. *Mol Cell Endocrinol.* 2014;393(1-2):1–7.
160. Lecointre C, Pichon O, Hamel A, Heloury Y, Michel-Calemard L, Morel Y, et al. Familial acampomelic form of campomelic dysplasia caused by a 960 kb deletion upstream of SOX9. *Am J Med Genet A.* 2009;149A(6):1183–1189.
161. Galazzi E, Dumino P, Moro M, Guizzardi F, Marazzi N, Sartorio A, et al. Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in Ulnar-Mammary syndrome. *Endocr Connect.* 2018;7(12):1432–1441.
162. Linden H, Williams R, King J, Blair E, Kini U. Ulnar Mammary syndrome and TBX3: expanding the phenotype. *Am J Med Genet A.* 2009;149A(12):2809–2812.
163. Denys P, Malvaux P, Van Den Berghe H, Tanghe W, Proesmans W. Association of an anatomo-pathological syndrome of male pseudohermaphroditism, Wilms' tumor, parenchymatous nephropathy and XX/XY mosaicism. *Arch Fr Pédiatr.* 1967;24(7):729–739.
164. Drash A, Sherman F, Hartmann WH, Blizzard RM. A syndrome of pseudohermaphroditism, Wilms' tumor, hypertension, and degenerative renal disease. *J Pediatr.* 1970;76(4):585–593.
165. Gomes NL, de Paula LCP, Silva JM, Silva TE, Lerário AM, Nishi MY, et al. A 46,XX testicular disorder of sex development caused by a Wilms' tumour Factor-1 (WT1) pathogenic variant. *Clin Genet.* 95(1), 172–176.
166. Larsson SH, Charliou JP, Miyagawa K, Engelkamp D, Rassoulzadegan M, Ross A, et al. Subnuclear localization of WT1 in splicing or transcription factor domains is regulated by alternative splicing. *Cell.* 1995;81(3):391–401.
167. Miller-Hodges E, Hohenstein P. WT1 in disease: shifting the epithelial-mesenchymal balance. *J Pathol.* 2012;226(2):229–240.
168. Barbosa AS, Hadjiathanasiou CG, Theodoridis C, Papathanasiou A, Tar A, Merksz M, et al. The same mutation affecting the splicing of WT1 gene is present on Frasier syndrome patients with or without Wilms' tumor. *Hum Mutat.* 1999;13(2):146–153.
169. Suri M, Kelehan P, O'Neill D, Vadeyar S, Grant J, Ahmed SF, et al. WT1 mutations in Meacham syndrome suggest a coelomic mesothelial origin of the cardiac and diaphragmatic malformations. *Am J Med Genet A.* 2007;143A(19):2312–2320.
170. Huyhn K, Renfree MB, Graves JA, Pask AJ. ATRX has a critical and conserved role in mammalian sexual differentiation. *BMC Dev Biol.* 2011;11:39.
171. McPherson EW, Clemens MM, Gibbons RJ, et al. X-linked alpha-thalassemia/mental retardation (ATR-X) syndrome: a new kindred with severe genital anomalies and mild hematologic expression. *Am J Med Genet.* 1995;55:302.
172. Tang P, Park DJ, Marshall Graves JA, Harley VR. ATRX and sex differentiation. *Trends Endocrinol Metab.* 2004;15:339.
173. Bouazzi H, Thakur S, Trujillo C, Alwasayah MK, Munnich A. Novel ATRX gene damaging missense mutation c.6740A>C segregates with profound to severe intellectual deficiency without alpha thalassaemia. *Indian J Med Res.* 2016;143(1):43–48.
174. Wada T, Kubota T, Fukushima Y, et al. Molecular genetic study of Japanese patients with X-linked alpha-thalassemia/mental retardation syndrome (ATR-X). *Am J Med Genet.* 2000;94:242.
175. De La Fuente R, Baumann C, Viveiros MM. Role of ATRX in chromatin structure and function: implications for chromosome instability and human disease. *Reproduction.* 2011;142(2):221–234.
176. O'Shea LC, Daly E, Hensley C, Fair T. ATRX is a novel progesterone-regulated protein and biomarker of low developmental potential in mammalian oocytes. *Reproduction.* 2017;153(5):671–682.
177. Dyer MA, Qadeer ZA, Valle-Garcia D, Bernstein E. ATRX and DAXX: mechanisms and mutations. *Cold Spring Harb Perspect Med.* 2017;7(3). pii: a026567.
178. Gibbons RJ, McDowell TL, Raman S, et al. Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. *Nat Genet.* 2000;24(4):368–371.
179. O'Shea LC, Fair T, Hensley C. X-linked α -thalassemia with mental retardation is downstream of protein kinase A in the meiotic cell cycle signaling cascade in *Xenopus* oocytes and is dynamically regulated in response to DNA damage. *Biol Reprod.* 2019;100(5):1238–1249.
180. Harrison SM, Campbell IM, Keays M, Granberg CF, Villanueva C, Tannin G, et al. Screening and familial characterization of copy-number variations in NR5A1 in 46,XY disorders of sex development and premature ovarian failure. *Am J Med Genet A.* 2013;161A(10):2487–2494.
181. Takasawa K, Igarashi M, Ono M, Takemoto A, Takada S, Yamataka A, et al. Phenotypic variation in 46,XX disorders of sex development due to the NR5A1 p.R92W variant: a sibling case report and literature review. *Sex Dev.* 2017;11(5-6):284–288.
182. Knarston IM, Robevska G, van den Bergen JA, Eggers S, Croft B, Yates J, et al. NR5A1 gene variants repress the ovarian-specific WNT signaling pathway in 46,XX disorders of sex development patients. *Hum Mutat.* 2019;40(2):207–216.
183. Baetens D, Stoop H, Peelman F, Todeschini AL, Rosseel T, Coppieters F, et al. NR5A1 is a novel disease gene for 46,XX testicular and ovotesticular disorders of sex development. *Genet Med.* 2017;19(4):367–376.
184. Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. *J Clin Endocrinol Metab.* 2016;101(1):284–292.
185. Swartz JM, Ciarlo R, Guo MH, Abrha A, Weaver B, Diamond DA, et al. A 46,XX ovotesticular disorder of sex development likely caused by a steroidogenic factor-1 (NR5A1) variant. *Horm Res Paediatr.* 2017;87(3):191–195.
186. Bashamboo A, Donohoue PA, Vilain E, Rojo S, Calvel P, Seneviratne SN, et al. A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. *Hum Mol Genet.* 2016;25(16):3446–3453.
187. Camats N, Fernández-Cancio M, Audí L, Schaller A, Flück CE. Broad phenotypes in heterozygous NR5A1 46,XY patients with a disorder of sex development: an oligogenic origin? *Eur J Hum Genet.* 2018;26(9):1329–1338.
188. Portnoi MF, Dumargne MC, Rojo S, Witchel SF, Duncan AJ, Eozenou C, et al. Mutations involving the SRY-related gene SOX8 are associated with a spectrum of human reproductive anomalies. *Hum Mol Genet.* 2018;27(7):1228–1240.
189. Sproll P, Eid W, Gomes CR, Mendonca BB, Gomes NL, Costa EM, Biason-Laubert A. Assembling the jigsaw puzzle: CBX2 isoform 2 and its targets in disorders/differences of sex development. *Mol Genet Genomic Med.* 2018;6(5):785–795.
190. Paris F, Flatters D, Caburet S, Legois B, Servant N, Lefebvre H, et al. A novel variant of DHH in a familial case of 46,XY disorder of sex development: Insights from molecular dynamics simulations. *Clin Endocrinol (Oxf).* 2017;87(5):539–544.
191. Ayers K, van den Bergen J, Robevska G, Listyasaki N, Raza J, Atta I, et al. Functional analysis of novel desert hedgehog gene variants improves the clinical interpretation of genomic data and provides a more accurate diagnosis for patients with 46,XY differences of sex development. *J Med Genet.* 2019;56(7):434–443.
192. Parmantier E, Lynn B, Lawson D, Turmaine M, Namini SS, Chakrabarti L, et al. Schwann cell-derived Desert hedgehog controls the development of peripheral nerve sheaths. *Neuron.* 1999;23(4):713–724.
193. Umehara F, Tate G, Itoh K, et al. A novel mutation of desert hedgehog in a patient with 46,XY partial gonadal dysgenesis accompanied by minifascicular neuropathy. *Am J Hum Genet.* 2000;67:1302.
194. Sato NS, Maekawa R, Ishiura H, Mitsui J, Naruse H, Tokushige SI, et al. Partial duplication of DHH causes minifascicular neuropathy: a novel mutation detection of DHH. *Ann Clin Transl Neurol.* 2017;4(6):415–421.

195. Werner R, Merz H, Birnbaum W, Marshall L, Schröder T, Reiz B, et al. 46,XY gonadal dysgenesis due to a homozygous mutation in desert hedgehog (DHH) identified by exome sequencing. *J Clin Endocrinol Metab.* 2015;100(7):E1022–E1029.
196. Muroya K, Okuyama T, Goishi K, Ogiso Y, Fukuda S, Kameyama J, et al. Sex-determining gene(s) on distal 9p: clinical and molecular studies in six cases. *J Clin Endocrinol Metab.* 2000;85(9):3094–3100.
197. Ounap K, Uibo O, Zordania R, et al. Three patients with 9p deletions including DMRT1 and DMRT2: a girl with XY complement, bilateral ovotestes, and extreme growth retardation, and two XX females with normal pubertal development. *Am J Med Genet A.* 2004;130A(4):415–423.
198. Marsudi BA, Kartapradja H, Paramayuda C, Batubara JRL, Harahap AR, Marzuki NS. Loss of *DMRT1* gene in a Mos 45,XY,-9[8]/46,XY,r(9)[29]/47,XY,+idic r(9)×2[1]/46,XY,idic r(9)[1]/46,XY[1] female presenting with short stature. *Mol Cytogenet.* 2018;11:28.
199. Molkentin JD. The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. *J Biol Chem.* 2000;275(50):38949–38952.
200. Martínez de LaPiscina I, de Mingo C, Riedl S, Rodríguez A, Pandey AV, Fernández-Cancio M, et al. GATA4 variants in individuals with a 46,XY disorder of sex development (dsd) may or may not be associated with cardiac defects depending on second hits in other DSD genes. *Front Endocrinol (Lausanne).* 2018;9:142.
201. Lourenço D, Brauner R, Rybczynska M, Nihoul-Fékété C, McElreavey K, Bashamboo A. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. *Proc Natl Acad Sci U S A.* 2011;108(4):1597–1602.
202. Laporte J, Kioschis P, Hu LJ, Kretz C, Carlsson B, Poustka A, et al. Cloning and characterization of an alternatively spliced gene in proximal Xq28 deleted in two patients with intersexual genitalia and myotubular myopathy. *Genomics.* 1997;41(3):458–462.
203. Ogata T, Laporte J, Fukami M. MAMLD1 (CXorf6): a new gene involved in hypospadias. *Horm Res.* 2009;71(5):245–252.
204. Camats N, Fernández-Cancio M, Audí L, Mullis PE, Moreno F, González Casado I, et al. Human MAMLD1 gene variations seem not sufficient to explain a 46,XY DSD phenotype. *PLoS One.* 2015;10(11):e0142831.
205. Miyado M, Nakamura M, Miyado K, Morohashi K, Sano S, Nagata E, et al. Maml1 deficiency significantly reduces mRNA expression levels of multiple genes expressed in mouse fetal Leydig cells but permits normal genital and reproductive development. *Endocrinology.* 2012;153(12):6033–6040.
206. Miyado M, Yoshida K, Miyado K, Katsumi M, Saito K, Nakamura S, et al. Knockout of murine Maml1 impairs testicular growth and daily sperm production but permits normal postnatal androgen production and fertility. *Int J Mol Sci.* 2017;18(6):pii: E1300.
207. Pearlman A, Loke J, Le Caignec C, White S, Chin L, Friedman A, et al. Mutations in MAP3K1 cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination. *Am J Hum Genet.* 2010;87(6):898–904.
208. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, et al. MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. *Am J Med Genet C Semin Med Genet.* 2017;175(2):253–259.
209. Le Caignec C, Baron S, McElreavey K, et al. 46,XY gonadal dysgenesis: evidence for autosomal dominant transmission in a large kindred. *Am J Med Genet A.* 2003;116A(1):37–43.
210. Chamberlin A, Huether R, Machado AZ, Groden M, Liu HM, Upadhyay K, et al. Mutations in MAP3K1 that cause 46,XY disorders of sex development disrupt distinct structural domains in the protein. *Hum Mol Genet.* 2019;28(10):1620–1628.
211. Loke J, Pearlman A, Radi O, Zuffardi O, Giussani U, Pallotta R, et al. Mutations in MAP3K1 tilt the balance from SOX9/FGF9 to WNT/ β -catenin signaling. *Hum Mol Genet.* 2014;23(4):1073–1083.
212. Selleri L, Zappavigna V, Ferretti E. 'Building a perfect body': control of vertebrate organogenesis by PBX-dependent regulatory networks. *Genes Dev.* 2019;33(5-6):258–275.
213. Le Tanno P, Breton J, Bidart M, Satre V, Harbuz R, et al. PBX1 haploinsufficiency leads to syndromic congenital anomalies of the kidney and urinary tract (CAKUT) in humans. *J Med Genet.* 2017;54(7):502–510.
214. Slavotinek A, Risolino M, Losa M, et al. De novo, deleterious sequence variants that alter the transcriptional activity of the homeoprotein PBX1 are associated with intellectual disability and pleiotropic developmental defects. *Hum Mol Genet.* 2017;26(24):4849–4860.
215. Eozenou C, Bashamboo A, Bignon-Topalovic J, Merel T, Zwermann O, Lourenco D, et al. The TALE homeodomain of PBX1 is involved in human primary testis-determination. *Hum Mutat.* 2019;40(8):1071–1076.
216. Guran T, Yesil G, Turan S, Atay Z, Bozkurtlar E, Aghayev A, et al. PPP2R3C gene variants cause syndromic 46,XY gonadal dysgenesis and impaired spermatogenesis in humans. *Eur J Endocrinol.* 2019;180(5):291–309.
217. Zhao L, Koopman P. SRY protein function in sex determination: thinking outside the box. *Chromosome Res.* 2012;20(1):153–162.
218. Schmitt-Ney M, Thiele H, Kaltwasser P, Bardoni B, Cisternino M, Scherer G. Two novel SRY missense mutations reducing DNA binding identified in XY females and their mosaic fathers. *Am J Hum Genet.* 1995;56(4):862–869.
219. Hines RS, Tho SP, Zhang YY, et al. Paternal somatic and germ-line mosaicism for a sex-determining region on Y (SRY) missense mutation leading to recurrent 46,XY sex reversal. *Fertil Steril.* 1997;67(4):675–679.
220. Hawkins JR, Taylor A, Goodfellow PN, Migeon CJ, Smith KD, Berkovitz GD. Evidence for increased prevalence of SRY mutations in XY females with complete rather than partial gonadal dysgenesis. *Am J Hum Genet.* 1992;51(5):979–984.
221. Jordan BK, Jain M, Natarajan S, Frasier SD, Vilain E. Familial mutation in the testis-determining gene SRY shared by an XY female and her normal father. *J Clin Endocrinol Metab.* 2002;87(7):3428–3432.
222. Filges I, Kunz C, Miny P, Boesch N, Szinnai G, Wenzel F, et al. A novel missense mutation in the high mobility group domain of SRY drastically reduces its DNA-binding capacity and causes paternally transmitted 46,XY complete gonadal dysgenesis. *Fertil Steril.* 2011;96(4):851–855.
223. Shahid M, Dhillon VS, Aslam M, Husain SA. Three new novel point mutations localized within and downstream of high-mobility group-box region in SRY gene in three Indian females with Turner syndrome. *J Clin Endocrinol Metab.* 2005;90(4):2429–2435.
224. Shahid M, Dhillon VS, Khalil HS, et al. A SRY-HMG box frame shift mutation inherited from a mosaic father with a mild form of testicular dysgenesis syndrome in Turner syndrome patient. *BMC Med Genet.* 2010;11:131.
225. White S, Hewitt J, Turbitt E, van der Zwan Y, Hersmus R, Drop S, et al. A multi-exon deletion within WWOX is associated with a 46,XY disorder of sex development. *Eur J Hum Genet.* 2012;20(3):348–351.
226. Mallaret M, Synofzik M, Lee J, Sagum CA, Mahajnah M, Sharkia R, et al. The tumour suppressor gene WWOX is mutated in autosomal recessive cerebellar ataxia with epilepsy and mental retardation. *Brain.* 2014;137(Pt 2):411–419.
227. Harris A, Siggers P, Corrochano S, Warr N, Sagar D, Grimes DT, et al. ZNRF3 functions in mammalian sex determination by inhibiting canonical WNT signaling. *Proc Natl Acad Sci U S A.* 2018;115(21):5474–5479.
228. Bashamboo A, Brauner R, Bignon-Topalovic J, Lortat-Jacob S, Karageorgou V, Lourenco D, et al. Mutations in the FOG2/ZFPM2 gene are associated with anomalies of human testis determination. *Hum Mol Genet.* 2014;23(14):3657–3665.
229. Finelli P, Pincelli AI, Russo S, Bonati MT, Recalcatti MP, Masciadri M, et al. Disruption of friend of GATA 2 gene (FOG-2) by a de novo t(8;10) chromosomal translocation is associated with heart defects and gonadal dysgenesis. *Clin Genet.* 2007;71(3):195–204.
230. Wiersma R, Ramdlal PK. The gonads of 111 South African patients with ovotesticular disorder of sex differentiation. *J Pediatr Surg.* 2009;44(3):556–560.
231. Nistal M, Paniagua R, González-Peramato P, Reyes-Múgica M. Perspectives in pediatric pathology, chapter 7. Ovotesticular DSD (true hermaphroditism). *Pediatr Dev Pathol.* 2015;18(5):345–352.

232. Dutta D, Shivaprasad KS, Das RN, et al. Ovotesticular disorder of sexual development due to 47,XXY/46,XY/45,X mixed gonadal dysgenesis in a phenotypic male presenting as cyclical haematuria: clinical presentation and assessment of long-term outcomes. *Andrologia*. 2014;46(2):191–193.
233. Verkauskas G, Jaubert F, Lortat-Jacob S, Malan V, Thibaud E, Nihoul-Fekete C. The long-term followup of 33 cases of true hermaphroditism: a 40-year experience with conservative gonadal surgery. *J Urol*. 2007;177:726.
234. Inoue H, Nomura M, Yanase T, Ichino I, Goto K, Ikuyama S, et al. A rare case of 46,XX true hermaphroditism with hidden mosaicism with sex-determining region Y chromosome-bearing cells in the gonads. *Intern Med*. 1998;37(5):467–471.
235. Ortenberg J, Oddoux C, Craver R, McElreavey K, Salas-Cortes L, Guillen-Navarro E, et al. SRY gene expression in the ovotestes of XX true hermaphrodites. *J Urol*, 167(4), 1828–1831.
236. Jiménez AL, Kofman-Alfaro S, Berumen J, et al. Partially deleted SRY gene confined to testicular tissue in a 46,XX true hermaphrodite without SRY in leukocytic DNA. *Am J Med Genet*. 2000;93(5):417–420.
237. Aleck KA, Argueso L, Stone J, Hackel JG, Erickson RP. True hermaphroditism with partial duplication of chromosome 22 and without SRY. *Am J Med Genet*. 1999;85(1):2–4.
238. Kuhnle U, Schwarz HP, Löhns U, Stengel-Ruthkowski S, Cleve H, Braun A. Familial true hermaphroditism: paternal and maternal transmission of true hermaphroditism (46,XX) and XX maleness in the absence of Y-chromosomal sequences. *Hum Genet*. 1993;92(6):571–576.
239. Slaney SF, Chalmers IJ, Affara NA, Chitty LS. An autosomal or X linked mutation results in true hermaphrodites and 46,XX males in the same family. *J Med Genet*. 1998;35(1):17–22.
240. Ramos ES, Moreira-Filho CA, Vicente YA, Llorach-Velludo MA, Tucci Jr S, Duarte MH, et al. SRY-negative true hermaphrodites and an XX male in two generations of the same family. *Hum Genet*. 1996;97(5):596–598.
241. Alonso G, Pasqualini T, Busaniche J, Ruiz E, Chemes H. True hermaphroditism in a phenotypic male without ambiguous genitalia: an unusual presentation at puberty. *Horm Res*. 2007;68:261.
242. Roth JD, Haddad NG, Albright EA, Cheng L, Rink RC, Kaefer M. An ovulating follicle presenting as a testicular mass in a teenage patient with ovotesticular DSD. *Urol Case Rep*. 2018;18:26–28.
243. Sugawara N, Kimura Y, Araki Y. Successful second delivery outcome using refrozen thawed testicular sperm from an infertile male true hermaphrodite with a 46, XX/46, XY karyotype: case report. *Hum Cell*. 2012;25(4):96–99.
244. Younis JS, Radin O, Kerner H, Ben-Ami M. Successful monozygotic twin pregnancy fathered by a male 46,XY true hermaphrodite. *Reprod Biomed Online*. 2011;22(1):80–82.
245. Schultz BA, Roberts S, Rodgers A, Ataya K. Pregnancy in true hermaphrodites and all male offspring to date. *Obstet Gynecol*. 2009;113(2 Pt 2):534–536.
246. Schoenhaus SA, Lentz SE, Saber P, Munro MG, Kivnick S. Pregnancy in a hermaphrodite with a male-predominant mosaic karyotype. *Fertil Steril*. 2008;90(5):e7–e10. 2016.
247. de la Chapelle A, Hastbacka J, Korhonen T, Maenpää J. The etiology of XX sex reversal. *Reprod Nutr Dev*. 1990;1:39S.
248. Majzoub A, Arafa M, Starks C, Elbardisi H, Al Said S, Sabanegh E. 46 XX karyotype during male fertility evaluation; case series and literature review. *Asian J Androl*. 2017;19(2):168–172.
249. McElreavey K, Cortes LS. X-Y translocations and sex differentiation. *Semin Reprod Med*. 2001;19(2):133–139.
250. Dauwerse JG, Hansson KB, Brouwers AA, Peters DJ, Breuning MH. An XX male with the sex-determining region Y gene inserted in the long arm of chromosome 16. *Fertil Steril*. 2006;86(2):e1–e5. 463.
251. Grinspon RP, Rey RA. Disorders of sex development with testicular differentiation in SRY-negative 46,XX individuals: clinical and genetic aspects. *Sex Dev*. 2016;10(1):1–11.
252. Huang WJ, Yen PH. Genetics of spermatogenic failure. *Sex Dev*. 2008;2(4-5):251–259.
253. Cox JJ, Willatt L, Homfray T, Woods CG. A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med*. 2011;364(1):91–93.
254. Hyon C, Chantot-Bastarud S, Harbuz R, Bhouri R, Perrot N, Peycelon M, et al. Refining the regulatory region upstream of SOX9 associated with 46,XX testicular disorders of Sex Development (DSD). *Am J Med Genet A*, 167A(8), 1851–1858.
255. Falah N, Posey JE, Thorson W, Benke P, Tekin M, Tarshish B, Lupski JR, Harel T. 22q11.2q13 duplication including SOX10 causes sex-reversal and peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease. *Am J Med Genet A*. 2017;173(4):1066–1070.
256. Rizzoti K, Brunelli S, Carmignac D, Thomas PQ, Robinson IC, Lovell-Badge R. SOX3 is required during the formation of the hypothalamo-pituitary axis. *Nat Genet*. 2004;36(3):247–255.
257. Grinspon RP, Nevado J, Mori Alvarez Mde L, Del Rey G, Castera R, Venara M, et al. 46,XX ovotesticular DSD associated with a SOX3 gene duplication in a SRY-negative boy. *Clin Endocrinol (Oxf)*. 2016;85(4):673–675.
258. Moalem S, Babul-Hirji R, Stavropoulos DJ, et al. XX male sex reversal with genital abnormalities associated with a de novo SOX3 gene duplication. *Am J Med Genet A*. 2012;158A(7):1759–1764.
259. Haines B, Hughes J, Corbett M, Shaw M, Innes J, Patel L, et al. Interchromosomal insertional translocation at Xq26.3 alters SOX3 expression in an individual with XX male sex reversal. *J Clin Endocrinol Metab*. 2015;100(5):E815–E820.
260. Sutton E, Hughes J, White S, Sekido R, Tan J, Arboleda V, et al. Identification of SOX3 as an XX male sex reversal gene in mice and humans. *J Clin Invest*. 2011;121(1):328–341.
261. Woods KS, Cundall M, Turton J, Rizzotti K, Mehta A, Palmer R, et al. Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet*, 76(5), 833–849.
262. Jagarlamudi K, Rajkovic A. Oogenesis: transcriptional regulators and mouse models. *Mol Cell Endocrinol*. 2012;356(1-2):31–39.
263. Patiño LC, Walton KL, Mueller TD, Johnson KE, Stocker W, Richani D, et al. BMP15 mutations associated with primary ovarian insufficiency reduce expression, activity, or synergy with GDF9. *J Clin Endocrinol Metab*. 2017;02(3):1009–1019.
264. Mila M, Alvarez-Mora MI, Madrigal I, Rodriguez-Revenga L. Fragile X syndrome: an overview and update of the FMR1 gene. *Clin Genet*. 2018;93(2):197–205.
265. Hunter J, Rivero-Arias O, Angelov A, Kim E, Fotheringham I, Leal J. Epidemiology of fragile X syndrome: a systematic review and meta-analysis. *Am J Med Genet A*. 2014;164A(7):1648–1658.
266. Bayne RA, Martins da Silva SJ, Anderson RA. Increased expression of the FIGLA transcription factor is associated with primordial follicle formation in the human fetal ovary. *Mol Hum Reprod*. 2004;10(6):373–381.
267. Bouilly J, Bachelot A, Broutin I, Touraine P, Binart N. Novel NOBOX loss-of-function mutations account for 6.2% of cases in a large primary ovarian insufficiency cohort. *Hum Mutat*. 2011;32(10):1108–1113.
268. França MM, Funari MFA, Lerario AM, et al. A novel homozygous 1-bp deletion in the NOBOX gene in two Brazilian sisters with primary ovarian failure. *Endocrine*. 2017;58(3):442–447.
269. Zhao H, Chen ZJ, Qin Y, Shi Y, Wang S, Choi Y, et al. Transcription factor FIGLA is mutated in patients with premature ovarian failure. *Am J Hum Genet*. 2008;82(6):1342–1348.
270. Chen B, Li L, Wang J, Li T, Pan H, Liu B, et al. Consanguineous familial study revealed biallelic FIGLA mutation associated with premature ovarian insufficiency. *J Ovarian Res*. 2018;11(1):48.
271. Ren Y, Diao F, Katari S, et al. Functional study of a novel missense single-nucleotide variant of NUP107 in two daughters of Mexican origin with premature ovarian insufficiency. *Mol Genet Genomic Med*. 2018;6(2):276–281.
272. Weinberg-Shukron A, Renbaum P, Kalifa R, Zeligson S, Ben-Neriah Z, Dreifuss A, et al. A mutation in the nucleoporin-107 gene causes XX gonadal dysgenesis. *J Clin Invest*. 2015;125(11):4295–4304.
273. Antic T, Hyjek EM, Taxy JB. The vanishing testis: a histomorphologic and clinical assessment. *Am J Clin Pathol*. 2011;136(6):872–880.
274. Law H, Mushtaq I, Wingrove K, Malone M, Sebire NJ. Histopathological features of testicular regression syndrome: Relation to patient age and implications for management. *Fetal Pediatr Pathol*. 2006;25:119.
275. Dangle P, Salgado C, Reyes-Mugica M, Schneck F, Ost M, Sims-Lucas S. Testicular hypoplasia is driven by defective vascular formation. *Urology*. 2017;101:94–98.

276. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;32(1):81–151.
277. Penning TM. The aldo-keto reductases (AKRs): overview. *Chem Biol Interact.* 2015;234:236–246.
278. Wilson JD, Auchus RJ, Leihy MW, Guryev OL, Estabrook RW, Osborn SM, et al. 5alpha-androstane-3alpha,17beta-diol is formed in tammar wallaby pouch young testes by a pathway involving 5alpha-pregnane-3alpha,17alpha-diol-20-one as a key intermediate. *Endocrinology.* 2003;144(2):575–580.
279. Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab.* 2004;15(9):432–438.
280. Turcu AF, Nanba AT, Auchus RJ. The rise, fall, and resurrection of 11-oxygenated androgens in human physiology and disease. *Horm Res Paediatr.* 2018;89(5):284–291.
281. Campana C, Rege J, Turcu AF, et al. Development of a novel cell based androgen screening model. *J Steroid Biochem Mol Biol.* 2016;156:17–22.
282. Flück CE, Meyer-Böni M, Pandey AV, et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet.* 2011;89(2):201–218.
283. O'Shaughnessy PJ, Antignac JP, Le Bizec B, Morvan ML, Svechnikov K, Söder O, et al. Alternative (backdoor) androgen production and masculinization in the human fetus. *PLoS Biol.* 2019;17(2). e3000002.
284. Voutilainen R, Miller WL. Developmental expression of genes for the steroidogenic enzymes P450sc (20,22-desmolase), P450c17 (17 alpha-hydroxylase/17,20-lyase), and P450c21 (21-hydroxylase) in the human fetus. *J Clin Endocrinol Metab.* 1986;63(5):1145–1150.
285. Escobar JC, Patel SS, Beshay VE, Suzuki T, Carr BR. The human placenta expresses CYP17 and generates androgens de novo. *J Clin Endocrinol Metab.* 2011;96(5):1385–1392.
286. Kremer H, Kraaij R, Toledo SPA, et al. Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet.* 1995;9:160.
287. Latronico AC, Arnhold JJ. Inactivating mutations of the human luteinizing hormone receptor in both sexes. *Semin Reprod Med.* 2012;30(5):382–386.
288. Richter-Unruh A, Korsch E, Hiort O, Holterhus PM, Themmen AP, Wudy SA. Novel insertion frameshift mutation of the LH receptor gene: problematic clinical distinction of Leydig cell hypoplasia from enzyme defects primarily affecting testosterone biosynthesis. *Eur J Endocrinol.* 2005;152:255.
289. Yan M, Dilihumu J, Luo Y, Reyilanmu B, Shen Y, Mireguli M. Novel compound heterozygous variants in the LHCGR gene in a genetically male patient with female external genitalia. *J Clin Res Paediatr Endocrinol.* 2019;11(2):211–217.
290. Lin D, Sugawara T, Strauss 3rd JF, Clark BJ, Stocco DM, Saenger P, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science.* 1995;267(5205):1828–1831.
291. Bose HS, Sugawara T, Strauss JF, 3rd, Miller WL. International Congenital Lipoid Adrenal Hyperplasia Consortium. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med.* 1996;335(25):1870–1878.
292. Miller WL. Disorders in the initial steps of steroid hormone synthesis. *J Steroid Biochem Mol Biol.* 2017;165(Pt A):18–37.
293. Mizuno Y, Ishii T, Hasegawa T. In vivo verification of the pathophysiology of lipoid congenital adrenal hyperplasia in the adrenal cortex. *Endocrinology.* 2019;160(2):331–338.
294. Hatabu N, Amano N, Mori J, Hasegawa Y, Matsuura H, Sumitomo N, et al. Pubertal development and pregnancy outcomes in 46,XX patients with nonclassic lipoid congenital adrenal hyperplasia. *J Clin Endocrinol Metab.* 2019;104(5):1866–1870.
295. Albarel F, Perrin J, Jegaden M, et al. Successful IVF pregnancy despite inadequate ovarian steroidogenesis due to congenital lipoid adrenal hyperplasia (CLAH): a case report. *Hum Reprod.* 2016;31(11):2609–2612.
296. Ishii T, Hori N, Amano N, Aya M, Shibata H, Katsumata N, Hasegawa T. Pubertal and adult testicular functions in nonclassic lipoid congenital adrenal hyperplasia: a case series and review. *J Endocr Soc.* 2019;3(7):1367–1374.
297. Metherell LA, Naville D, Halaby G, Begeot M, Huebner A, Nürnberg G, et al. Nonclassic lipoid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab.* 2009;94(10):3865–3871.
298. Tee MK, Abramssohn M, Loewenthal N, Harris M, Siwach S, Kaplinsky A, et al. Varied clinical presentations of seven patients with mutations in CYP11A1 encoding the cholesterol side-chain cleavage enzyme, P450sc. *J Clin Endocrinol Metab.* 2013;98(2):713–720.
299. Kolli V, Kim H, Torky A, Lao Q, Tatsi C, Mallappa A, Merke DP. Characterization of the CYP11A1 nonsynonymous variant p. E314K in children presenting with adrenal insufficiency. *J Clin Endocrinol Metab.* 2019;104(2):269–276.
300. Maharaj A, Buonocore F, Meimaridou E, Ruiz-Babot G, Guasti L, Peng HM, et al. Predicted benign and synonymous variants in CYP11A1 cause primary adrenal insufficiency through missplicing. *J Endocr Soc.* 2018;3(1):201–221.
301. Gucev ZS, Tee MK, Chitayat D, Wherrett DK, Miller WL. Distinguishing deficiencies in the steroidogenic acute regulatory protein and the cholesterol side chain cleavage enzyme causing neonatal adrenal failure. *J Pediatr.* 2013;162(4):819–822.
302. El-Maouche D, Arlt W, Merke DP. Congenital adrenal hyperplasia. *Lancet.* 2017;390(10108):2194–2210.
303. Witchel SF. Congenital adrenal hyperplasia. *J Pediatr Adolesc Gynecol.* 2017;30(5):520–534.
304. Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab.* 2004;15(9):432–438.
305. Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative “backdoor” pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab.* 2012;97(3):E367–E375.
306. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;32(1):81–151.
307. Thil'en A, Nordenström A, Hagenfeldt L, von Döbeln U, Guthenberg C, Larsson A. Benefits of neonatal screening for congenital adrenal hyperplasia (21-hydroxylase deficiency) in Sweden. *Pediatrics.* 1998;101(4). E11.
308. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103(11):4043–4088.
309. Prader A. Der genitalbefund beim pseudohermaphroditismus femininus der kengenitalen adrenogenitalen syndroms. *Helv Paediatr Acta.* 1954;9:231.
310. Grumbach MM, Ducharme JR. The effects of androgens on fetal sexual development: Androgen-induced female pseudohermaphroditism. *Fertil Steril.* 1960;11:157.
311. Witchel SF, Nayak S, Suda-Hartman M, Lee PA. Newborn screening for 21-hydroxylase deficiency: results of CYP21 molecular genetic analysis. *J Pediatr.* 1997;131(2):328–331.
312. Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative “backdoor” pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab.* 2012;97(3):E367–E375.
313. Miller WL, Merke DP. Tenascin-X, Congenital Adrenal Hyperplasia, and the CAH-X Syndrome. *Horm Res Paediatr.* 2018;89(5):352–361.
314. Concolino P, Costella A. Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency: a comprehensive focus on 233 pathogenic variants of CYP21A2 gene. *Mol Diagn Ther.* 2018;22(3):261–280.
315. Hannah-Shmouni F, Chen W, Merke DP. Genetics of congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am.* 2017;46(2):435–458.
316. Rösler A, Leiberman E, Cohen T. High frequency of congenital adrenal hyperplasia (classic 11 beta-hydroxylase deficiency) among Jews from Morocco. *Am J Med Genet.* 1992;42(6):827–834.
317. Reisch N, Högler W, Parajes S, Rose IT, Dhir V, Göttinger J, Arlt W, Krone N. A diagnosis not to be missed: nonclassic steroid 11β-hydroxylase deficiency presenting with premature

- adrenarche and hirsutism. *J Clin Endocrinol Metab.* 2013;98(10):E1620–E1625.
318. Tee MK, Miller WL. Phosphorylation of human cytochrome P450c17 by p38 α selectively increases 17,20 lyase activity and androgen biosynthesis. *Biol Chem.* 2013;288(33):23903–23913.
 319. Miller WL. The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab.* 2012;97(1):59–67.
 320. Kok RC, Timmerman MA, Wolffbuttel KP, Drop SL, de Jong FH. Isolated 17,20-lyase deficiency due to the cytochrome b5 mutation W27X. *J Clin Endocrinol Metab.* 2010;95(3):994–999.
 321. Idkowiak J, Randell T, Dhir V, Patel P, Shackleton CH, Taylor NF, et al. A missense mutation in the human cytochrome b5 gene causes 46,XY disorder of sex development due to true isolated 17,20 lyase deficiency. *J Clin Endocrinol Metab.* 2012;97(3):E465–E475.
 322. Flück CE, Meyer-Böni M, Pandey AV, et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet.* 2011;89(2):201–218.
 323. Shackleton C, Marcos J, Arlt W, Hauffa BP. Prenatal diagnosis of P450 oxidoreductase deficiency (ORD): a disorder causing low pregnancy estriol, maternal and fetal virilization, and the Antley-Bixler syndrome phenotype. *Am J Med Genet A.* 2004;129A(2):105–112.
 324. Reisch N, Idkowiak J, Hughes BA, Ivison HE, Abdul-Rahman OA, Hendon LG, et al. Prenatal diagnosis of congenital adrenal hyperplasia caused by P450 oxidoreductase deficiency. *J Clin Endocrinol Metab.* 2013;98(3):E528–E536.
 325. Flück CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet.* 2004;36(3):228–230.
 326. Porter FD, Herman GE. Malformations syndromes caused by disorders of cholesterol synthesis. *J Lipid Res.* 2011;52(1):6–34.
 327. Saez JM, De Peretti E, Morera AM, David M, Bertrand J. Familial male pseudohermaphroditism with gynecomastia due to a testicular 17-ketosteroid reductase defect. *I Studies in vivo J Clin Endocrinol Metab.* 1971;32(5):604–610.
 328. Mendonca BB, Gomes NL, Costa EM, Inacio M, Martin RM, Nishi MY, et al. 46,XY disorder of sex development (DSD) due to 17 β -hydroxysteroid dehydrogenase type 3 deficiency. *J Steroid Biochem Mol Biol.* 2017;165(Pt A):79–85.
 329. Mendonca BB, Arnhold IJ, Bloise W, Andersson S, Russell DW, Wilson JD. 17 β -hydroxysteroid dehydrogenase 3 deficiency in women. *J Clin Endocrinol Metab.* 1999;84(2):802–804.
 330. Abacı A, Çatlı G, Kırbayık Ö, Şahin NM, Abalı ZY, Ünal E, et al. Genotype-phenotype correlation, gonadal malignancy risk, gender preference, and testosterone/dihydrotestosterone ratio in steroid 5 α -reductase type 2 deficiency: a multicenter study from Turkey. *J Endocrinol Invest.* 2019;42(4):453–470.
 331. Mendonca BB, Batista RL, Domenice S, Costa EM, Arnhold IJ, Russell DW, Wilson JD. Steroid 5 α -reductase 2 deficiency. *J Steroid Biochem Mol Biol.* 2016;163:206–211.
 332. Marumudi E, Ammini AC. Fertility in patients with 5 α -reductase-2 deficiency. *Fertil Steril.* 2011;95(7):e45.
 333. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab.* 1999;84(12):4677–4694.
 334. Brinkman AO, Faber PW, van Rooy HJC, et al. The human androgen receptor: Domain structure, genomic organization and regulation of expression. *J Steroid Biochem Molec Biol.* 1989;34:307–310.
 335. Davey RA, Grossmann M. Androgen receptor structure, function and biology: from bench to bedside. *Clin Biochem Rev.* 2016;37(1):3–15.
 336. Hughes IA, Davies JD, Bunch TI, Pasterski V, Mastroyannopoulou K, Macdougall J. Androgen insensitivity syndrome. *Lancet.* 2012;380(9851):1419–1428.
 337. Kohler B, Lumbroso S, Leger J, Audran F, Grau ES, Kurtz F, et al. Androgen insensitivity syndrome: Somatic mosaicism of the androgen receptor in seven families and consequences for sex assignment and genetic counseling. *J Clin Endocrinol Metab.* 2005;90:106.
 338. Bouvattier C, Carel JC, Lecoindre C, David A, Sultan C, Bertrand AM, et al. Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab.* 2002;87:29.
 339. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M. The androgen receptor gene mutations database: 2012 update. *Hum Mutat.* 2012;33(5):887–894.
 340. Ahmad Z, Xing C, Panach K, Kittler R, McPhaul MJ, Wilson JD. Identification of the underlying androgen receptor defect in the Dallas Reifenshtein family. *J Endocr Soc.* 2017;1(7):836–842.
 341. Hornig NC, de Beaufort C, Denzer F, Cools M, Wabitsch M, Ukata M, et al. A recurrent germline mutation in the 5'UTR of the androgen receptor causes complete androgen insensitivity by activating aberrant uORF Translation. *PLoS One.* 2016;11(4):e0154158.
 342. Känkäkoski J, Jääskeläinen J, Jääskeläinen T, Tommiska J, Saarinen L, Lehtonen R, et al. Complete androgen insensitivity syndrome caused by a deep intronic pseudoexon-activating mutation in the androgen receptor gene. *Sci Rep.* 2016;6:32819.
 343. Askew EB, Bai S, Hnat AT, Minges JT, Wilson EM. Melanoma antigen gene protein-A11 (MAGE-11) F-box links the androgen receptor NH2-terminal transactivation domain to p160 coactivators. *J Biol Chem.* 2009;284(50):34793–34808.
 344. Lagarde WH, Blackwelder AJ, Minges JT, Hnat AT, French FS, Wilson EM. Androgen receptor exon 1 mutation causes androgen insensitivity by creating phosphorylation site and inhibiting melanoma antigen-A11 activation of NH2- and carboxyl-terminal interaction-dependent transactivation. *J Biol Chem.* 2012;287(14):10905–10915.
 345. Breza M, Koutsis G. Kennedy's disease (spinal and bulbar muscular atrophy): a clinically oriented review of a rare disease. *J Neurol.* 2019;266(3):565–573.
 346. Bott LC, Salomons FA, Maric D, Liu Y, Merry D, Fischbeck KH, Dantuma NP. The polyglutamine-expanded androgen receptor responsible for spinal and bulbar muscular atrophy inhibits the APC/C(Cdh1) ubiquitin ligase complex. *Sci Rep.* 2016;6:27703.
 347. Picard JY, Cate RL, Racine C, Josso N. The Persistent Müllerian Duct Syndrome: an update based upon a personal experience of 157 cases. *Sex Dev.* 2017;11(3):109–125.
 348. Ledig S, Wieacker P. Clinical and genetic aspects of Mayer-Rokitansky-Küster-Hauser syndrome. *Med Genet.* 2018;30(1):3–11.
 349. Katsanis N, Beales PL, Woods MO, Lewis RA, Green JS, Parfrey PS, et al. Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. *Nat Genet.* 2000;26(1):67–70.
 350. Schaefer E, Durand M, Stoetzel C, Doray B, Viville B, Hellé S, et al. Molecular diagnosis reveals genetic heterogeneity for the overlapping MKKS and BBS phenotypes. *Eur J Med Genet.* 2011;54(2):157–160.
 351. Williams LS, Demir Eksi D, Shen Y, Lossie AC, Chorich LP, Sullivan ME, et al. Genetic analysis of Mayer-Rokitansky-Küster-Hauser syndrome in a large cohort of families. *Fertil Steril.* 2017;108(1):145–151.e2.
 352. Zhang W, Zhou X, Liu L, Zhu Y, Liu C, Pan H, et al. Identification and functional analysis of a novel LHX1 mutation associated with congenital absence of the uterus and vagina. *Oncotarget.* 2017;8(5):8785–8790.
 353. Philibert P, Biason-Lauber A, Gueorguieva I, Stuckens C, Pienkowski C, Lebon-Labich B, et al. Molecular analysis of WNT4 gene in four adolescent girls with müllerian duct abnormality and hyperandrogenism (atypical Mayer-Rokitansky-Küster-Hauser syndrome). *Fertil Steril.* 2011;95(8):2683–2686.
 354. Brady AF, Winter RM, Wilson LC, Tatnall FM, Sheridan RJ, Garrett C. Hemifacial microsomia, external auditory canal atresia, deafness and Müllerian anomalies associated with acroosteolysis: a new autosomal recessive syndrome? *Clin Dysmorphol.* 2002;11(3):155–161.
 355. Morcel K, Watrin T, Pasquier L, Rochard L, Le Caignec C, Dubourg C, et al. Utero-vaginal aplasia (Mayer-Rokitansky-Küster-Hauser syndrome) associated with deletions in known DiGeorge or DiGeorge-like loci. *Orphanet J Rare Dis.* 2011;6:9.
 356. Li S, Qayyum A, Coakley FV, Hricak H. Association of renal agenesis and müllerian duct anomalies. *J Comput Assist Tomogr.* 2000;24:829.
 357. Boruah DK, Sanyal S, Gogoi BB, Mahanta K, Prakash A, Augustine A, et al. Spectrum of MRI appearance of Mayer-

- Rokitansky-Kuster-Hauser (MRKH) syndrome in primary amenorrhea patients. *J Clin Diagn Res.* 2017;11(7):TC30–TC35.
358. Fiaschetti V, Taglieri A, Gisone V, Coco I, Simonetti G. Mayer-Rokitansky-Kuster-Hauser syndrome diagnosed by magnetic resonance imaging. Role of imaging to identify and evaluate the uncommon variation in development of the female genital tract. *J Radiol Case Rep.* 2012;6(4):17–24.
359. Baskin L. What is hypospadias? *Clin Pediatr (Phila).* 2017;56(5):409–418.
360. Mottet N, Cabrol C, Metz JP, Toubin C, Arbez-Gindre F, Valduga M, et al. Autopsy findings of ectodermal dysplasia and sex development disorder in a fetus with 19q12q13 microdeletion. *Eur J Med Genet.* 2018;103539.
361. Cortes D, Thorup JM, Visfeldt J. Cryptorchidism: aspects of fertility and neoplasms. A study including data of 1,335 consecutive boys who underwent testicular biopsy simultaneously with surgery for cryptorchidism. *Horm Res.* 2001;55(1):21–27.
362. Urh K, Kolenc Ž, Hrovat M, Svet L, Dovč P, Kunej T. Molecular mechanisms of syndromic cryptorchidism: data synthesis of 50 studies and visualization of gene-disease network. *Front Endocrinol (Lausanne).* 2018;9:425.
363. Huff DS, Fenig DM, Canning DA, Carr MG, Zderic SA, Snyder 3rd HM. Abnormal germ cell development in cryptorchidism. *Horm Res.* 2001;55(1):11–17.
364. Barteczko KJ, Jacob MI. The testicular descent in human. Origin, development and fate of the gubernaculum Hunteri, processus vaginalis peritonei, and gonadal ligaments. *Adv Anat Embryol Cell Biol.* 2000;156:1–98. III-X.
365. Bay K, Main KM, Toppari J, Skakkebaek NE. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol.* 2011;8(4):187–196.
366. Lee PA, Coughlin MT. Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone, and semen data. *Horm Res.* 2001;55(1):28–32.
367. Foresta C, Zuccarello D, Garolla A, Ferlin A. Role of hormones, genes, and environment in human cryptorchidism. *Endocr Rev.* 2008;29(5):560–580.
368. Kollin C, Stukenborg JB, Nurmio M, Sundqvist E, Gustafsson T, Söder O, et al. Boys with undescended testes: endocrine, volumetric and morphometric studies on testicular function before and after orchidopexy at nine months or three years of age. *J Clin Endocrinol Metab.* 97(12), 4588–4595.
369. Rohayem J, Luberto A, Nieschlag E, Zitzmann M, Kliesch S. Delayed treatment of undescended testes may promote hypogonadism and infertility. *Endocrine.* 2017;55(3):914–924.
370. Tomboc M, Lee PA, Mitwally MF, Schneck FX, Bellinger M, Witchel SF. Insulin-like 3/relaxin-like factor gene mutations are associated with cryptorchidism. *J Clin Endocrinol Metab.* 2000;85(11):4013–4018.
371. Kolon TF, Wiener JS, Lewitton M, Roth DR, Gonzales Jr ET, Lamb DJ. Analysis of homeobox gene HOXA10 mutations in cryptorchidism. *J Urol.* 1999;161(1):275–280.
372. Ferlin A, Zuccarello D, Garolla A, Selice R, Vinanzi C, Ganz F, et al. Mutations in INSL3 and RXFP2 genes in cryptorchid boys. *Ann N Y Acad Sci.* 2009;1160:213–214.
373. Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab.* 95(6), 2857–2867.
374. Balasubramanian R, Crowley Jr WF. Reproductive endocrine phenotypes relating to CHD7 mutations in humans. *Am J Med Genet C Semin Med Genet.* 2017;175(4):507–515.
375. Maione L, Dwyer AA, Francou B, Guiochon-Mantel A, Binart N, Bouligand J, Young J. Genetics in endocrinology: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenesis and next-generation sequencing. *Eur J Endocrinol.* 2018;178(3):R55–R80.
376. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A.* 2010;107(34):15140–15144.
377. Stillman RJ. In utero exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance and male and female offspring. *Am J Obstet Gynecol.* 1982;142(7):905–921.
378. Troisi R, Hatch EE, Palmer JR, Titus L, Robboy SJ, Strohshitter WC, et al. Prenatal diethylstilbestrol exposure and high-grade squamous cell neoplasia of the lower genital tract. *Am J Obstet Gynecol.* 2016;215(3):322. e1–8.
379. Paris F, Jeandel C, Servant N, Sultan C. Increased serum estrogenic bioactivity in three male newborns with ambiguous genitalia: a potential consequence of prenatal exposure to environmental endocrine disruptors. *Environ Res.* 2006;100(1):39–43.
380. Kalfa N, Philibert P, Baskin LS, Sultan C. Hypospadias: interactions between environment and genetics. *Mol Cell Endocrinol.* 2011;335(2):89–95.
381. Reutter H, Keppler-Noreuil K, Keegan C, Thiele H, Yamada G, Ludwig M. Genetics of Bladder-Exstrophy-Epispadias Complex (BEEC): systematic elucidation of mendelian and multifactorial phenotypes. *Curr Genomics.* 2016;17(1):4–13.
382. Boyadjiev SA, Dodson JL, Radford CL, Ashrafi GH, Beatty TH, Mathews RI, et al. Clinical and molecular characterization of the bladder exstrophy-epispadias complex: analysis of 232 families. *BJU Int.* 2004; 94(9), 1337–1343.
383. Lundin J, Markljung E, Baranowska Körberg I, Hofmeister W, Cao J, et al. Further support linking the 22q11.2 microduplication to an increased risk of bladder exstrophy and highlighting LZTR1 as a candidate gene. *Mol Genet Genomic Med.* 2019;7(6). e666.
384. Ahmed SF, Achermann JC, Arlt W, Balen A, Conway G, Conway G, et al. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). *Clin Endocrinol (Oxf).* 2016; 84(5), 771–788.
385. McNamara ER, Swartz JM, Diamond DA. Initial management of disorders of sex development in newborns. *Urology.* 2017;101:1–8.
386. Nixon R, Cerqueira V, Kyriakou A, Lucas-Herald A, McNeilly J, McMillan M, et al. Prevalence of endocrine and genetic abnormalities in boys evaluated systematically for a disorder of sex development. *Hum Reprod.* 2017;32(10), 2130–2137.
387. Al-Juraibah FN, Lucas-Herald AK, Alimussina M, Ahmed SF. The evaluation and management of the boy with DSD. *Best Pract Res Clin Endocrinol Metab.* 2018;32(4):445–453.
388. Feldman KW, Smith DW. Fetal phallic growth and penile standards for newborn male infants. *J Pediatr.* 1975;86(3):395–398.
389. Oberfield SE, Mondok A, Shahrivar F, Klein JF, Levine LS. Clitoral size in full-term infants. *Am J Perinatol.* 1989;6(4):453–454.
390. Sane K, Pescovitz OH. The clitoral index: a determination of clitoral size in normal girls and in girls with abnormal sexual development. *J Pediatr.* 1992;120(2 Pt 1):264–266.
391. Ahmed SF, Khwaja O, Hughes IA. The role of a clinical score in the assessment of ambiguous genitalia. *BJU Int.* 2000;85(1):120–124.
392. Habeshian K, Fowler K, Gomez-Lobo V, Marathe K. Guidelines for pediatric anogenital examination: Insights from our vulvar dermatology clinic. *Pediatr Dermatol.* 2018;35(5):693–695.
393. Guerra-Junior G, Andrade KC, Barcelos IHK, Maciel-Guerra AT. Imaging techniques in the diagnostic journey of disorders of sex development. *Sex Dev.* 2018;12(1-3):95–99.
394. Yatsenko SA, Witchel SF. Genetic approach to ambiguous genitalia and disorders of sex development: What clinicians need to know. *Semin Perinatol.* 2017;41(4):232–243.
395. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM, et al. The EU COST Action. Genetics in endocrinology: Approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol.* 2018;179(4):R197–R206.
396. Hughes LA, McKay Bounford K, Webb E, Dasani P, Clokie S, Chandran H, et al. Next generation sequencing (NGS) to improve the diagnosis and management of patients with disorders of sex development (DSD). *Endocr Connect.* 2019;8(2):100–110.
397. Baetens D, Mendonça BB, Verdin H, Cools M, De Baere E. Non-coding variation in disorders of sex development. *Clin Genet.* 2017;91(2):163–172.

398. Achermann JC, Domenice S, Bachega TA, Nishi MY, Mendonca BB. Disorders of sex development: effect of molecular diagnostics. *Nat Rev Endocrinol*. 2015;11(8):478–488.
399. Strande NT, Brnich SE, Roman TS, Berg JS. Navigating the nuances of clinical sequence variant interpretation in Mendelian disease. *Genet Med*. 2018;20(9):918–926.
400. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424.
401. Wenger AM, Guturu H, Bernstein JA, Bejerano G. Systematic reanalysis of clinical exome data yields additional diagnoses: implications for providers. *Genet Med*. 2017;19(2):209–214.
402. Botkin JR, Belmont JW, Berg JS, Berkman BE, Bombard Y, Holm IA, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 2015;97(1):6–21.
403. Byers HM, Neufeld-Kaiser W, Chang EY, Tsuchiya K, Oehler ES, Adam MP. Discordant sex between fetal screening and postnatal phenotype requires evaluation. *J Perinatol*. 2019;39(1):28–33.
404. Neufeld-Kaiser WA, Cheng EY, Liu YJ. Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. *BMC Med*. 2015;13:129.
405. Badeau M, Lindsay C, Blais J, Nshimyumukiza L, Takwoingi Y, Langlois S, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. *Cochrane Database Syst Rev*. 2017;11. CD011767.
406. Aksglaede L, Sørensen K, Boas M, Mouritsen A, Hagen CP, Jensen RB, et al. Changes in anti-Müllerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab*. 2010;95(12):5357–5364.
407. Hagen CP, Aksglaede L, Sørensen K, Main KM, Boas M, Cleemann L, et al. Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab*. 2010;95(11):5003–5010.
408. Josso N, Rey RA, Picard JY. Anti-müllerian hormone: a valuable addition to the toolbox of the pediatric endocrinologist. *Int J Endocrinol*. 2013;2013:674105.
409. Bertelloni S, Russo G, Baroncelli GI. Human chorionic gonadotropin test: old uncertainties, new perspectives, and value in 46, XY disorders of sex development. *Sex Dev*. 2018;12(1-3):41–49.
410. Boehmer AL, Brinkmann AO, Sandkuijl LA, Halley DJ, Niermeijer MF, Andersson S, et al. 17Beta-hydroxysteroid dehydrogenase-3 deficiency: diagnosis, phenotypic variability, population genetics, and worldwide distribution of ancient and de novo mutations. *J Clin Endocrinol Metab*. 1999;84(12):4713–4721.
411. White PC. Neonatal screening for congenital adrenal hyperplasia. *Nat Rev Endocrinol*. 2009;5(9):490–498.
412. Witchel SF. Newborn screening for congenital adrenal hyperplasia: beyond 17-hydroxyprogesterone concentrations. *J Pediatr (Rio J)*. 2019;95(3):257–259.
413. White PC. Optimizing newborn screening for congenital adrenal hyperplasia. *J Pediatr*. 2013;163(1):10–12.
414. Pearce M, DeMartino L, McMahon R, Hamel R, Maloney B, Stansfield DM, et al. Newborn screening for congenital adrenal hyperplasia in New York State. *Mol Genet Metab Rep*. 2016;7:1–7.
415. Fiet J, Le Bouc Y, Guéchet J, Hélin N, Maubert MA, Farabos D, Lamazière A. A liquid chromatography/tandem mass spectrometry profile of 16 serum steroids, including 21-deoxycortisol and 21-deoxycorticosterone, for management of congenital adrenal hyperplasia. *J Endocr Soc*. 2017;1(3):186–201.
416. Travers S, Martinier L, Bouvattier C, Boileau P, Lombès M, Pussard E. Multiplexed steroid profiling of gluco- and mineralocorticoids pathways using a liquid chromatography tandem mass spectrometry method. *J Steroid Biochem Mol Biol*. 2017;165(Pt B), 202–211.
417. Held PK, Shapira SK, Hinton CF, Jones E, Hannon WH, Ojodu J. Congenital adrenal hyperplasia cases identified by newborn screening in one- and two-screen states. *Mol Genet Metab*. 2015;116:133–138.
418. Tajima T, Fukushi M. Neonatal mass screening for 21-hydroxylase deficiency. *Clin Pediatr Endocrinol*. 2016;25:1–8.
419. Tieh PY, Yee JK, Hicks RA, Mao CS, Lee WN. Utility of a precursor-to-product ratio in the evaluation of presumptive positives in newborn screening of congenital adrenal hyperplasia. *J Perinatol*. 2017;37:283–287.
420. Kamrath C, Hartmann MF, Boettcher C, Zimmer KP, Wudy SA. Diagnosis of 21-hydroxylase deficiency by urinary metabolite ratios using gas chromatography-mass spectrometry analysis: Reference values for neonates and infants. *J Steroid Biochem Mol Biol*. 2016;156:10–16.
421. Miller WL. Congenital adrenal hyperplasia: time to replace 17OHP with 21-Deoxycortisol. *Horm Res Paediatr*. 2019;91(6):416–420.
422. Ernst MM, Liao LM, Baratz AB, Sandberg DE. Disorders of sex development/intersex: gaps in psychosocial care for children. *Pediatrics*. 2018;142(2). pii: e20174045.
423. Zainuddin AA, Mahdy ZA. The Islamic perspectives of gender-related issues in the management of patients with disorders of sex development. *Arch Sex Behav*. 2017;46(2):353–360.
424. Liao LM, Green H, Creighton SM, Crouch NS, Conway GS. Service users' experiences of obtaining and giving information about disorders of sex development. *BJOG*. 2010;117(2):193–199.
425. Wilson JD, Rivarola MA, Mendonca BB, et al. Advice on the management of ambiguous genitalia to a young endocrinologist from experienced clinicians. *Semin Reprod Med*. 2012;30(5):339–350.
426. Islam R, Lane S, Williams SA, Becker CM, Conway GS, Creighton SM. Establishing reproductive potential and advances in fertility preservation techniques for XY individuals with differences in sex development. *Clin Endocrinol (Oxf)*. 2019;91(2):237–244.
427. Finney EL, Johnson EK, Chen D, Lockart BA, Yerkes EB, Rowell EE, et al. Gonadal tissue cryopreservation for a girl with partial androgen insensitivity syndrome. *J Endocr Soc*. 2019;3(5):887–891.
428. Timmermans S, Yang A, Gardner M, Keegan CE, Yashar BM, Fechner PY, et al. Gender destinies: assigning gender in Disorders of Sex Development-Intersex clinics. *Sociol Health Illn*. 2019;41(8):1520–1534.
429. Delozier AM, Gamwell KL, Sharkey C, Bakula DM, Perez MN, Wolfe-Christensen C, et al. Uncertainty and posttraumatic stress: differences between mothers and fathers of infants with disorders of sex development. *Arch Sex Behav*. 2019;48(5):1617–1624.
430. Słowikowska-Hilczner J, Hirschberg AL, Claahsen-van der Grinten H, Reisch N, Bouvattier C, Thyen U, et al. dsd-LIFE Group. Fertility outcome and information on fertility issues in individuals with different forms of disorders of sex development: findings from the dsd-LIFE study. *Fertil Steril*. 2017;108(5):822–831.
431. Rapp M, Mueller-Godeffroy E, Lee P, Roehle R, Kreukels BPC, Köhler B, et al. dsd-LIFE group. Multicentre cross-sectional clinical evaluation study about quality of life in adults with disorders/differences of sex development (DSD) compared to country specific reference populations (dsd-LIFE). *Health Qual Life Outcomes*. 2018;16(1):54.
432. Bakula DM, Mullins AJ, Sharkey CM, Wolfe-Christensen C, Mullins LL, Wisniewski AB. Gender identity outcomes in children with disorders/differences of sex development: Predictive factors. *Semin Perinatol*. 2017;41(4):214–217.
433. Kreukels BPC, Köhler B, Nordenström A, Roehle R, Thyen U, Bouvattier C, et al. dsd-LIFE group. Gender dysphoria and gender change in disorders of sex development/intersex conditions: results from the dsd-LIFE study. *J Sex Med*. 2018;15(5):777–785.
434. Pritisini F, Kanakis GA, Kyrgios I, Kotanidou EP, Litou E, Mouzaki K, et al. Psychological aspects of androgen insensitivity syndrome: two cases illustrating therapeutic challenges. *Case Rep Endocrinol*. 2017;2017:8313162.
435. Imperato-McGinley J, Miller M, Wilson JD, Peterson RE, Shackleton C, Gajdusek DC. A cluster of male pseudohermaphrodites with 5 alpha-reductase deficiency in Papua New Guinea. *Clin Endocrinol (Oxf)*. 1991;34(4):293–298.
436. Cohen-Kettenis PT. Gender change in 46,XY persons with 5alpha-reductase-2 deficiency and 17beta-hydroxysteroid dehydrogenase-3 deficiency. *Arch Sex Behav*. 2005;34(4):399–410.

437. Houk CP, Damiani D, Lee PA. Choice of gender in 5alpha-reductase deficiency: a moving target. *J Pediatr Endocrinol Metab.* 2005;18(4):339–345 R.
438. Lee PA, Houk CP. Review of outcome information in 46,XX patients with congenital adrenal hyperplasia assigned/reared male: what does it say about gender assignment? *Int J Pediatr Endocrinol.* 2010;2010:982025.
439. Houk CP, Lee PA. Approach to assigning gender in 46,XX congenital adrenal hyperplasia with male external genitalia: replacing dogmatism with pragmatism. *J Clin Endocrinol Metab.* 2010;95(10):4501–4508.
440. McCarthy MM. Is sexual differentiation of brain and behavior epigenetic? *Curr Opin Behav Sci.* 2019;25:83–88.
441. McCarthy MM, Herold K, Stockman SL. Fast, furious and enduring: Sensitive versus critical periods in sexual differentiation of the brain. *Physiol Behav.* 2018;187:13–19.
442. Pasterski V, Geffner ME, Brain C, Hindmarsh P, Brook C, Hines M. Prenatal hormones and childhood sex segregation: playmate and play style preferences in girls with congenital adrenal hyperplasia. *Horm Behav.* 2011;59(4):549–555.
443. Gondim R, Teles F, Barroso Jr U. Sexual orientation of 46, XX patients with congenital adrenal hyperplasia: a descriptive review. *J Pediatr Urol.* 2018;14(6):486–493.
444. Berenbaum SA, Korman Bryk K, Duck SC, Resnick SM. Psychological adjustment in children and adults with congenital adrenal hyperplasia. *J Pediatr.* 2004;144(6):741–746.
445. Arnold AP, Burgoyne PS. Are XX and XY brain cells intrinsically different? *Trends Endocrinol Metab.* 2004;15(1):6–11.
446. Balthazart J. Minireview: hormones and human sexual orientation. *Endocrinology.* 2011;152(8):2937–2947.
447. Bonifacio HJ, Rosenthal SM. Gender variance and dysphoria in children and adolescents. *Pediatr Clin North Am.* 2015;62(4):1001–1016.
448. Kyriakou A, Dessens A, Bryce J, Iotova V, Juul A, Krawczynski M, et al. Current models of care for disorders of sex development - results from an International survey of specialist centres. *Orphanet J Rare Dis.* 2016;11(1):155.
449. Shankar RK, Bäckeljauw PF. Current best practice in the management of Turner syndrome. *Ther Adv Endocrinol Metab.* 2018;9(1):33–40.
450. Donaldson M, Kriström B, Ankarberg-Lindgren C, Verlinde S, van Alfen-van der Velden J, Gawlik A, et al. on behalf of the European Society for Paediatric Endocrinology Turner Syndrome Working Group. Optimal pubertal induction in girls with Turner syndrome using either oral or transdermal estradiol: a proposed modern strategy. *Horm Res Paediatr.* 2019;91(3):153–163.
451. Bäckeljauw P, Klein K. Sex hormone replacement therapy for individuals with Turner syndrome. *Am J Med Genet C Semin Med Genet.* 2019;181(1):13–17.
452. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103(11):4043–4088.
453. Chaudhari M, Johnson EK, DaJusta D, Nahata L. Testicular adrenal rest tumor screening and fertility counseling among males with congenital adrenal hyperplasia. *J Pediatr Urol.* 2018;14(2):155.e1–155.e6.
454. Krege S, Eckoldt F, Richter-Unruh A, Köhler B, Leuschner I, Mentzel HJ, et al. Variations of sex development: The first German interdisciplinary consensus paper. *J Pediatr Urol.* 2019;15(2):114–123.
455. Siminoff LA, Sandberg DE. Promoting shared decision making in disorders of sex development (DSD): decision aids and support tools. *Horm Metab Res.* 2015;47(5):335–339.
456. Almasri J, Zaiem F, Rodriguez-Gutierrez R, Tamhane SU, Iqbal AM, Prokop LJ, et al. Genital reconstructive surgery in females with congenital adrenal hyperplasia: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2018;103(11):4089–4096.
457. Baskin LS. Restoring normal anatomy in female patients with atypical genitalia. *Semin Perinatol.* 2017;41(4):227–231.
458. Creighton SM, Minto CL, Steele SJ. Objective cosmetic and anatomical outcomes at adolescence of feminising surgery for ambiguous genitalia done in childhood. *Lancet.* 2001;358(9276):124–125.
459. Ismail-Pratt IS, Bikoo M, Liao LM, Conway GS, Creighton SM. Normalization of the vagina by dilator treatment alone in Complete Androgen Insensitivity Syndrome and Mayer-Rokitansky-Kuster-Hauser Syndrome. *Hum Reprod.* 2007;22(7):2020–2024.
460. Köhler B, Kleinemeier E, Lux A, Hiort O, Grüters A, Thyen U, DSD Network Working Group. Satisfaction with genital surgery and sexual life of adults with XY disorders of sex development: results from the German clinical evaluation study. *J Clin Endocrinol Metab.* 2012;97(2):577–588.
461. Mouriquand, P.D., Gorduza, D.B., Gay, C.L., Meyer-Bahlburg, H. F., Baker, L., Baskin, L.S., et al. Surgery in disorders of sex development (DSD) with a gender issue: If (why), when, and how? *J Pediatr Urol.* 2016;12(3), 139–149.
462. Bougnères P, Bouvattier C, Cartigny M, Michala L. Deferring surgical treatment of ambiguous genitalia into adolescence in girls with 21-hydroxylase deficiency: a feasibility study. *Int J Pediatr Endocrinol.* 2017;2017:3.
463. Nordenskjöld A, Holmdahl G, Frisé L, Falhammar H, Filipsson H, Thorén M, et al. Type of mutation and surgical procedure affect long-term quality of life for women with congenital adrenal hyperplasia. *J Clin Endocrinol Metab.* 2008;93(2):380–386.
464. Fagerholm R, Santtila P, Miettinen PJ, Mattila A, Rintala R, Taskinen S. Sexual function and attitudes toward surgery after feminizing genitoplasty. *J Urol.* 2011;185(5):1900–1904.
465. Casteràs A, De Silva P, Rumsby G, Conway GS. Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate. *Clin Endocrinol (Oxf).* 2009;70(6):833–837.
466. Thyen U, Ittermann T, Flessa S, Muehlan H, Birnbaum W, Rapp M, et al. dsd-LIFE group. Quality of health care in adolescents and adults with disorders/differences of sex development (DSD) in six European countries (dsd-LIFE). *BMC Health Serv Res.* 2018;18(1):527.
467. Buell-Gutbrod R, Ivanovic M, Montag A, Lengyel E, Fadare O, Gwin K. FOXL2 and SOX9 distinguish the lineage of the sex cord-stromal cells in gonadoblastomas. *Pediatr Dev Pathol.* 2011;14(5):391–395.
468. Jørgensen A, Lindhardt Johansen M, Juul A, Skakkebaek NE, Main KM, Rajpert-De Meyts E. Pathogenesis of germ cell neoplasia in testicular dysgenesis and disorders of sex development. *Semin Cell Dev Biol.* 2015;45:124–137.
469. Baroni T, Arato I, Mancuso F, Calafiore R, Luca G. On the origin of testicular germ cell tumors: from gonocytes to testicular cancer. *Front Endocrinol (Lausanne).* 2019;10:343.
470. Mazzanti L, Cicognani A, Baldazzi L, Bergamaschi R, Scarano E, Strocchi S, et al. Gonadoblastoma in Turner syndrome and Y-chromosome-derived material. *Am J Med Genet A.* 2005;135:150.
471. Kwon A, Hyun SE, Jung MK, Chae HW, Lee WJ, Kim TH, et al. Risk of gonadoblastoma development in patients with Turner syndrome with cryptic Y chromosome material. *Horm Cancer.* 2017;8(3):166–173.
472. Ezaki J, Hashimoto K, Asano T, Kanda S, Akioka Y, Hattori M, et al. Gonadal tumor in Frasier syndrome: a review and classification. *Cancer Prev Res (Phila).* 2015;8(4):271–276.
473. Chaudhry S, Tadokoro-Cuccaro R, Hannema SE, Acerini CL, Hughes IA. Frequency of gonadal tumours in complete androgen insensitivity syndrome (CAIS): A retrospective case-series analysis. *J Pediatr Urol.* 2017;13(5):498.e1–498.e6.
474. Cools M, Wolffenbuttel KP, Hersmus R, Mendonca BB, Kaprová J, Drop SLS, et al. Malignant testicular germ cell tumors in post-pubertal individuals with androgen insensitivity: prevalence, pathology and relevance of single nucleotide polymorphism-based susceptibility profiling. *Hum Reprod.* 2017;32(12):2561–2573.
475. Tack LJW, Maris E, Looijenga LHJ, Hannema SE, Audi L, Köhler B, et al. Management of Gonads in Adults with Androgen Insensitivity: An International Survey. *Horm Res Paediatr.* 2018;90(4):236–246.
476. Döhnert U, Wünsch L, Hiort O. Gonadectomy in complete androgen insensitivity syndrome: why and when? *Sex Dev.* 2017;11(4):171–174.
477. Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, et al. United Kingdom Congenital Adrenal Hyperplasia Adult Study

- Executive (CaHASE). Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab.* 2010;95(11), 5110–5121.
478. Conway GS. Congenital adrenal hyperplasia: adolescence and transition. *Horm Res.* 2007;68(Suppl 5):155–157.
479. Bachelot A, Vialon M, Baptiste A, Tejedor I, Elie C, Polak M, Touraine P. Impact of transition on quality of life in patients with congenital adrenal hyperplasia diagnosed during childhood. *Endocr Connect.* 2017;6(7):422–429.
480. Nordenström A. Adult women with 21-hydroxylase deficient congenital adrenal hyperplasia, surgical and psychological aspects. *Curr Opin Pediatr.* 2011;23(4):436–442.
481. Berglund A, Johannsen TH, Stochholm K, Viuff MH, Fedder J, Main KM, Gravholt CH. Morbidity, mortality, and socioeconomic in females With 46,XY disorders of sex development: a nationwide study. *J Clin Endocrinol Metab.* 2018;103(4):1418–1428.
482. Falhammar H, Claahsen-van der Grinten H, Reisch N, Slowikowska-Hilczler J, Nordenström A, Roehle R, et al. dsd-LIFE group. Health status in 1040 adults with disorders of sex development (DSD): a European multicenter study. *Endocr Connect.* 2018;7(3):466–478.
483. Slowikowska-Hilczler J, Hirschberg AL, der Grinten Classhsen-van, et al. Fertility outcome and information on fertility issues in individuals with different forms of disorders of sex development; findings from the dsd-LIFE study. *Fertil Steril.* 2017;108: 822–831.
484. Van Batavia JP, Kolon TF. Fertility in disorders of sex development: a review. *J Pediatr Urol.* 2016;12:418–425.
485. Pippi Salle JL, Sayed S, Salle A, et al. Proximal hypospadias: a persistent challenge. Single institution outcome analysis of three surgical techniques over a 10-year period. *J Pediatr Urol.* 2015;1. e1-le7.
486. Nordenvall AS, Norrby C, Butwicka A, et al. Psychosocial outcomes in adult men born with hypospadias: a register-based study. *PLoS One.* 2017;12. e0174923.
487. Lucas-Herald A, Bertelloni S, Jull A, et al. The long-term outcome of boys with partial androgen insensitivity syndrome and a mutation in the androgen receptor gene. *J Clin Endocrinol Metab.* 2016;101. 3959–3957.
488. Greenhalgh H. *Intersex Babies Caught in Surgery Battle Over Medical Risks, Ethical Rights. The Thomson Reuters Trust Principles;* 2018.
489. Baratz A, Karkazis K. Cris de coeur and the moral imperative to listen to and learn from intersex people. *Narrat Inq Bioeth.* 2015;5(2):127–132.

7

Hypoglycemia in the Newborn and Infant

Diva D. De Leon, Paul Thornton, Charles A. Stanley, Mark A. Sperling

CHAPTER OUTLINE

INTRODUCTION	175
PRINCIPLES OF GLUCOSE METABOLISM	175
PHYSIOLOGY OF PERINATAL GLUCOSE HOMEOSTASIS	176
Glucose Metabolism in the Fetus	176
Changes at Birth: Transition Phase	176
HORMONAL AND METABOLIC SYSTEMS OF FASTING ADAPTATION	178
DEFINITION HYPOGLYCEMIA IN NEONATES AND INFANTS	180
CLINICAL SYMPTOMS AND SIGNS ASSOCIATED WITH HYPOGLYCEMIA	182
DIAGNOSTIC APPROACH	182
CLASSIFICATION OF CAUSES OF PERSISTENT HYPOGLYCEMIA IN THE NEONATE AND INFANT	183
Hyperinsulinism and Similar Disorders	183
Defects in Counterregulatory Response	192
Defects in Glycogenolysis and Gluconeogenesis	192
Disorders of Fatty Acid Oxidation: Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency	194
Defects of Glucose Transporters	195
TREATMENT	196
CONCLUSIONS	196
References	196

INTRODUCTION

One of the most important metabolic events to mark the transition from fetal to neonatal life is the adaptation from an environment that has a readily available and continuous source of glucose—transplacental passage of maternal blood—to an environment in which glucose is provided in a limited and intermittent supply via feeding. At birth, after separation from the placenta, the complex mechanisms involved in the maintenance of plasma glucose concentration must be activated and coordinated to avoid hypoglycemia and resultant damage to the central nervous system. A newborn or infant with hypoglycemia presents an urgent diagnostic and therapeutic challenge. The clinical features must be rapidly assessed and a plan of action developed based on the infant's age, maternal and parturition history, severity and persistence of hypoglycemia, and all other relevant clinical clues for definitive diagnosis and treatment. In this chapter, we review the normal physiology of glucose metabolism and the pathophysiology of the most common disorders resulting in hypoglycemia in the neonate (first 4 weeks of life) and infant (1 month to 1 year of life) with an emphasis on the approach to the diagnosis and management of these pathologies.

PRINCIPLES OF GLUCOSE METABOLISM

A systematic approach to hypoglycemia in the newborn, infant, or child requires an appreciation of the central role of glucose in the body's fuel economy.¹ Glucose metabolism accounts for approximately half of basal daily energy needs and is the principal metabolic fuel of the human brain. Glucose can be stored for energy in the form of glycogen and fat, although only glucose stored in liver glycogen is available for release into the circulation, and fatty acids cannot be converted back into glucose; glucose carbon can be used for synthesis of protein and for structural components (such as cell membranes). The aerobic oxidation of glucose yields high energy by producing 36 mols of adenosine triphosphate (ATP) for each mol of glucose. Although glucose is the predominant metabolic fuel for both the brain and the body in the period immediately after a meal, during longer term fasting, glucose utilization must be restricted to glycolytic tissues, such as red blood cells and the brain and the rest of the body must rely on oxidation of fatty acids released from adipose tissue triglyceride stores.²

All glucose extracted by the brain is oxidized, and thus cerebral glucose utilization parallels cerebral oxygen uptake. In 5-week-old infants, cerebral glucose utilization already represents 71% to 93% of the adult level in most brain regions (ranging from 13 to 25 $\mu\text{mol}/100\text{ g}/\text{min}$). Adult levels of cerebral glucose utilization (19–33 $\mu\text{mol}/100\text{ g}/\text{min}$) are reached by 2 years of age, and they continue to increase until 3 to 4 years of age—when they reach values ranging from 49 to 65 $\mu\text{mol}/100\text{ g}/\text{min}$, which are maintained to approximately 9 years of age and then gradually decline to adult levels by the end of the second decade.³

Glucose uptake by the brain occurs by means of a carrier-mediated facilitated diffusion process that is glucose concentration dependent, as well as energy, Na^+ , and insulin independent.⁴ This process is mediated by facilitative glucose transporter (GLUT) proteins. Several members of the GLUT family have been detected in the brain. GLUT1 is located at the blood-brain barrier,⁵ and although some neurons express GLUT2 and GLUT4, the majority use GLUT3 as their primary transporter.⁶ The main isoform present in insulin-responsive tissues is GLUT4.^{7,8}

Of major importance is that glucose entry into brain cells and its subsequent metabolism are not dependent on insulin but rather are dependent on circulating arterial plasma glucose concentration.⁹ Therefore a decrease in arterial glucose concentration or a defect in the glucose transport mechanism of the brain will result in intracerebral glucopenia and low cerebrospinal fluid glucose concentration (hypoglycorrhachia)—with attendant symptoms and signs of cerebral glucopenia as subsequently described.^{9–11} For example, genetic deficiency of the GLUT1 glucose transporter causes hypoglycorrhachia, seizures, and severe developmental delay.¹²

In normal adult humans and in the normal newborn infant after the first few days of life, plasma glucose concentrations range from 3.9 to 5.6 mmol/L (70–100 mg/dL). In experiments using rats, brain glucose consumption outstrips glucose transport, leading to a brain glucose concentration near zero at a plasma glucose

concentration of 2 mmol/L (36 mg/dL).¹³ To prevent circulating arterial plasma glucose from decreasing precipitously under normal physiologic conditions, and therefore to prevent impairment of vital function that depends on cerebral glucose metabolism, an elaborate defense mechanism has evolved.¹ This defense against hypoglycemia is integrated by the autonomic nervous system and by hormones that act to enhance glucose production through enzymatic modulation of glycogenolysis and gluconeogenesis, while simultaneously limiting peripheral glucose use.^{14,15} Similar defense mechanisms against hypoglycemia function in the normal full term human newborn after separation from the placenta.¹⁶ Thus hypoglycemia is the result of a defect in one of these systems that maintain a normal range of plasma glucose concentration, preventing its fall to less than 3.9 mmol/L (70 mg/dL) during fasting and its rise to more than 7.8 mmol/L (140 mg/dL) during feeding.

These mechanisms, most importantly, the regulation of β -cell insulin secretion, are not fully mature at birth, when there is an abrupt transition from intrauterine life to extrauterine life,¹⁶ and mean plasma glucose concentrations transiently fall by 25% before recovering by 2 days of life to normal adult values. Neonates who are born with various risk factors, including birth asphyxia, intrauterine growth restriction (IUGR), maternal toxemia, and prematurity may be particularly vulnerable to hypoglycemia,¹⁷ often with consequences to subsequent cerebral development or function that can be prevented by early detection and treatment.^{18,19}

PHYSIOLOGY OF PERINATAL GLUCOSE HOMEOSTASIS

Glucose Metabolism in the Fetus

Glucose provides 80% of fetal energy, with the remaining 20% derived mostly from oxidation of lactate and amino acids.²⁰ Free fatty acids are transported across the placenta in limited amounts sufficient for fetal growth, but do not contribute significantly to fetal energy demands. The glucose used by the fetus is derived entirely from the maternal circulation through facilitated diffusion across the placenta using primarily the GLUT1 glucose transporter.²¹ Placental glucose transport does not involve the GLUT4 glucose transporter, and therefore is not regulated by either maternal or fetal insulin concentrations. Because placental glucose transport is so rapid, the maternal and fetal glucose concentrations behave as a single pool and there is no endogenous glucose production in the fetus. This has important clinical consequences, for example, acute hypoglycemia in a mother will result in acute hypoglycemia in the fetus—with little or no ability of the fetus to acutely compensate for the abrupt reduction in glucose supply.

From early gestation to the time of delivery, maternal arterial and fetal umbilical vein concentrations of plasma glucose are very similar. The mean maternal-fetal plasma glucose concentration difference is essentially zero at 20 weeks of gestation and increases only slightly to 0.75 mmol/L (13 mg/dL) at term.²² The small increase in maternal-fetal gradient may reflect increased utilization by the growing placenta, because approximately 50% to 60% of maternal glucose taken up is consumed by oxidation in the placenta and only 40% to 50% transported to the fetus.²³ As gestation progresses, the increasing demand of the fetus for glucose oxidation and storage is accomplished by increasing uterine blood flow. There is excess capacity in uterine blood flow, and it may be reduced as much as 50% without causing any fetal harm. However, reducing blood flow from the placenta to the fetus causes significant impact on delivery of glucose and other nutrients to the fetus, with resultant IUGR.

In the second half of pregnancy, the fetal-placental unit secretes large quantities of placental lactogen, progesterone,

and estrogen, which cause increased maternal insulin resistance and may, in susceptible mothers, result in gestational diabetes. Maternal insulin secretion is responsible for controlling plasma glucose concentrations in the mother and, while insulin does not cross the placenta (unless bound by antibodies),²⁴ the maternal insulin also controls fetal glucose concentrations, because of the rapid facilitated transfer of glucose across the placenta. Although fetal insulin does not control fetal glucose concentrations, fetal insulin secretion is responsive to changes in fetal glucose concentrations. The role of fetal insulin is to maintain the rapid growth rate of the fetus. For example, maternal hyperglycemia (e.g., maternal diabetes) leads to fetal hyperinsulinemia and increased fetal growth; in contrast, maternal hypoglycemia (as in maternal malnutrition or in maternal hyperinsulinism caused by a glucokinase activating mutation) can suppress fetal insulin secretion, resulting in impaired fetal growth.²⁵

Changes at Birth: Transition Phase

The abrupt interruption of maternal glucose transfer to the fetus at delivery imposes a need for the newborn infant to independently control plasma glucose concentrations by adjusting insulin secretion and mobilizing counterregulatory responses. There is evidence that most of the counterregulatory mechanisms, including glucagon and epinephrine secretion and activation of hepatic glycogenolysis and gluconeogenesis, are functional at birth.²⁶⁻²⁸ Nevertheless, there is a transitional period immediately after delivery when mean plasma glucose concentrations fall in normal newborn infants from 70 to 80 mg/dL (close to maternal glucose values) to 55 to 60 mg/dL before returning by 2 to 3 days of life to the range of 70 to 100 mg/dL, which is normal for extrauterine life.^{17,29} This phenomenon, known as *transitional neonatal hypoglycemia*, in normal newborns has been recognized for many years, although its clinical significance remains uncertain, indeed controversial. There is increasing evidence that the mechanism of transitional hypoglycemia involves pancreatic regulation of insulin secretion and reflects persistence of the lower glucose threshold for β -cell insulin secretion in the fetus, which is required for maintaining fetal growth.³⁰ Evidence that transitional neonatal hypoglycemia should be considered as “transitional neonatal hyperinsulinism” includes observations that, during the period that plasma glucose is low in normal newborns, lipolysis and ketogenesis are suppressed and liver glycogen reserves are maintained, as shown by the large glycemic responses elicited by administration of glucagon or epinephrine.³⁰ As discussed later in this chapter, these findings are diagnostic hallmarks of insulin-induced hypoglycemia. The lower concentrations of plasma glucose in normal newborns are quite stable and relatively unaffected by durations of postnatal fasting of up to 24 hours, suggesting that transitional neonatal hypoglycemia reflects a lower glucose threshold for insulin secretion.³⁰ Recent studies in mice confirm a lower glucose threshold for insulin secretion in the immediate postnatal period. The mechanism responsible for the low glucose threshold in fetal beta-cells remains unclear, but suggested explanations include expression of genes that are normally “disallowed” in the beta-cell, such as hexokinase-1, the plasma membrane transporter for pyruvate and lactate, and lactate dehydrogenase or heightened sensitivity of insulin granule release to low glucose.³¹⁻³⁵ The phenomenon of transitional neonatal hyperinsulinism in normal newborns appears to usually be benign, but often complicates the recognition of infants who have persistent, pathological hypoglycemia disorders (see later).

Other challenges to the regulation of plasma glucose concentrations after delivery include the need to switch from a constant supply of glucose and amino acids provided from the maternal circulation to a variable and intermittent oral intake of nutrients. In breastfed infants, the composition of feedings

also changes over the first days of life, with colostrum being lower in carbohydrates and higher in fat than mature breast milk. Breastfed babies frequently may not achieve full nourishment initially and, beyond the period of transitional neonatal hyperinsulinism, may develop mild fasting hyperketonemia. Although this hyperketonemia was previously often attributed to the high fat content of colostrum (“suckling ketosis”), it appears to reflect a response to mild fasting stress.^{36–38} Impairments in adaptation to these changes in fuel supply in newborns may be manifested as symptomatic hypoglycemia, as described in later sections on specific hypoglycemia disorders in neonates and early infancy. Insulin secretion is glucose-responsive during late fetal development, but maximum capacity may be limited, especially in extremely premature infants who require intravenous (IV) feedings.

In summary, plasma glucose concentrations during intrauterine life are constant and are maintained to greater than 3.9 mmol/L (70 mg/dL). Following birth, plasma glucose falls to a mean glucose of 3.1 to 3.3 mmol/L (56–60 mg/dL), during the first 12 hours as a consequence of the low beta-cell glucose threshold for insulin secretion. As insulin regulation adapts to extrauterine life, glucose concentration rises to a mean of 3.5 mmol/L (63 mg/dL) by 12 to 24 hours of life; by 48 to 72 hours plasma glucose rises to a mean of 4.1 mmol/L (74 mg/dL).³⁹ The distribution of plasma glucose concentrations in neonates immediately after birth is not “normal” but is skewed toward lower values because of babies with various risk factors for hypoglycemia, as described later. For this reason, approximately 30% of “normal” newborns will have plasma glucose concentrations lower than 2.8 mmol/L (50 mg/dL) in the first 8 to 12 hours of life, but thereafter the frequency of glucose concentrations greater than 50 mg/dL drops to only 0.5% in newborns older than 24 hours of age.¹⁷ This drop in plasma glucose levels in the first 24 hours of life is *transitional hypoglycemia (transitional neonatal hyperinsulinism)*, which by definition occurs in normal healthy newborns. It should be differentiated from both transient and persistent pathologic causes of hypoglycemia.

Abnormalities of Transition

Studies of plasma glucose concentrations immediately after delivery in normal newborns show not only a lower mean concentration of glucose, but also a wider variance and, as noted earlier, a skewing of the distribution toward glucose values. This means that the simple mean \pm 2 standard deviations (SDs) is not a valid measure of the normal range of glucose in newborns, although this is used as the basis for many guidelines on neonatal hypoglycemia.^{40,41} The excess of low glucose concentrations reflects the fact that a large proportion of neonates are at increased risk of potentially pathologic hypoglycemia during the period of transitional neonatal hyperinsulinism. These at-risk infants have more severe and more prolonged hypoglycemia after delivery, which is caused by pancreatic insulin secretion and has been termed *perinatal stress-induced hyperinsulinism*. Perinatal stress-induced hyperinsulinism occurs with a number of fetal and maternal disorders, such as IUGR, birth asphyxia, maternal diabetes, and maternal hypertension (Box 7.1).^{42–44} Whether perinatal stress-induced hyperinsulinism is simply an exaggerated and more protracted form of transitional neonatal hyperinsulinism is not known. Affected neonates may require treatment to control hypoglycemia for several weeks or months after birth. Premature infants may not have developed adequate hepatic glycogen stores and may also have immaturities of liver enzyme systems for gluconeogenesis and ketone synthesis, which increase their risk of hypoglycemia but to a lesser extent than in infants with perinatal stress. It is important for the clinician to recognize these at-risk neonates and neonates whose glucose control is out of the

BOX 7.1 Neonates at Risk of Persistent Hypoglycemia (modified from PES guide)

Infants of Diabetic Mothers (including gestational diabetes)
 Large for Gestational Age (LGA) (even without maternal diabetes)
 Perinatal Stress-induced Hyperinsulinism
 Intrauterine Growth Restriction (IUGR), Small for Gestational Age (SGA)
 Birth Asphyxia; C-section for fetal distress
 Maternal preeclampsia or hypertension
 Meconium aspiration syndrome
 Erythroblastosis fetalis
 Polycythemia
 Premature or Postmature Delivery
 Family History of a Genetic Form of Hypoglycemia
 Congenital Syndromes Associated With Hypoglycemia
 Beckwith-Wiedemann Syndrome (especially 11pUPD)
 Turner Syndrome
 Kabuki Syndrome
 Midline facial malformations, microphallus

range of transitional hypoglycemia during this period and treat appropriately. In particular, it is important to recognize conditions in which insulin secretion is increased, as these infants will neither mobilize glycogen nor be able to oxidize fatty acids, thus putting their brains at risk of damage because of the combination of hypoglycemia and hypoketonemia.

Management of Hypoglycemia in the First 24 to 48 Hours

Management of hypoglycemia in the first 24 hours of life is important from two points of view. First, the management plan should avoid treatment of infants who do not need treatment. Second, it should include a plan for those who either have or are at risk of having a persistent hypoglycemic disorder, which both identifies those neonates and treats them adequately. It is not currently recommended that all newborns have plasma glucose concentrations measured. However, if an infant has symptoms consistent with hypoglycemia—such as lethargy, apnea, or seizures, or is unwell or in any of the at-risk categories (e.g., infants with siblings with known hypoglycemic disorders, preterm infants, large for gestational age infants, small for gestational age infants, infants of a diabetic mother, infants who had birth asphyxia, and other infants with disorders outlined in Box 7.1)—the infant’s glucose levels should be measured.

The recent recommendations of the Pediatric Endocrine Society suggest that beyond the period of normal transitional neonatal hypoglycemia in the first 24 to 48 hours after birth, the glucose thresholds and goals for management of low plasma glucose concentrations should be the same in neonates as in older children.⁴⁵ In addition, these same standards are recommended for neonates who either have or are at increased risk of having a persistent hypoglycemia disorder (e.g., family history or physical features of a hypoglycemia disorder, such as large for gestational age birthweight, suggesting hyperinsulinism or macroglossia, and hemihypertrophy, suggesting Beckwith-Wiedemann syndrome [BWS]). For neonates who are suspected to not have a persistent disorder and who are receiving normal feedings, the recommended glucose target for management is to maintain plasma glucose above 50 mg/dL during the first 48 hours of life and above 60 mg/dL beyond 48 hours, if the hypoglycemia is likely to resolve within a day or two.

Emergency treatment of hypoglycemia in neonates should be tailored to the presence or absence of symptoms and the degree of hypoglycemia. For mild asymptomatic hypoglycemia in the

first 48 hours of life, early feeding or oral glucose may be tried initially. However, symptomatic or severe hypoglycemia should be treated with IV dextrose (e.g., 0.2 g/kg bolus, followed by a glucose infusion rate [GIR] of at least 4–6 mg/kg/min initially, and adjusted as needed to maintain glucose >70 mg/dL). If the hypoglycemia is known to be caused by hyperinsulinism, glucagon may also be considered (0.5–1 mg IV, subcutaneous [SQ], or intramuscular [IM]). Follow-up frequent monitoring of plasma glucose should be done to determine whether formal diagnostic evaluation is required.

By 48 hours of age, the period of transitional neonatal hypoglycemia should normally be over and plasma glucose concentrations should be similar to older children and adults: a normal range of 70 to 100 mg/dL. Thus, formal evaluation to diagnose the cause of hypoglycemia should be considered after 48 hours of age for neonates in whom the possibility of having a persistent hypoglycemia disorder cannot be completely excluded. Such an evaluation to diagnose the cause of hypoglycemia should be undertaken before the baby is allowed to be discharged home. To address the issue of babies who may not have achieved complete resolution of transitional hypoglycemia before the time of discharge, the Pediatric Endocrine Society recommendations suggest considering a fasting challenge to ensure that plasma glucose can be safely maintained above 60 mg/dL for over 6 to 8 hours (the “skip a feed” test). This should only be considered for neonates who have no risk features for a persistent hypoglycemia disorder (i.e., no episodes of symptomatic hypoglycemia, no need for IV dextrose, no features to suggest genetic or syndromic form of hypoglycemia, or perinatal stress-induced hypoglycemia, etc.). For all other neonates, a complete formal fasting study should be considered. One should note that there is no evidence that proves these protocols recommended by the Pediatric Endocrine Society will identify 100% of infants, so caution should be made if

clinical circumstances warrant further thought. Finally, it is important to emphasize to anxious parents the consequences of missing a diagnosis of a hypoglycemic disorder. In our experience, most parents are willing to lengthen the hospital stay by 6 to 9 hours to ensure the long-term safety of their child.

HORMONAL AND METABOLIC SYSTEMS OF FASTING ADAPTATION

Hypoglycemia in neonates, infants, and children is essentially always a problem with fasting adaptation. Postprandial hypoglycemia is exceedingly rare and is limited to a few unusual situations, such as postprandial hypoglycemia after Nissen fundoplication, hereditary fructose intolerance, or the protein-induced hypoglycemia seen in some forms of congenital hyperinsulinism. Therefore a consideration of the four major hormonal and metabolic pathways that maintain fuel homeostasis during fasting provides an important framework for understanding the causes, diagnosis, and treatment of different forms of hypoglycemia.

Three metabolic systems regulate the physiologic response to fasting: (1) hepatic glycogenolysis, (2) hepatic gluconeogenesis, and (3) hepatic ketogenesis. The key enzymatic steps in these pathways are shown in Fig. 7.1. These metabolic systems are coordinated by (4) the endocrine system, consisting of suppression of insulin (the most important endocrine response to fasting, as insulin suppresses all three metabolic systems), and the secretion of the counterregulatory hormones: glucagon, epinephrine (a marker of sympathetic nervous system activation), cortisol, and growth hormone (GH). Table 7.1 summarizes the counterbalancing effects of these counterregulatory hormones on the three key metabolic systems. There is a hierarchic redundancy in the interaction of the counterregulatory hormones that provides a margin of safety (“fail-safe mechanism”) if only

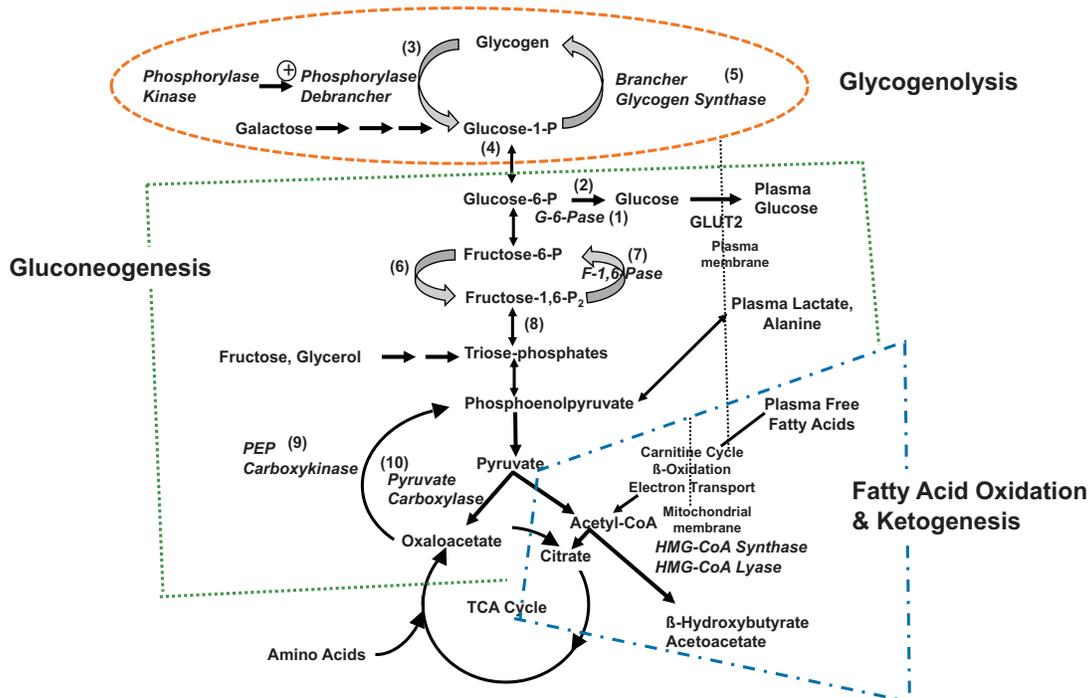


Fig. 7.1 Key metabolic pathways of intermediary metabolism. Disruption of the elements of these pathways may be pathogenetic in the development of hypoglycemia. Not shown is the hormonal control of these pathways. Indicated are (1) glucose 6-phosphatase, (2) glucokinase, (3) phosphorylase, (4) phosphoglucumutase, (5) glycogen synthetase, (6) phosphofructokinase, (7) fructose 1,6-diphosphatase, (8) fructose 1,6-diphosphate aldolase, (9) phosphoenolpyruvate carboxykinase, and (10) pyruvate carboxylase. (From Pagliara, A.S., Karl, I.E., Haymond, M., Kipnis, D.M. (1973). Hypoglycemia in infancy and childhood. *J Pediatr*, 82, 365–379 and 82, 558–577.)

TABLE 7.1 Hormonal Regulation of Fasting Metabolic Systems

Counterregulatory Hormone	Glycogenolysis	Gluconeogenesis	Lipolysis	Ketogenesis
Insulin	Inhibits	Inhibits	Inhibits	Inhibits
Glucagon	Stimulates	Stimulates		Stimulates
Cortisol		Stimulates		
Growth hormone			Stimulates	
Epinephrine	Stimulates		Stimulates	Stimulates

one counterregulatory hormone is impaired. Epinephrine (sympathetic activation) and glucagon are quick acting, each signaling its effects by stimulation of cyclic adenosine monophosphate (AMP). Deficiencies of glucagon, as occurs in long-standing type 1 diabetes mellitus, can be largely compensated for by an intact autonomic nervous system with appropriate α - and β -adrenergic, and cholinergic effects. Conversely, autonomic failure can be largely compensated for if glucagon secretion remains intact.¹

Hepatic glycogenolysis is sufficient to meet energy requirements for only a few hours. Beyond that time, glucose must be produced by hepatic gluconeogenesis from precursors, such as amino acids, glycerol, and lactate recycled from glycolysis. The major source of gluconeogenic precursors is amino acids from muscle protein. Although the pool of muscle protein is large, it is required for body function and thus in contrast to stores of glycogen in liver and fat in adipose tissue, there are no "reserves" of protein to draw on during fasting. To spare the use of essential protein during extended fasting, glucose consumption must be suppressed by switching on the mobilization of fatty acids from adipose triglyceride stores for oxidation in muscle and other tissues and of glycerol as substrate for hepatic gluconeogenesis. Fatty acids are also oxidized in liver to produce the ketone bodies, β -hydroxybutyrate and acetoacetate, which can be used by the brain as an alternative substrate to further spare glucose consumption.

The essential function of fasting adaptation is to maintain fuel supply to the brain. Glucose homeostasis is very limited in neonates and infants compared with adults, in part because of their smaller reserves of liver glycogen and muscle protein, but chiefly because of their relatively larger rates of glucose consumption because of their larger brain-to-body-mass ratio. For example, the fuel stores of a 10-kg infant are only 15% of those of an adult. However, the caloric needs are 60% of those of an adult and glucose utilization rates per kilogram body weight are 2- to 3-fold greater. As shown in Fig. 7.2, early in fasting after absorption of a meal is completed, glucose is the primary brain fuel and accounts for over 90% of total body oxygen consumption. Glucose is initially provided chiefly from hepatic glycogenolysis, supplemented by hepatic gluconeogenesis using amino acids released by muscle protein turnover and lactate from glycolytic tissues, such as red blood cells. After 8 to 12 hours in normal infants (24–36 hours in adults), glucose production declines, because the supply of liver glycogen is depleted and the rate of gluconeogenesis from amino acids remains constant. At this time, a transition to fat as the major fuel for the body begins, with accelerated adipose tissue lipolysis and increased fatty acid oxidation in muscle and ketogenesis in liver. Lipolysis also generates glycerol, which becomes an important gluconeogenic substrate once fasting adaptation is fully active. The brain cannot directly use fatty acids, because they do not pass the blood-brain barrier. However, the brain can substitute glucose consumption with the ketones acetoacetate and β -hydroxybutyrate, which are released by the liver as the end product of hepatic fatty acid oxidation. In late stages of fasting adaptation, fatty acid oxidation and ketone utilization account for 90% of total body oxygen consumption.

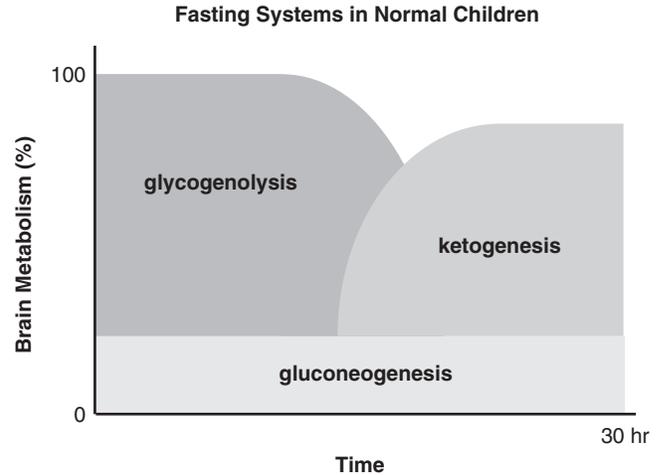


Fig. 7.2 Contribution of major fasting systems to brain metabolism over time in a typical normal infant. Note that glycogen stores are depleted by 8 to 12 hours and that ketogenesis becomes the major source of brain substrate by 24 hours.

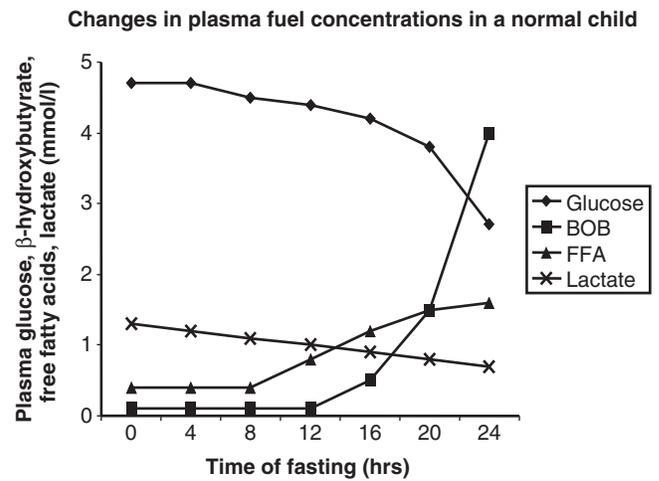


Fig. 7.3 In infants, a 24-hour fast is accompanied by a gradual fall in plasma glucose levels as hepatic glycogen stores are depleted, a progressive fall in concentrations of gluconeogenic substrate (e.g., lactate, alanine), as they are used for hepatic gluconeogenesis, a brisk rise in free fatty acids as lipolysis is activated, and a dramatic rise in β -hydroxybutyrate (the major ketone) as hepatic ketogenesis is turned on.

The operation of the metabolic and endocrine systems of fasting adaptation is evidenced by the changes in circulating levels of metabolic fuels and hormones during a fast. As shown in Fig. 7.3, in young infants, a 24-hour fast is accompanied by a gradual fall in plasma glucose levels as hepatic glycogen stores are depleted, a progressive decline

in concentrations of gluconeogenic substrate (e.g., lactate, alanine) as they are used for hepatic gluconeogenesis, a brisk rise in free fatty acids as lipolysis is activated, and a dramatic rise in β -hydroxybutyrate (the major ketone) as hepatic ketogenesis is turned on.⁴⁶

By the time that plasma glucose concentration has fallen to less than 2.8 mmol/L (50 mg/dL), all the metabolic systems and the hormonal responses described earlier will have fully engaged. A “critical sample” drawn at this time will therefore provide a snapshot of the integrity of the four metabolic and endocrine fasting systems (Table 7.2). The use of these critical samples in diagnosing the cause of hypoglycemia is discussed later.

DEFINITION HYPOGLYCEMIA IN NEONATES AND INFANTS

The definition of hypoglycemia is an important concept to assist in diagnosis and management of patients with suspected or proven hypoglycemic disorders. For this reason, we recommend using various different plasma glucose thresholds for different definitions. The diagnostic threshold is the level of glucose below or at which a diagnosis of the etiology of hypoglycemia may be made. The therapeutic threshold is the level of

glucose that indicates adequate therapy of a disorder of glucose dysregulation. Finally, there is the very controversial issue of what level of glucose causes brain damage.

A number of potential artifacts can interfere with measuring glucose levels in neonates and infants (Box 7.2). Whole-blood glucose concentrations are 10% to 15% lower than plasma glucose concentrations because erythrocytes have a higher concentration of protein (hemoglobin) versus plasma with its higher water content, and hence higher dissolved glucose concentrations. The difference may be greater in neonates with higher hematocrits. Blood samples that are not processed promptly can have erroneously low glucose levels, owing to glycolysis by red and white blood cells. At room temperature, the decline of whole-blood glucose can be 5 to 7 mg/dL/h. The use of inhibitors, such as fluoride, in collection tubes avoids this problem.

Hospital bedside glucose meters and similar home glucose meters are less precise than clinical laboratory methods and can be expected to have an error range of 10% to 15%. These methods are also prone to errors, such as outdated strips or inadequate sampling—most of which result in falsely low glucose values. For this reason, bedside monitors can be used for screening purposes—but any glucose value below 3.3 mmol/L (60 mg/dL) should be verified in the clinical laboratory. Falsely

TABLE 7.2 Differential Diagnosis of Hypoglycemia in Neonates and Infants

Disorder	Plasma Fuels at End of Fast (mmol/L)				Plasma Hormones at End of Fast				Physical Examination
	Glucose	Lactate	Free fatty acids	β -hydroxybutyrate	Insulin (μ mol/L)	Cortisol (ug/dL)	Growth hormone (ng/mL)	Glycemic response to glucagon (mg/dL)	
Normal Infants	2.8	0.7–1.5	1.5–2.5	2–4	<2	>20	>10	<30	
Hyperinsulinism	2.8	N	<1.7	<1.8	>1	N	N	>30	LGA
Cortisol deficiency	2.8	N	N	N	N	Low	Low	N	
GH deficiency	2.8	N	N	N	N	N	Low	N	Short stature
Panhypopituitarism	2.8	N	N	N	N	Low	Low	N	Short stature, midline facial malformation, optic hypoplasia, micropenis
Epinephrine deficiency (beta-blocker)	2.8	N	<1.5	<2	N	N	N	N	
Debrancher deficiency (GSD III)	2.8	N	N	N	N	N	N	N	Hepatomegaly 4+
Phosphorylase deficiency (GSD VI)	2.8	N	N	N	N	N	N	N	Hepatomegaly 2+
Phosphorylase kinase deficiency (GSD IX)	2.8	N	N	N	N	N	N	N	Hepatomegaly 2+
Glycogen synthase deficiency (GSD 0)	2.8	N	N	N	N	N	N	N	Hepatomegaly 1+
Glucose 6-phosphatase deficiency (GSD Ia and Ib)	2.8	4–8+	N	<2	N	N	N	(lactate)	Hepatomegaly 4+
Fructose 1,6-diphosphatase deficiency	2.8	4–8+	N	N	N	N	N	N	Hepatomegaly 1+
Pyruvate carboxylase deficiency	2.8	4–8+	N	N	N	N	N	N	
Lipolysis									
Congenital lipodystrophy, familial dysautonomia, beta-blockers	2.8	N	<1.5	<2	N	N	N	N	
Fatty acid oxidation defect	2.8	N	>2.5	<1.5	N	N	N	N	

GH, Growth hormone; GSD, glycogen storage disease; LGA, large for gestational age.

BOX 7.2 Factors Affecting Measurement of Plasma Glucose Concentration

- Whole-blood versus plasma glucose concentration (plasma is 10%–15% higher)
- Duration between sample collection and sample measurement
- Presence or absence of glycolytic inhibitors in collection tubes
- Sample collection from indwelling lines without adequate flushing

(Modified from Sacks, D.B. (1994). Carbohydrates. In: Burtis, C.A., Ashwood, E.R. (eds.), Tietz Textbook for Clinical Chemistry, 2nd ed. WB Saunders, Philadelphia.)

low (or high) glucose values may occur with samples drawn from indwelling lines without adequate flushing of the saline (or glucose) infusate. Falsely low levels may also occur in critically ill infants with poor perfusion and use of bedside meters in the critical care setting is recommended only for venous or arterial samples.⁴⁷ Modern bedside monitors use whole-blood samples but generally are calibrated to give a result expressed as a plasma glucose.

Diagnostic Glucose Threshold

The classic definition of symptomatic hypoglycemia is Whipple's triad—symptoms, signs or both consistent with hypoglycemia, a low plasma glucose concentration, and resolution of symptoms/signs after the plasma glucose concentration is raised. These three criteria were originally used for diagnosing insulinomas in adults. However, in neonates and infants, who cannot dependably communicate their symptoms and in whom clinical signs of hypoglycemia are not specific, it may not be possible to satisfy Whipple's triad. In these cases, recognition of hypoglycemia may require confirmation by repeated measurements of plasma glucose concentrations, either in association with symptoms or signs of hypoglycemia or during screening of at-risk infants. If levels are found below the level of glucose that causes neurogenic symptoms (<3.3 mmol/L or <60 mg/dL), after the first 48 hours of life, further investigations should be carried out to determine the etiology.⁴⁵ In these instances, formal provocative testing should be performed to determine if the neonate is safe for discharge or to determine the etiology of hypoglycemia.

Beyond the first 48 hours of life, normal plasma glucose concentration in neonates and infants are not different than in older children and adults. Thus plasma glucose concentrations in the postabsorptive state range between 3.9 and 5.6 mmol/L (70 and 100 mg/dL), with a mean of 4.4 to 4.7 mmol/L (80–85 mg/dL). A plasma glucose level of 2.8 mmol/L (50 mg/dL) is conventionally used as an end point for provocative diagnostic tests for hypoglycemia. This value is low enough to strongly stimulate the endocrine and metabolic defenses against hypoglycemia for identifying the mechanism responsible for hypoglycemia. Falling glucose levels elicit a typical sequence of responses: plasma insulin levels begin to decrease when plasma glucose falls to the range of 4.4 to 4.7 mmol/L (80–85 mg/dL) and insulin secretion is generally "switched off" at glucose concentrations below 2.5 to 3 mmol/L (45–54 mg/dL); glucagon secretion increases when plasma glucose levels are in the range of 3.6 to 3.9 mmol/L (65–70 mg/dL); epinephrine, cortisol, and GH responses are activated in the range of 3.6 to 3.9 mmol/L (65–70 mg/dL). As the plasma glucose falls below 3.3 mmol/L (59 mg/dL), auditory and visual reaction time is prolonged and cognitive function begins to decline as

the plasma glucose concentration declines below 2.5 to 3.5 mmol/L (45–63 mg/dL), with some of this variability dependent of the testing used.^{48,49}

Therapeutic Threshold

Once a diagnosis of the etiology of hypoglycemia has been made or suspected, the goal of therapy is to maintain the plasma glucose concentration >3.8 mmol/L (>70 mg/dL). This target allows patients with hypoglycemia disorders to maintain glucose levels above the threshold for counterregulatory responses and prevent the development of hypoglycemia unawareness caused by repetitive hypoglycemia. For disorders of fatty acid oxidation and gluconeogenesis, maintaining glucose concentration in this range will prevent buildup of free fatty acids and lactate, respectively.

Plasma glucose concentrations between 2.8 and 3.9 mmol/L (50 and 70 mg/dL) should be regarded as suboptimal and below the goal for therapy for hypoglycemia and should lead one to evaluate if a therapy escalation is needed.

Hypoglycemic Brain Damage

"What level of glucose causes brain damage?" is one of the most frequently asked questions. Unfortunately, the answer is more complex than the lower the glucose, and the longer the glucose is low, the worse the risk. Brain damage likely occurs because of fuel deficiency in neurons. Neurons have many sources of energy, the most common of which are glucose, lactate, and β -hydroxybutyrate. Studies of normal newborn infants have shown that after 6 hours of fasting, ketones can account for 12% of energy production by the brain.

To fully oxidize these compounds to energy, the cell needs oxygen and adequate blood flow. Indeed, one of the early brain adjustments to hypoglycemia (at approximately 30 mg/dL) is to increase cerebral blood flow delivering more glucose and oxygen to the brain. Studies have shown that the addition of hypoxemia, asphyxia, or ischemia to hypoglycemia dramatically increase the extent of brain damage compared with hypoglycemia alone, and the presence of hypoglycemia increases the risk of brain damage from less degrees of hypotension. There are many reports attempting to address the long-term outcomes associated with a glucose concentration of 45 mg/dL versus 47 mg/dL versus 50 mg/dL and all are flawed because none of them look at all three fuels. Thus one cannot draw any firm conclusions from papers currently published about what level of glucose causes brain damage. However, there is evidence to suggest that severe hypoglycemia is associated with a bad outcome with studies that look at patients with a combination of brain imaging abnormalities and poor neurologic outcome finding that 95% of patients had glucose less than 30 mg/dL.

The same may be true for the development of neuroglycopenic symptoms, which typically occur before brain damage would occur. In some disorders, such as defects in ketogenesis, signs and symptoms may begin to appear during fasting at plasma glucose levels of 3.3 mmol/L (60 mg/dL). On the other hand, some patients (those with glucose 6-phosphatase deficiency causing GSD1) may have few symptoms of neuroglycopenia at plasma glucose levels as low as 1.1 to 1.7 mmol/L (20–30 mg/dL) because their high plasma levels of lactate provide an alternative substrate for the brain.

Conditions, such as hyperinsulinism have a very high rate (30%–50%) of brain damage,^{50–54} as they are deficient in glucose and β -hydroxybutyrate and lactate, whereas neonates

with GSD1 are often not even diagnosed until they are toddlers and rarely have significant brain damage.

In conclusion, there is no one cutoff for what level of hypoglycemia causes brain damage; rather, it is a complex interplay of fuel availability, oxygen levels, and blood flow that all combine to maintaining the integrity of the brain.

CLINICAL SYMPTOMS AND SIGNS ASSOCIATED WITH HYPOGLYCEMIA

The clinical features of hypoglycemia in infants may be associated with both neurogenic and neuroglycopenic components (Box 7.3). Symptoms are often subtle and nonspecific, therefore a high index of clinical suspicion must be maintained. Any alteration in clinical status in a newborn that suggests a change in neurologic behavior, fall in temperature, change in feeding pattern, or presence of tremors must be considered a possible initial presentation of a hypoglycemic episode. A seizure or apnea must always be considered a possible manifestation of hypoglycemia and plasma glucose should be measured immediately.

BOX 7.3 Hypoglycemia Signs in Neonates

NEUROGENIC (ADRENERGIC OR CHOLINERGIC)

- Jitteriness
- Tachycardia
- Pallor
- Hypothermia

NEUROGLYCOPENIC

- Lethargy
- Irritability
- Poor feeding
- Cyanosis
- Tachypnea
- Apneic episodes
- Weak/high-pitched cry
- Floppiness
- Eye-rolling
- Lip smacking
- Twitching, convulsions

DIAGNOSTIC APPROACH

Determining the specific underlying cause of hypoglycemia is critical for the establishment of specific treatment and thus prevention of further episodes of hypoglycemia. The diagnosis should be based on the combination of data obtained from the history, physical examination, laboratory findings, and, especially, the hormonal and fuel responses at the time of hypoglycemia.

Important elements from the pregnancy and perinatal history, in addition to the family history, are critical to determine the existence of risk factors that could point to specific diagnoses, for example perinatal stress-induced hyperinsulinism in a neonate with IUGR. Information regarding the duration of fasting that provoked hypoglycemia is also helpful, for example, onset within a few hours of a meal would be consistent with hyperinsulinism or GSD I, whereas onset after 10 to 12 hours would be consistent with a defect in fatty acid oxidation. Pituitary deficiency with GH or adrenocorticotropic hormone (ACTH)-cortisol deficiency might be suspected by the presence of midline facial malformations, microphthalmia, or microphallus (follicle-stimulating hormone [FSH]/luteinizing hormone [LH] deficiency in utero). Growth failure is also a prominent feature of GSD I or debrancher deficiency glycogen storage disease after the first 3 months of life. Both of these disorders are associated with massive hepatomegaly. Abnormal results of liver function tests (transaminases) and hyperammonemia, with or without elevated creatine kinase level, would suggest a possible fatty acid oxidation disorder (see Table 7.2).

A logical diagnostic approach is to analyze a hypoglycemic event as a maladaptation to fasting, and thus the most important information required for diagnosis, comes from tests on the blood and urine specimens obtained at the time of hypoglycemia (also known as the *critical samples*). Fig. 7.4 outlines an algorithm for diagnosis of different forms of hypoglycemia based on readily available laboratory tests on the critical samples. The first discriminant is a measure of acidemia at the time of hypoglycemia using the serum bicarbonate. If the acidemia is caused by elevations of the ketoacids (β -hydroxybutyrate and acetoacetate), possibilities include a normal child fasted for too long, a defect in glycogenolysis (glycogen storage disease type 3), or counterregulatory hormone deficiency (hypopituitarism). If the acidemia is caused by an elevation of lactic acid, a block of gluconeogenesis should be suspected (GSD I or fructose 1,6-diphosphatase deficiency or ethanol ingestion). If

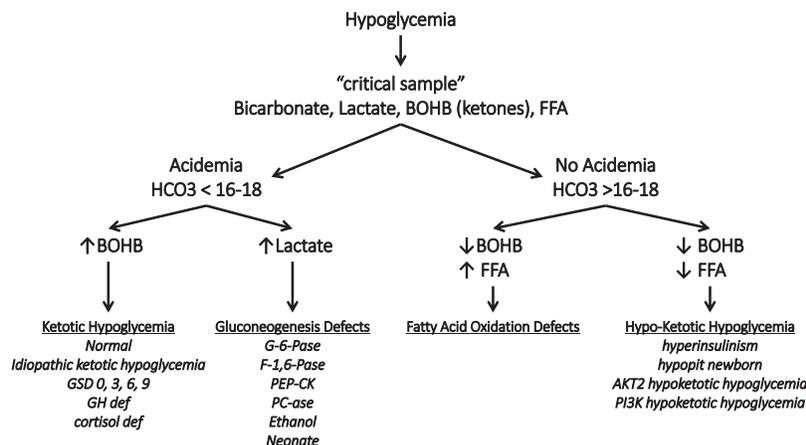


Fig. 7.4 Algorithm for diagnosis of hypoglycemia based on "critical" blood tests obtained during an episode of hypoglycemia. *BOHB*, β -hydroxybutyrate; *FFA*, free fatty acids; *GSD*, glycogen storage disorder; *HCO₃*, bicarbonate. (Modified from Stanley, C.A., Baker, L. (1978). Hypoglycemia. In: Kaye, R., Oski, F.A., Barness, L.A. (eds.), *Core Textbook of Pediatrics*. JB Lippincott, Philadelphia, p. 280–305.)

there is no acidemia (i.e., absence of the normal elevation of ketones) but free fatty acid levels are high, a defect in fatty acid oxidation and ketogenesis should be suspected (e.g., medium-chain acyl-CoA dehydrogenase deficiency [MCAD] deficiency). If ketones are not appropriately increased but the free fatty acid concentrations are also suppressed, hyperinsulinism should be suspected. In the neonatal period, the features of hyperinsulinism can be mimicked by congenital pituitary hormonal deficiency.

Further tests can then be planned using the initial critical specimens to confirm the suspected diagnosis. These may include physiologic tests (such as the glucagon stimulation test at a time of hypoglycemia to confirm hyperinsulinism) or specialized laboratory tests (such as a plasma acylcarnitine profile) to identify a defect in mitochondrial β -oxidation.

If a critical sample obtained during a spontaneous episode of hypoglycemia is not available, a formal fasting test may be necessary to diagnose the cause of hypoglycemia. The goal of this test is to reproduce the setting in which hypoglycemia occurs to identify the underlying cause. The fasting test should be considered a method of testing a hypothesis that has already been developed, based on available clinical and laboratory data about the cause of the hypoglycemia. Thus the test can be modified with additions or deletions to the basic protocol. This is important, because challenging an infant with fasting is not without risk—particularly if a genetic defect in fatty acid oxidation or adrenal insufficiency is present. Therefore fasting or other diagnostic challenges should be done only in the hospital under carefully controlled settings with an experienced physician and nursing staff readily available. Infants younger than 1 year are usually fasted for up to 24 hours, whereas in older children the maximum fast may be up to 36 hours. The fast is terminated when the plasma glucose falls below 2.8 mmol/L (50 mg/dL), but it may be ended sooner if plasma β -hydroxybutyrate rises to greater than 2.5 mmol/L or if there are any adverse signs or symptoms. Periodic blood samples are obtained for analysis of major fuels and hormones and for appropriate ancillary tests (e.g., serum total carnitine, acylcarnitine profile, liver transaminases, creatine phosphokinase, or urinary organic acids). If hyperinsulinism is suspected, the fasting test may be ended with glucagon (1 mg given IV) to evaluate the glycemic response.

Special note should be made that the most frequent cause of hypoglycemia in neonates, infants, and children (hyperinsulinism) often cannot be diagnosed based solely on the plasma insulin concentration. With very sensitive assays, serum insulin concentrations will be less than 1 to 2 μ U/mL at times of hypoglycemia (i.e., below the sensitivity of most insulin assays). However, serum C-peptide concentrations may be a useful marker with values of 0.5 ng/mL or higher strongly suggestive of hyperinsulinism, as shown in Table 7.3. Therefore the

diagnosis must often be made based on evidence of inappropriate insulin effects: hypoketonemia, hypo-free fatty acidemia, and a positive glycemic response to glucagon (see Table 7.3).

CLASSIFICATION OF CAUSES OF PERSISTENT HYPOGLYCEMIA IN THE NEONATE AND INFANT (BOX 7.4)

Hyperinsulinism and Similar Disorders

Suppression of insulin secretion is critical for maintaining euglycemia during fasting through the activation of hepatic glycogenolysis, gluconeogenesis, and fatty acid oxidation. Indeed, failure to suppress insulin secretion is the most common cause of persistent hypoglycemia in neonates and children. The clinical and biochemical hallmarks of increased insulin secretion/actions in the neonate are (1) increased birth weight because of the growth-promoting effects of insulin in utero; (2) increased glucose utilization; and (3) evidence that glycogenolysis, lipolysis, and ketogenesis are suppressed, as inferred by the finding of a glycemic response to glucagon (an increase of >30 mg/dL), and the suppression of plasma free fatty acids

BOX 7.4 Classification of Causes of Persistent Hypoglycemia in the Neonate and Infant

- A. Disorders of Insulin Excess or Actions
 1. Hyperinsulinism
 - a. Perinatal Stress-Induced Hyperinsulinism
 - b. Monogenic Hyperinsulinism
 - K*_{ATP} Hyperinsulinism
 - Focal
 - Diffuse
 - GDH Hyperinsulinism
 - SCHAD Hyperinsulinism
 - GCK Hyperinsulinism
 - HNF4A and HNF1A Hyperinsulinism
 - UCP2 Hyperinsulinism
 - FOXA2
 - c. Syndromic Hyperinsulinism
 - Beckwith-Wiedemann Syndrome
 - Kabuki Syndrome
 - Turner Syndrome
 2. Postprandial Hyperinsulinemic hypoglycemia after Fundoplication
 3. Hypoketotic Hypoglycemia Caused by Activating Mutations in Insulin Signaling Pathway
 - AKT2-hypoketotic hypoglycemia
 - PI3K-hypoketotic hypoglycemia
- B. Defects of Counterregulatory Response
 1. Hypopituitarism
 2. Congenital ACTH Deficiency (TBX19)
- C. Defects in Glycogenolysis and Gluconeogenesis
 - GSD Type 1
- D. Defects in Fatty Acid Oxidation
 - MCAD
- E. Defects of Glucose Transporters
 - GLUT1 Deficiency
 - GLUT2 Deficiency

ACTH, Adrenocorticotropic hormone; *FOXA2*, forkhead box A2; *GCK*, glucokinase; *GDH*, glutamate dehydrogenase; *GLUT*, glucose transporter; *GSD*, glycogen storage disease; *HNF*, hepatocyte nuclear factor; *MCAD*, medium-chain acyl-CoA dehydrogenase deficiency; *SCHAD*, short-chain 3-hydroxyacyl-CoA dehydrogenase.

TABLE 7.3 Criteria for Diagnosing Hyperinsulinism Based on “Critical” Samples (Drawn when Plasma Glucose is Less Than 50 mg/dL)

Parameter	Sensitivity (%)	Specificity (%)
Insulin >2 μ U/mL ^a	82.2	100
C-Peptide ≥ 0.5 ng/mL	88.5	100
β -hydroxybutyrate <1.8 mmol/L	100	100
Free Fatty Acids <1.7 mmol/L	87	100
IGFBP-1 ≤ 110 ng/mL	85	96.6
Glycemic response to glucagon >30 mg/dL	89	100

^aDepends on sensitivity of insulin assay. *IGFBP*, Insulin-like growth factor binding-protein.

and β -hydroxybutyrate concentrations during hypoglycemia (see Table 7.3).

The lack of alternative fuels (ketones) during hypoglycemia heightens the risk of brain damage in infants with these conditions, thus, it is essential that the diagnosis and establishment of treatment are expedited. The differential diagnosis and mechanisms of disease are broad, but they can be grouped in two categories: (1) hyperinsulinism caused by dysregulated insulin secretion, and (2) mimickers of hyperinsulinism, in which insulin secretion by the pancreatic beta cells is appropriately suppressed during hypoglycemia, but the effects of insulin on insulin-responsive tissues is inappropriately turned on.

Hyperinsulinism

Hyperinsulinism is the most common cause of persistent hypoglycemia in neonates, infants, and children.⁵⁵ Hyperinsulinism is caused by dysregulated insulin secretion from the pancreatic β -cells and can be the result of perinatal factors (perinatal stress-induced hyperinsulinism); monogenic, because of mutations in genes important for the regulation of insulin secretion; or syndromic (as in BWS).⁵⁶

Transient Hyperinsulinism Resulting from Maternal Factors

Transient hyperinsulinism is a well-recognized complication in neonates after a pregnancy complicated by gestational diabetes. At birth, infants born to these mothers may be large and plethoric—and their body stores of glycogen, protein, and fat are replete. The classic clinical description of the effect of hyperinsulinism relates to the infant of the diabetic mother:⁵⁷

These infants are remarkable not only because like fetal versions of Shadrack, Meshack, and Abednego, they emerge at least alive from within the fiery metabolic furnace of diabetes mellitus, but because they resemble one another so closely they might well be related. They are plump, sleek, liberally coated with vernix caseosa, full-faced, and plethoric. The umbilical cord and placenta share in the gigantism. During their first 24 or more extrauterine hours, they lie on their backs, bloated and flushed, their legs flexed and abducted, their lightly closed hands on either side of the head, the abdomen prominent and their respiration sighing. They convey a distinct impression of having had such a surfeit of both food and fluid pressed upon them by an insistent hostess that they desire only peace so that they may recover from their excesses. On the second day, their resentment of the slightest noise improves the analogy when their trembling anxiety seems to speak of intrauterine indiscretions of which we know nothing.

The hypoglycemia in infants of diabetic mothers is caused by hyperinsulinemia triggered by maternal hyperglycemia.^{58,59} Infants born to diabetic mothers also have a subnormal surge in plasma glucagon immediately after birth, subnormal glucagon secretion in response to stimuli, and (initially) excessive sympathetic activity that may lead to adrenomedullary exhaustion because urinary excretion of epinephrine is diminished.^{60,61} Thus despite their abundance of tissue stores of available substrate, the normal plasma hormonal pattern of low insulin, high glucagon, and catecholamines is reversed. Their endogenous glucose production is inhibited and glucose utilization is increased compared with that in normal infants, thus predisposing them to hypoglycemia.

Mothers whose diabetes has been well controlled during pregnancy in general, have near-normal-sized infants who are less likely to develop neonatal hypoglycemia and other complications formerly considered typical of maternal diabetes. Nevertheless, treatment of infants born to mothers with diabetes

commonly requires provision of IV glucose for a few days, until the hyperinsulinemia abates.

During labor and delivery, maternal hyperglycemia should be avoided because it may result in fetal hyperglycemia—which predisposes to rebound hypoglycemia when the glucose supply is interrupted at birth. Other maternal factors that can result in transient neonatal hyperinsulinism include oral hypoglycemics (such as sulfonylureas) or other medications (terbutaline or propranolol).

By definition, transient hyperinsulinism, as a cause of neonatal hypoglycemia in an infant of a diabetic mother, should abate in 1 or 2 days. If the condition persists, prolonged or congenital hyperinsulinism must be considered.

Perinatal Stress-Induced Hyperinsulinism

The process of beta cell maturation after birth may be impacted by perinatal factors resulting in perinatal stress-induced hyperinsulinism, a distinct form of hyperinsulinism that spontaneously resolves within the first few weeks of life. Perinatal factors associated with perinatal stress-induced hyperinsulinism include birth asphyxia, maternal preeclampsia, prematurity, intrauterine growth retardation, and other peripartum stress.^{42–44} Up to 50% of neonates in these at-risk categories may be affected.⁶² The estimated incidence of perinatal stress-induced hyperinsulinism is 1:6,000 to 12,000 live births.⁴⁴

The clinical presentation of perinatal stress-induced hyperinsulinism is characterized by high glucose utilization, and the response to fasting hypoglycemia shows inappropriately detectable plasma insulin, low plasma β -hydroxybutyrate and free fatty acid concentrations, and inappropriate glycemic response to glucagon at the time of hypoglycemia. Unlike the transient hyperinsulinism seen in the infant of the diabetic mother, perinatal stress-induced hyperinsulinism may persist for several weeks. In a series of neonates with perinatal stress-induced hyperinsulinism, the median age of resolution was 6 months.⁴⁴ The mechanism responsible for the dysregulated insulin secretion is not known. Acute insulin responses show that, in general, the patterns of insulin response to secretagogues (calcium, tolbutamide, glucose, and leucine) in infants with perinatal stress-induced hyperinsulinism resembled those of normal controls.⁴⁴

Infants with perinatal stress-induced hyperinsulinism usually respond very well to medical therapy with diazoxide.^{42–44} Previously, it was common practice to use pharmacologic doses of glucocorticoids to treat such neonates with persistent hypoglycemia. However, the use of glucocorticoids as nonspecific therapy for neonatal hypoglycemia is not recommended because they are not only ineffective, but also can suppress the hypothalamic-pituitary-adrenal axis.

Monogenic Hyperinsulinism

Congenital hyperinsulinism, also known as *persistent hyperinsulinemic hypoglycemia of infancy*, represents a group of clinically and genetically heterogeneous disorders characterized by dysregulated insulin secretion and resulting in severe and persistent hypoglycemia. First described in 1954 by MacQuarrie⁶³ as “idiopathic hypoglycemia of infancy,” congenital hyperinsulinism is the most common cause of persistent hypoglycemia in neonates, infants, and children. Worldwide, the incidence of congenital hyperinsulinism is estimated at 1 in 50,000 live births, with higher incidence of up to 1 in 2500 in areas of high consanguinity.⁶⁴

To understand the pathophysiology of congenital hyperinsulinism, knowledge of the major pathways regulating insulin secretion by pancreatic β -cells is critical (outlined in Fig. 7.5). Glucose-stimulated insulin secretion involves glucose entry

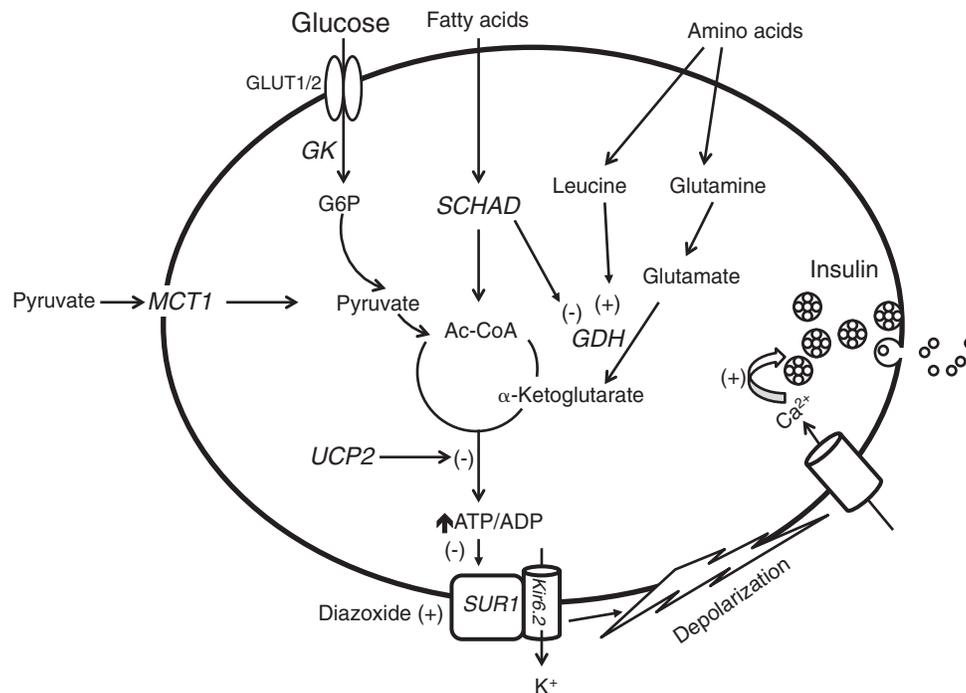


Fig. 7.5 Current model of mechanisms of insulin secretion by the beta cell of the pancreas. Glucose transported into the beta cell by the insulin-independent glucose transporter GLUT2/GLUT1 undergoes phosphorylation by glucokinase and is then metabolized, resulting in an increase in the adenosine triphosphate/adenosine diphosphate (ATP/ADP) ratio. The increase in the ATP/ADP ratio closes the K_{ATP} channel and initiates the cascade of events characterized by increase in intracellular potassium concentration, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (GDH) and by increasing the oxidation of glutamate, thereby increasing the ATP/ADP ratio and closure of the K_{ATP} channel. Diazoxide inhibits insulin secretion by stimulating the K_{ATP} channel. The gene defects known to cause hyperinsulinism are shown in italics. Six are inactivating mutations: *SUR1* (sulfonylurea receptor), *Kir6.2* (potassium channel), *SCHAD* (short-chain 3-OH acyl-CoA dehydrogenase), *UCP2* (uncoupling protein 2), *HNF4A* (hepatic nuclear transcription factor 4 alpha), and *HNF1A* (hepatic nuclear transcription factor 1 alpha). Three are activating mutations: *GK* (glucokinase), *GDH* (glutamate dehydrogenase), and *MCT1* (monocarboxylate transporter 1). (–), Inhibition; (+), stimulation.

to the beta cell by facilitated diffusion through glucose transporters (mainly GLUT1 in human beta cells)⁶⁵ and phosphorylation by glucokinase (GK), leading to glucose oxidation and a rise in the ATP/adenosine diphosphate (ADP) ratio that results in closure of the plasma membrane ATP-dependent potassium (K_{ATP}) channel. The β -cell K_{ATP} channel is a heteroameric complex consisting of two subunits: a K^+ -selective pore-forming subunit (*Kir6.2*), and a regulatory subunit (sulfonylurea receptor 1 [*SUR-1*]). Four *Kir6.2* subunits form the central pore, coupled to four *SUR-1* subunits. The K_{ATP} channel is inhibited (closed) by sulfonylurea drugs (used therapeutically to stimulate insulin secretion in type 2 diabetes), and activated (opened) by diazoxide (the first-line medical treatment for congenital hyperinsulinism). In the unstimulated state, the β -cell ATP-sensitive potassium channels are open—keeping a resting membrane potential of approximately -65 mV. Following the uptake and metabolism of glucose, increase in the intracellular ATP/ADP ratio results in closure of ATP-sensitive potassium channels, depolarization of the cell membrane, opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx, rise in cytosolic free Ca^{2+} concentration, and activation of the exocytotic machinery.⁶⁶ Stimulation of insulin secretion by amino acids occurs through allosteric activation of glutamate dehydrogenase (GDH) by leucine, which results in increased oxidation of glutamate—leading to a rise in the ATP/ADP ratio, inhibition of K_{ATP} -channel activity, and membrane depolarization.

Mutations in genes encoding 10 factors involved in different steps of this pathway have been associated with congenital hyperinsulinism: *SUR-1*, a member of the superfamily of ATP-binding cassette proteins, encoded by *ABCC8*;⁶⁷ *Kir6.2*,

encoded by *KCNJ11*;⁶⁸ *GK*, encoded by *GCK*;⁶⁹ *GDH*, encoded by *GLUD1*;⁷⁰ the mitochondrial enzyme short-chain 3-hydroxyacyl-CoA dehydrogenase (*SCHAD*), encoded by *HADH*;⁷¹ monocarboxyl transporter-1 (*MCT-1*), encoded by *SLC16A1*;⁷² uncoupling protein-2 (*UCP2*), encoded by *Ucp2*;⁷³ hepatocyte nuclear factor 4 alpha (*HNF4A*), encoded by *HNF4A*;⁷⁴ hepatocyte nuclear factor 1 alpha (*HNF1A*), encoded by *HNF1A*;⁷⁵ and forkhead box A2 (*Foxa2*), encoded by *FOXA2*.⁷⁶ In this chapter, we review the forms more likely to present in the neonatal period. For a discussion of *MCT-1* hyperinsulinism, see Chapter 23.

K_{ATP} Hyperinsulinism. Inactivating mutations of the beta cell K_{ATP} channels are responsible for the most common and most severe form of congenital hyperinsulinism. According to the severity of the molecular defect and the phenotype, K_{ATP} HI can be classified into three subtypes: (1) recessive, diazoxide-unresponsive; (2) dominant, diazoxide-unresponsive; and (3) dominant, diazoxide-responsive. Recessive mutations interfere with protein expression or channel trafficking, resulting essentially in a complete absence of channels on the plasma membrane, and therefore diazoxide is not effective. Recessive mutations are the most common defects identified in children with congenital hyperinsulinism. Dominant mutations allow normal trafficking of the channels to the plasma membrane, but impair channel activity either completely or partially, resulting in a spectrum of phenotypes that go from severe, diazoxide-unresponsive to mild, diazoxide-responsive.⁷⁷

The clinical presentation of K_{ATP} HI then depends on the severity of the mutation effect. More commonly, these children are large for gestational age and have severe neonatal

hypoglycemia that does not respond to diazoxide, except for the cases with a dominant diazoxide-responsive subtype in which birth weight is normal and the clinical presentation tends to occur later in life.⁷⁸

The pathophysiology of K_{ATP} -HI has been illustrated by functional evaluation of islet function *in vivo* and *in vitro*.^{79–82} The absence of functional K_{ATP} channels results in uncoupling between plasma glucose and insulin secretion or β -cell “glucose blindness.” Thus insulin secretion is not turned off, as plasma glucose decreases and fails to increase in response to a rapid raise in plasma glucose (Fig. 7.6). The former defect dominates the clinical manifestations in early infancy, whereas the latter may play a role in later development of glucose intolerance and, possibly, diabetes. In marked contrast with the failure to respond to changing concentrations of plasma glucose, these β -cells are hyperresponsive to stimulation with amino acids,⁸² which results in protein-induced hypoglycemia.⁸³

Other clinical features of infants with K_{ATP} -HI include extremely high glucose requirements, frequently 4 to 5 times higher than normal to control plasma glucose (although in some cases the requirement may be normal), feeding aversion, and gastroesophageal reflux, likely consequences of force feeding. Treatment options for the diazoxide-unresponsive cases are limited. These infants may require pancreatectomy, within the first few weeks after birth, to manage the hypoglycemia. On the other hand, diazoxide is very effective for controlling hypoglycemia in cases with dominant diazoxide-responsive K_{ATP} -HI. Octreotide, a long-acting somatostatin analog, is a second-line medical therapy for infants unresponsive to diazoxide, although the US Food and Drug Administration (FDA) has not approved it for this indication, and because of concerns with a possible association with necrotizing enterocolitis, its use should be carefully considered.^{84,85} A nonsurgical approach in diazoxide-unresponsive hyperinsulinism consists of octreotide in combination with continuous or frequent enteral feedings or continuous dextrose through a gastrostomy.^{86,87} This approach is challenging for home management of these children, and it requires close supervision and frequent glucose testing.

There are two distinctive histologic forms of K_{ATP} -hyperinsulinism, focal and diffuse hyperinsulinism.

Focal K_{ATP} -Hyperinsulinism (Focal Adenomatosis). Approximately 40% to 60% of the cases of K_{ATP} -HI (which require surgery) have focal disease.⁸⁸ Focal lesions arise by a “two-hit” mechanism: (1) a paternally inherited mutation of the *ABCC8* or *KCNJ11* gene (located on the 11p15 chromosome), and (2) a deletion of maternally inherited 11p15 chromosomal region, compensated by a paternal uniparental disomy, as observed in some cases of BWS. The 11p15 region, which carries the *ABCC8* and *KCNJ11* genes, contains several imprinted genes: insulin-like growth factor 2 (*IGF2*), a growth-promoting protein is expressed from the paternal chromosome, whereas, *H19*, a long noncoding ribonucleic acid (RNA) growth suppressor and *CDKN1C*, a cell cycle regulator and growth inhibitor, are expressed from the maternal chromosome. Loss of these growth-suppressing genes, while the paternal growth-promoting gene is active, play an important permissive role in the clonal expansion of the cells expressing the mutated channel.⁸⁹

Diffuse Hyperinsulinism. In diffuse hyperinsulinism, all β -cells in the pancreas are affected. It results from the inheritance of two recessive mutations in *ABCC8* or *KCNJ11* or a dominant mutation in these genes.

Neonates with the diffuse hyperinsulinism are more likely to present at birth, whereas those with focal form may fail detection in the neonatal period and present at several weeks to months of life with hypoglycemia seizures.⁹⁰ However, because of significant overlap in clinical presentation, clinical features alone cannot be used to distinguish between the two forms. Genetic testing offers the best means of identifying infants with focal K_{ATP} -HI: a single recessive paternally inherited mutation in *ABCC8* or *KCNJ11* has a positive predictive value of 94% for focal hyperinsulinism.⁹¹

Histologically, diffuse and focal hyperinsulinism are distinct.⁹⁰ Diffuse hyperinsulinism is characterized by the presence of abnormally large islet cell nuclei distributed throughout the pancreas. In contrast, the pancreatic histology in focal hyperinsulinism is characterized by a lesion formed by the confluence of hyperplastic islets occupying more than 40% of the cross-sectional area of pancreatic lobules. In contrast to true adenomas, the focal adenomatous hyperplasia includes exocrine acinar cells intermixed within the lesion. The morphology of islets away from the focal lesion is

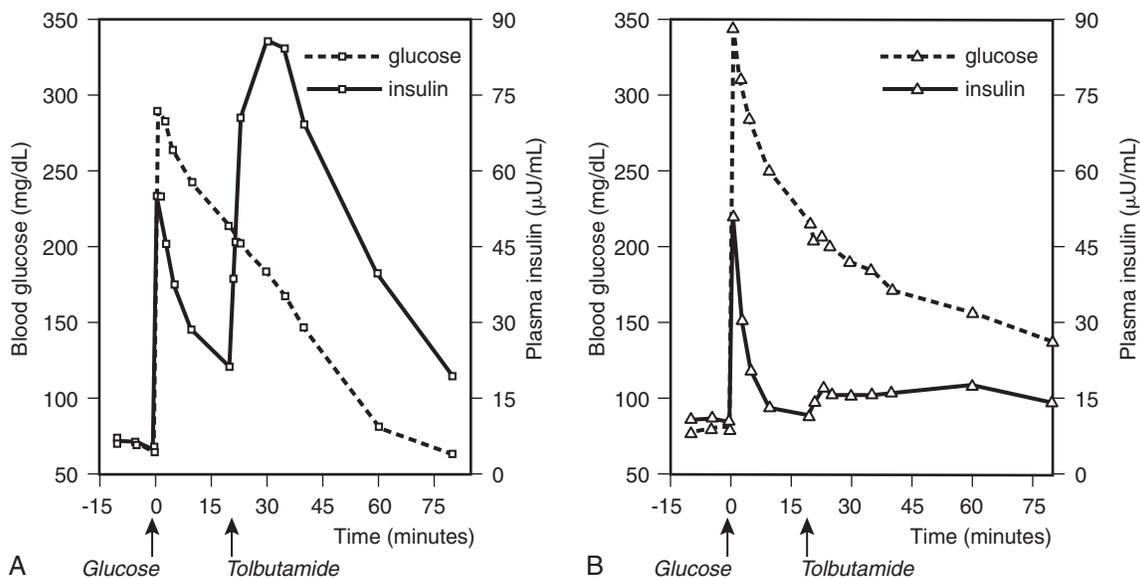


Fig. 7.6 Acute insulin response to glucose and tolbutamide in children with diffuse K_{ATP} hyperinsulinism (mean 11- and 13-minute increments). **A**, Normal adult control. **B**, Patient with diffuse K_{ATP} hyperinsulinism. (From Grimberg, A., Ferry, R.J., Kelly, A., et al. (2000). Dysregulation of insulin secretion in children with congenital hyperinsulinism caused by sulfonylurea receptor mutations. *Diabetes*, 50, 322.)

normal.⁹² The ability to interpret these histologic characteristics requires specialized training and is only available at centers with dedicated multidisciplinary teams to evaluate and treat hyperinsulinism.

Focal K_{ATP} HI is curable by surgery, whereas surgery is only palliative for diffuse K_{ATP} HI. Therefore efforts to diagnose and localize focal lesions in infants with diazoxide-unresponsive hyperinsulinism before surgery are critical. Conventional imaging techniques, such as computed tomography (CT) or magnetic resonance imaging, are unable to detect focal lesions. Interventional radiology studies, such as transhepatic portal venous insulin sampling⁹³ and selective pancreatic arterial calcium stimulation,⁹⁴ have only modest success, are technically difficult, and are highly invasive. The gold standard technique to localize the focal lesions is positron emission tomography (PET) scanning with fluorine-18 L-3, 4-dihydroxyphenylalanine (¹⁸F-fluoro-L-DOPA).^{95–97} Pancreatic β -cells take up L-DOPA,⁹⁸ and DOPA decarboxylase is active in pancreatic islet cells.⁹⁹ In children with focal hyperinsulinism, there is localized accumulation of ¹⁸F-fluoro-L-DOPA (Fig. 7.7). Coregistration of PET and CT images allows the anatomic localization of the lesion (Fig. 7.8).

Glutamate Dehydrogenase-Hyperinsulinism: The Hyperinsulinism Hyperammonemia Syndrome. Congenital hyperinsulinism caused by gain-of-function mutations of *GLUD1* (encoding GDH) is the second most common form of genetic hyperinsulinism and the most common form that responds to treatment with diazoxide.^{70,100–103} In approximately 70% of the cases, the mutations are de novo.¹⁰⁴ Among the 30% of familial cases, a clear autosomal dominant pattern of inheritance is evident.

GDH is a mitochondrial matrix enzyme that is a key regulator of amino acid and ammonia metabolism in pancreatic β -cells, liver, kidney, and brain. As shown in Fig. 7.5, GDH is allosterically activated by leucine to oxidize glutamate to α -ketoglutarate, which enters the Krebs cycle, resulting in an increased ATP/ADP ratio—which triggers insulin release. The HI/HA mutations affect either the inhibitory guanosine-5'-triphosphate (GTP)-binding site or the antenna loop of the enzyme, which communicates between adjacent subunits, impairing the inhibitory allosteric effect of GTP on GDH enzyme activity, thus leading to excessive insulin release.

The clinical presentation of HI/HA is characterized by fasting and protein-induced hypoglycemia and persistent but

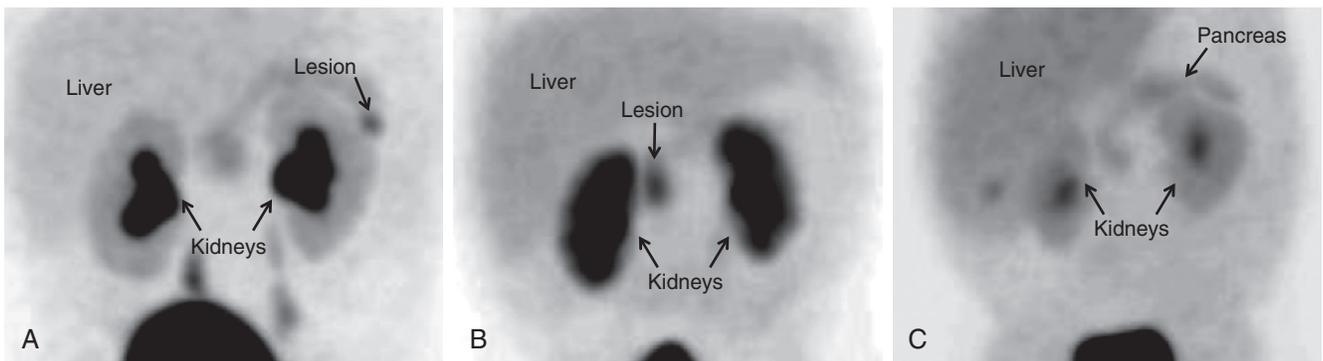


Fig. 7.7 ¹⁸Fluoro-L-DOPA PET scan images showing L-DOPA uptake in the liver, kidneys, and pancreas. Note increased uptake in the tail (A) and head (B) of the pancreas demonstrating focal lesions. In contrast, diffuse hyperinsulinism uptake throughout the pancreas is uniform (C).

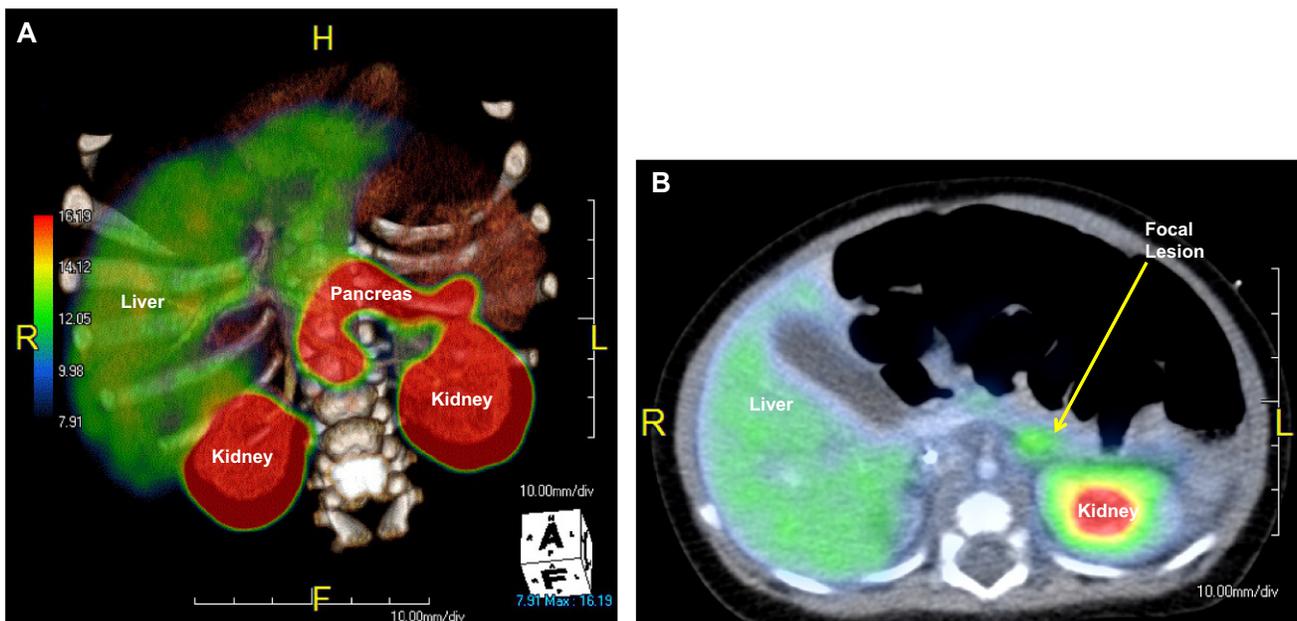


Fig. 7.8 A, Frontal view of fused positron emission tomography (PET)/computed tomography (CT) scan image of a diffuse case of hyperinsulinism showing L-3, 4-dihydroxyphenylalanine (L-DOPA) uptake in the liver, in the kidneys, and throughout the pancreas with equal intensity. **B**, Axial image of a contrast-enhanced CT scan fused with an ¹⁸Fluoro-L-DOPA PET scan showing a focus of increased uptake in the posterior body of the pancreas. Note the normal tracer uptake in the liver and left kidney. F, Feet; H, head; L, left; R, right. (From Dr. Lisa States.)

asymptomatic elevation of plasma ammonia.¹⁰⁵ Infants with HI/HA have normal birth weight and typically are not diagnosed until later in infancy. Plasma ammonia levels in HI/HA are elevated 3 to 5 times above the normal range to approximately 60 to 150 $\mu\text{mol/L}$. Ammonia levels are quite constant, and in contrast to the urea cycle enzyme defects, do not increase with protein feeding. The hyperammonemia does not appear to cause symptoms and does not require treatment.

The clinical phenotype of HI/HA is dominated by the effects of the activating mutations in the pancreatic β -cells. Isolated islets from transgenic mice expressing a mutated human GDH exhibit normal glucose-stimulated insulin secretion but enhanced leucine- and amino-acid-stimulated insulin secretion.¹⁰⁶ In children with HI/HA, there is a dramatic increase in insulin following an IV bolus of leucine, but in contrast to children with K_{ATP} -HI, they do not respond to calcium stimulation.¹⁰⁷ The hypoglycemia in children with HI/HA is provoked by fasting and by protein-rich meals. The fasting hypoglycemia may be relatively mild. Children may be able to fast for 8 to 12 hours before experiencing hypoglycemia. However, these patients have dramatic protein-induced hypoglycemia—with plasma glucose concentration dramatically decreasing 30 to 90 minutes after a protein meal¹⁰⁵ (Fig. 7.9). Diazoxide therapy, 5 to 15 mg/kg/day, is usually effective in controlling both fasting and protein-induced hypoglycemia in HI/HA. Carbohydrate preloading may be helpful in avoiding the latter.¹⁰⁴

Initially, the hyperammonemia was presumed to be caused by the effects of the mutated enzyme in the liver; however, an alternative mechanism involving activation of renal GDH seems to be more likely.¹⁰⁸ The hyperammonemia is persistent and not associated to the classic signs of ammonia toxicity. It occurs in both the fed and fasted states and is unaffected by plasma glucose concentrations or protein intake.

In addition to hypoglycemia and hyperammonemia, patients with HI/HA syndrome have an increased frequency of seizures, learning disability, and behavioral disorders that are not directly linked to hypoglycemia.^{109,110} Studies describing the neurologic manifestations of HI/HA are limited; however, available evidence suggests that 42% to 64% of patients have generalized epilepsy that is characterized by high-amplitude generalized spike and wave discharges on

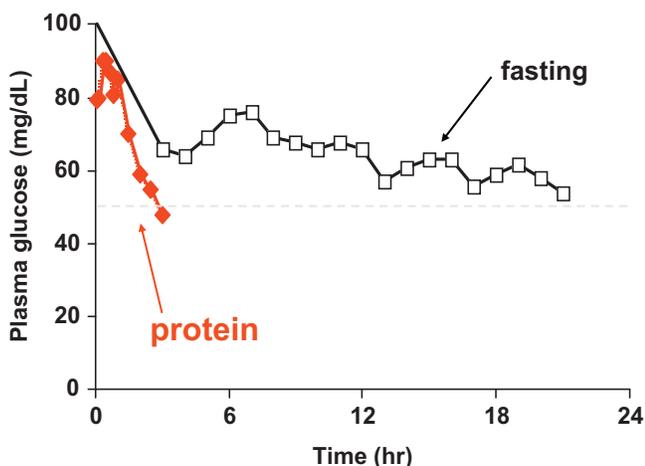


Fig. 7.9 Plasma glucose responses to fasting (open squares) and protein feeding (solid diamonds) in a 16-year-old girl with the hyperinsulinism/hyperammonemia syndrome caused by a dominantly expressed R269H regulatory mutation of glutamate dehydrogenase. (From Hsu, B.Y., Kelly, A., Thornton, P.S., et al. (2001). Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr*, 138, 383.)

electroencephalogram (EEG).^{109,111} The pathophysiology of the neurologic manifestations has not been elucidated. A proposed mechanism involves GDH overactivity in brain cells, leading to disruption of central nervous system glutamate pools, resulting in an imbalance between the major excitatory and inhibitory neurotransmitters, glutamine and γ -aminobutyric acid (GABA), respectively.¹¹¹

SCHAD-Hyperinsulinism. A less common form of congenital hyperinsulinism, also involving loss of regulation of GDH activity, is caused by inactivating mutations in *HADH* (the gene encoding the mitochondrial SCHAD).^{71,112,113} SCHAD-HI is an autosomal recessive disorder characterized by fasting hypoglycemia caused by inappropriate insulin regulation. The cause of dysregulated insulin secretion was elucidated with the discovery that in the β cell, SCHAD plays an inhibitory role in GDH activity.¹¹⁴ Thus the insulin dysregulation observed in SCHAD deficiency is caused by loss of this inhibition, explaining the clinical similarities between GDH-HI and SCHAD-HI.

In contrast to all other defects in fatty acid oxidation, children with SCHAD-HI have no signs of hepatic dysfunction or cardiomyopathy, or of effects on skeletal muscles. The clinical presentation of SCHAD-HI is heterogeneous, ranging from late onset of mild hypoglycemia to severe early onset of hypoglycemia in the neonatal period. In addition to fasting hypoglycemia, children with SCHAD-HI exhibit protein-induced hypoglycemia, similar to the children with GDH-HI.¹¹⁵ The biochemical hallmarks, in addition to markers of increased insulin action, are increased concentration of plasma 3-hydroxybutyryl-carnitine and increased urine concentration of 3-hydroxyglutarate, findings consistent with reduced SCHAD enzyme activity. In contrast to GDH-HI, children with SCHAD-HI do not have elevated ammonia, probably because of the lower expression of SCHAD in other tissues where GDH is expressed. Affected children are responsive to medical therapy with diazoxide.

Glucokinase-Hyperinsulinism. A less frequent form of congenital hyperinsulinism is caused by activating mutations in *GCK* (encoding glucokinase-GK). Glucokinase is a hexokinase that serves as the glucose sensor in pancreatic β -cells¹¹⁶ (see Fig. 7.5). In contrast to other hexokinases, GK has a much lower affinity for its substrate with half-maximal activity ($S_{0.5}$) at a glucose concentration of 7.6 mM. The properties of GK and the positive cooperative action of glucose on enzyme activity, with a curve that is steep in the range of 5 mM glucose, make GK well suited to tightly control plasma glucose in the normal physiologic range of 70 to 100 mg/dL.²⁵ Mutations in *GCK* alter the kinetics of glucokinase by increasing the enzyme affinity for glucose and cause hypoglycemia by lowering the threshold for glucose-stimulated insulin secretion in the beta cell.^{69,117,118} In contrast, heterozygous inactivating *GCK* mutations lead to maturity-onset diabetes of youth type 2 (MODY 2)¹¹⁹ and homozygous inactivating *GCK* mutations are a cause of permanent neonatal diabetes.¹²⁰ Some GK-HI cases are sporadic, and some are dominantly inherited. All of the mutations have reduced glucose $S_{0.5}$ ranging from 1.1 to 4.5 mM.

The clinical presentation of GK-HI is characterized by large for gestational age birth weight, reflecting the growth-promoting effects of increased fetal insulin secretion. Hypoglycemia may present in the neonatal period, but often it is not recognized until later in infancy or childhood. The severity of the phenotype is variable, with some mutations having a mild phenotype, with fasting hypoglycemia responsive to diazoxide, whereas others seem to lower the glucose threshold further and may be more difficult to treat. Based on our experience, diazoxide is uniformly unable to prevent hypoglycemia in children with GK-HI. Treatment with somatostatin analogues has been tried in some

children with GK-HI, with various success. Although some cases have undergone pancreatectomy, most children have continued to have hypoglycemia and required additional medical management after surgery. In some cases, it is necessary to provide continuous support with overnight feedings or glucose to prevent hypoglycemia.

From the few cases that have undergone a pancreatectomy, pancreatic histology has been reported to have little or no abnormal features.^{121–123} Others have reported nuclear enlargement and increased islet size and β -cell proliferation.^{124,125}

Uncoupling Protein-2 Hyperinsulinism. Loss-of-function mutations in the mitochondrial uncoupling protein UCP2 are associated with a dominant, diazoxide-responsive form of congenital hyperinsulinism.⁷³ UCP2 transports protons and phosphate from the cytosol across the inner mitochondrial membrane, in exchange for oxaloacetate and malate. By depleting the pool of mitochondrial tricarboxylic acid cycle intermediates, UCP2 activity restricts glucose oxidation in favor of the oxidation of glutamine and amino acids.¹²⁶ Inactivating mutations of UCP2 then, enhance the oxidation of glucose in pancreatic beta cells, leading to amplification of the insulin response to glucose. Indeed, the phenotype of UCP2-HI is characterized by glucose-induced hypoglycemia in addition to fasting hypoglycemia.¹²⁷

Hepatocyte Nuclear Factors and Hyperinsulinism: HNF1A and HNF4A-Hyperinsulinism. HNF1A and 4A are transcription factors expressed in hepatocytes, pancreatic β -cells, intestinal epithelial cells, and renal tubular cells. In β -cells and hepatocytes, they form a feed-forward loop, such that the haploinsufficiency of either causes decreased expression of the other.¹²⁸ Both HNF1A and HNF4A play an important role on pancreatic β -cell function and heterozygous mutations in the genes encoding them: *HNF1A* and *HNF4A*, respectively, are known to cause familial monogenic diabetes (MODY 3 and MODY 1). These autosomal dominant forms of diabetes are characterized by progressive impairment in glucose-stimulated insulin secretion leading to frank hyperglycemia, usually manifesting before the age of 25 years.¹²⁹

More recently, it has been demonstrated that the phenotype in individuals with inactivating mutations in *HNF1A* and *HNF4A* is biphasic, characterized by persistent hypoglycemia caused by hyperinsulinism early in life, followed by reduced insulin secretion leading to diabetes in early adolescence and young adulthood.^{74,75,130–133} Together, mutations in *HNF1A* and *HNF4A* account for approximately 5.9% of all cases of hyperinsulinism in which a mutation is identified.¹³³

The clinical phenotype of HNF1A- and HNF4A-HI is characterized by large birth weight, although some cases have normal birth weight, and persistent hypoglycemia that is for the most part diazoxide-responsive.¹³⁴ The hyperinsulinism is transient, with some cases resolving within the first few months of life,

whereas in others the hyperinsulinism phase may persist into childhood.

The phenotype in children with a particular mutation in *HNF4A* (p.Arg76Trp) involves extrapancreatic features, including hepatomegaly with elevated transaminases and renal Fanconi tubulopathy, similar to Fanconi-Bickel syndrome.^{75,135,136}

The precise mechanism by which mutations in *HNF1A* and *HNF4A* lead to hyperinsulinism is currently unknown, but it is likely that the defects in these transcription factors alter different gene expression patterns during early and later in life.

FOXA2 Hyperinsulinism. A novel form of hyperinsulinism associated with hypopituitarism was recently described in individuals with inactivating mutations in *FOXA2*.^{76,137} Foxa2 is a transcription factor that is involved in the development of multiple tissues, including the pituitary gland, and also plays an important role in insulin secretion by mature beta cells. The reported cases presented with hypoketotic hypoglycemia in the neonatal period that responded to replacement of pituitary hormones and treatment with diazoxide.

Syndromic Forms of Hyperinsulinism

Several genetic syndromes are now recognized to be associated with hyperinsulinism, including BWS, Kabuki syndrome, and Turner syndrome. Typical clinical features that distinguish these syndromes may be more subtle in the neonatal period, therefore a high index of suspicion and careful clinical evaluation are essential to make the diagnosis.

Hyperinsulinism in Beckwith-Wiedemann Syndrome. BWS is an overgrowth and cancer predisposition syndrome that results from various genetic and epigenetic changes involving two imprinted control centers on chromosome 11p15.5. This region can be divided into two distinct imprinted domains separated by a nonimprinted region. The distal domain 1 contains *IGF2*, a growth-promoting protein which is paternally expressed, and *H19*, a long noncoding RNA growth suppressor expressed from the maternal allele. In the proximal domain 2, the imprinted region contains three genes that have been implicated in BWS: *KCNQ1OT1*, paternally expressed untranslated transcript; *CDKN1C*, which is maternally expressed and encodes a negative regulator of cell proliferation; and *KCNQ1*, a maternally expressed potassium repolarizing channel (Fig. 7.10). Fifty percent of all BWS cases are caused by loss of methylation at the imprinted control region 2, 20% of cases are caused by paternal uniparental isodisomy involving both imprinted gene clusters, 5% are caused by gain of methylation at imprinted control region 1, and 5% are caused by maternally inherited mutations in *CDKN1C*.¹³⁸

The classical features of BWS include macrosomia, macroglossia, visceromegaly, abdominal wall defects, ear creases/pits, body asymmetry, and an increased risk for embryonal tumor development. Hypoglycemia caused by hyperinsulinism is

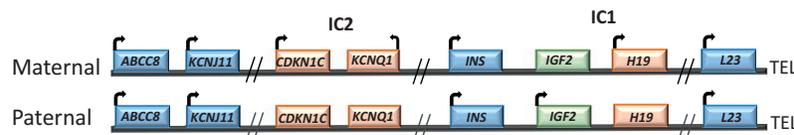


Fig. 7.10 Beckwith-Wiedemann syndrome locus on 11p15.5 outlining the normal parent of origin-specific allelic expression. Expressed genes are noted with an arrow. The genes encoding the K_{ATP} channels, *ABCC8* and *KCNJ11*, as well as insulin (*INS*) and *L23* are biallelically expressed, while *CDKN1C*, *KCNQ1*, *IGF2*, and *H19* are imprinted. IC1 is methylated on the paternal allele, resulting in paternal expression of *IGF2* and unmethylated on the maternal allele resulting in maternal expression of *H19*. IC2 is methylated on the maternal allele resulting in *KCNQ1* and *CDKN1C* expression and unmethylated on the paternal allele. *ABCC8*, ATP-binding cassette, subfamily C, member 8; *CDKN1C*, cyclin-dependent kinase inhibitor 1C; *IC*, imprinting center; *IGF2*, insulin-like growth factor 2; *INS*, insulin; *KCNJ11*, potassium channel, inwardly rectifying, subfamily J, member 11; *KCNQ1*, potassium channel, voltage-gated, KQT-like subfamily, member 1; *L23*, ribosomal protein L23-like; *TEL*, telomere.

present in up to 50% of cases.¹³⁹ The mechanism responsible for hyperinsulinism in BWS has not been elucidated, but may involve dysregulated insulin secretion and beta cell hyperplasia.^{140,141}

The hyperinsulinism in BWS may be mild and transient, although in some cases, it can be severe and persistent.¹³⁹ Some BWS infants respond to diazoxide, whereas others require pancreatectomy to control the hypoglycemia. Because the hyperinsulinism will resolve within the first year of life in most cases, pancreatectomy should be reserved for those cases in which the hypoglycemia cannot be medically controlled.

Hyperinsulinism in Kabuki Syndrome. Kabuki syndrome is the second most common cause of syndromic hyperinsulinism.¹⁴² Some 70% to 75% of cases of Kabuki syndrome are caused by autosomal recessive mutations in *KMT2D* (*lysine-specific methyltransferase 2D*), and X-linked mutations in *KDM6A* (*lysine-specific demethylase 6A*) account for 1% to 9% of cases.¹⁴³ Cardinal features of Kabuki syndrome include the typical “Kabuki Mask” facial characteristics (long palpebral fissure, arched eyebrows, eversion of the external third of the lower lid); skeletal abnormalities; dermatoglyphic abnormalities; neurodevelopmental delay and intellectual disabilities; and postnatal growth retardation. Endocrine manifestations include GH deficiency (22% of cases), hypothyroidism ($\leq 10\%$ of cases), premature thelarche (20%), and neonatal hypoglycemia (11%). The mechanism responsible for the neonatal hypoglycemia may be multifactorial: including hyperinsulinism and GH deficiency.^{144,145} Hyperinsulinism is a feature in up to 6% of Kabuki cases,¹⁴⁶ it is typically present from birth, diazoxide-responsive, and often appears to resolve during the first decade of life.

Hyperinsulinism in Turner Syndrome. It is well known that girls with Turner syndrome have a higher predisposition to both type 1 and type 2 diabetes,¹⁴⁷ but it has been less recognized that they are also predisposed to hypoglycemia.¹⁴⁸ There is increasing evidence that hypoglycemia in girls with Turner syndrome is caused by hyperinsulinism.^{149–153} Indeed, a higher than expected frequency of Turner syndrome among children with hyperinsulinism has been reported.¹⁵¹

Similar to other forms of hyperinsulinism, hyperinsulinism in girls with Turner syndrome more frequently presents shortly after birth and it is usually responsive to treatment with diazoxide, although, some of the reported cases have been unresponsive and severe requiring pancreatectomy. Although the mechanism responsible for hyperinsulinism in Turner syndrome has not been clearly established, a potential candidate gene on the X chromosome is *KDM6A*, a histone demethylase associated with Kabuki syndrome. Histological examination of the pancreas of a girl with Turner syndrome and hyperinsulinism showed increase islet cell nucleomegaly, similar to diffuse hyperinsulinism caused by inactivating K_{ATP} channel mutations. Islets isolated from this pancreas revealed an abnormal but distinct functional pattern, with increased basal cytosolic calcium and insulin, lower threshold for glucose-stimulated insulin secretion, increased sensitivity to stimulation by a mixture of amino acids, and preserved response to stimulation with glyburide.¹⁵¹

MANAGEMENT AND TREATMENT OF HYPERINSULINISM

Treatment of neonates with suspected congenital hyperinsulinism should be initiated promptly, as their risk of brain damage is high because their brain is deprived not only of glucose but also alternative fuels (ketones). Reports from multiple centers have demonstrated a high prevalence of developmental delays in patients with congenital hyperinsulinism, ranging from 30%

to 50%.^{50–54} Patients with hyperinsulinism, requiring surgical therapy, have a higher incidence of neurodevelopmental problems than diazoxide-responsive patients⁵³; however, abnormal neurodevelopment is also seen in up to 30% of infants with transient hyperinsulinism.¹⁵⁴ In general, the high risk of brain damage appears to be caused by delays in diagnosis and treatment rather than a consequence of the molecular defect itself, and thus is potentially preventable.

The goal of therapy is to restore plasma glucose to the normal range (>70 mg/dL) and to maintain it there. IV glucose at high rates, up to 5 times the normal glucose requirement, may be required and central lines may be needed for administration of highly concentrated dextrose. Glucagon, given as a continuous IV infusion (1 mg/day), can be used in infants requiring high glucose infusion rates as a temporary measure to reduce the risk of fluid overload.¹⁵⁵

The first line of therapy for hyperinsulinism is diazoxide, a K_{ATP} -channel opener that requires functional channels to be present at the cell surface to have an effect; thus most patients with K_{ATP} -HI do not respond to diazoxide. In contrast, patients with GDH-HI, HNF1A-HI, HNF4A-HI, SCHAD-HI, and those with perinatal stress-induced hyperinsulinism do respond to diazoxide. Patients with GK-HI are partially responsive to diazoxide and most require additional therapy. The pharmacologic dose range of diazoxide is 5 to 15 mg/kg/day, given orally twice a day. The major adverse event with diazoxide in neonates is fluid retention and therefore concomitant use of a diuretic (chlorothiazide or furosemide) is recommended, especially in infants receiving IV fluids. Pulmonary hypertension is the most serious potential complication; however, its occurrence is rare if appropriate steps are put into place to prevent it, particularly, therapy with diuretics. Other side effects associated with diazoxide include neutropenia, thrombocytopenia, and hyperuricemia.¹⁵⁶ Diazoxide therapy is also associated with significant hypertrichosis and coarsening of facial features.

Evaluation of whether the hyperinsulinism is responsive to diazoxide or not is critical because this determines the treatment path and also offers clues to the potential genetic cause of the hyperinsulinism. The half-life of diazoxide is 24 to 36 hours in adults; limited data indicate that in children, the half-life is 9.5 to 24 hours,¹⁵⁷ therefore the response to diazoxide should be evaluated after at least 5 days of therapy. Successful response should be demonstrated by showing maintenance of plasma glucose above 3.9 mmol/L (70 mg/dL) after fasting. The duration of the fast should be determined on a case-by-case basis, based in part on the age of the child.

Failure to respond to diazoxide suggests the possibility of K_{ATP} -HI and therefore genetic testing is critical to determine the likelihood of focal K_{ATP} -HI.⁹¹ Thus once the diagnosis is established and initial therapy is initiated, samples for the child and the child's parents should be obtained for deoxyribonucleic acid molecular analysis. Fig. 7.11 depicts the diagnostic and treatment approach for children with hyperinsulinism. A cardiac evaluation should also be considered in children with hyperinsulinism, as a large proportion of the cases have cardiac structural abnormalities, more commonly ventricular hypertrophy.¹⁵⁸

For children with diazoxide-unresponsive hyperinsulinism in whom focal hyperinsulinism has been ruled out, medical therapy with second-line agents should be considered, including somatostatin analogues. Somatostatin analogues inhibit insulin secretion by inducing hyperpolarization of β -cells, direct inhibition of voltage-dependent calcium channels, and more distal events in the insulin secretory pathway. The most frequently used somatostatin analogue is octreotide, which is administered SQ every 6 to 8 hours, at 5 to 20 μ g/kg/day. The initial response to octreotide is good in most infants with hyperinsulinism, but tachyphylaxis develops after a few doses,

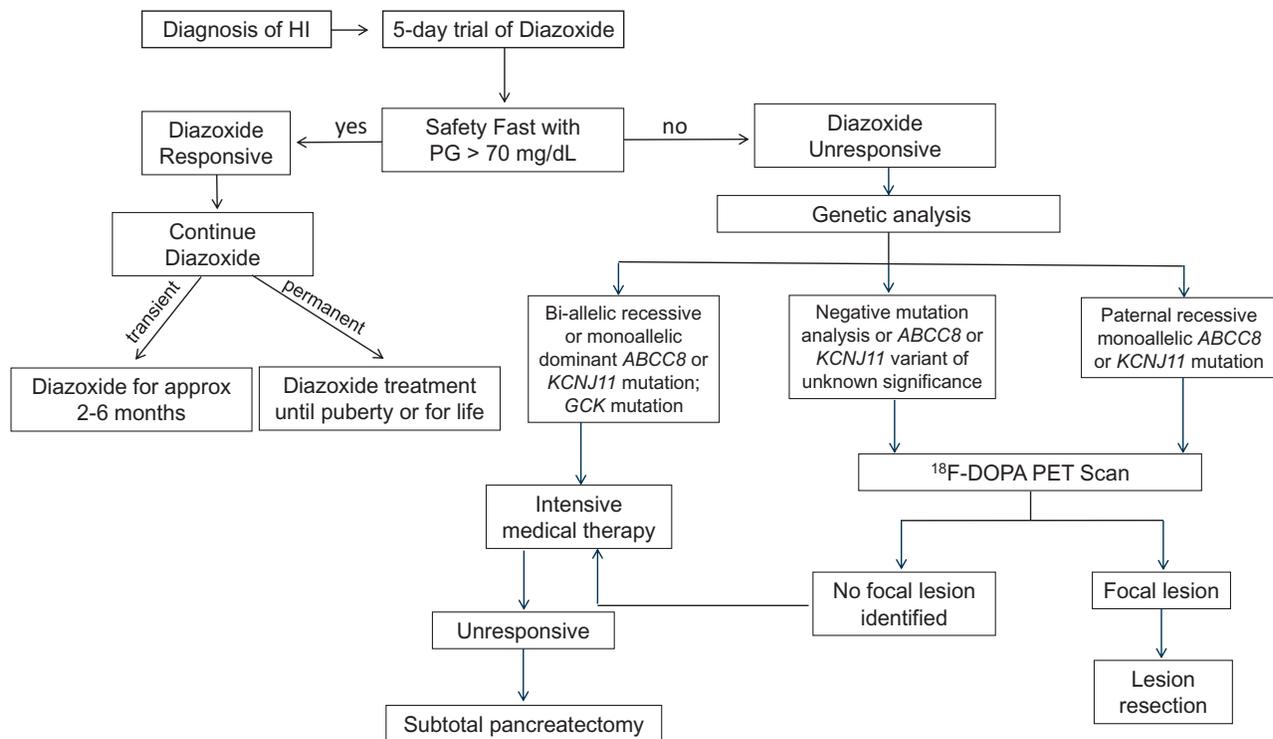


Fig. 7.11 Diagnostic and treatment approach in cases of hyperinsulinism.

rendering therapy inadequate for long-term use in most cases. An approach to avoid the need for multiple injections has been the use of SQ pumps for continuous administration of octreotide (e.g., off-label use of insulin pumps filled with octreotide).¹⁵⁹ A different approach limits dosing of octreotide to twice a day, used in combination with continuous overnight dextrose through a gastrostomy, in an effort to avoid tachyphylaxis.¹⁶⁰ The most frequent side effects of octreotide are transient elevation of liver enzymes, gall bladder pathology, and GH suppression.¹⁶¹ More serious side effects have also been reported, including necrotizing enterocolitis.^{85,162,163} Because of this association, the use of octreotide in infants less than 4 weeks of age or infants with other risk factors should be avoided. Long-acting somatostatin analogues, such as octreotide long-acting release and lanreotide autogel is favored for treatment of older children.^{164–166} Their use in younger children has been limited because dosing cannot be easily titrated with current formulations.

Hypoketotic Hypoglycemia Caused by Activating Mutations in Insulin Signaling Pathway

AKT2-Hypoketotic Hypoglycemia

Recessive gain-of-function mutations in *AKT2*, a key component of the insulin signaling cascade, cause a phenotype similar to hyperinsulinemic hypoglycemia, but without detectable plasma insulin levels, despite classical signs of increased insulin action (suppressed free fatty acids and ketones).¹⁶⁷ The phenotype in these children is characterized by severe, persistent hypoglycemia unresponsive to medical therapy and requiring continuous intragastric feedings. These children also had overgrowth with body asymmetry.

PI3K-Hypoketotic Hypoglycemia

Mosaic activating mutations in class 1A phosphatidylinositol-3-kinase (PI3K), which is upstream from *AKT2* in insulin

signaling, have also been described as a cause of hypoketotic hypoglycemia. The phenotype of the reported cases is characterized by early onset and severe hypoketotic hypoglycemia with undetectable insulin.¹⁶⁸

Acquired Postprandial Hyperinsulinemic Hypoglycemia after Fundoplication

The Nissen fundoplication and other modified fundoplication procedures, frequently performed for the treatment of gastroesophageal reflux in neonates and children, have been associated with late dumping syndrome, particularly postprandial hyperinsulinemic hypoglycemia. In a study in neonates, it was estimated that approximately 24% of those undergoing fundoplication developed postprandial hypoglycemia after the surgery.¹⁶⁹ Classically, dumping syndrome is characterized by early symptoms or “early dumping,” thought to be caused by the fluid shifts provoked by the osmotic load in the small bowel,¹⁷⁰ and “late dumping” or postprandial hypoglycemia.¹⁷¹ In our experience, dumping syndrome in children is characterized by severe postprandial hypoglycemia without the significant gastrointestinal symptoms observed in adults.¹⁷² Very frequently, children with postprandial hyperinsulinemic hypoglycemia, after fundoplication, experience seizures, lethargy, and other symptoms of hypoglycemia for several months before the diagnosis is made. In the neonate particularly, the hypoglycemia may not be recognized unless these infants are screened with postfeeding plasma glucose monitoring after the surgery.¹⁶⁹

The pathophysiology of hyperinsulinemic hypoglycemia after fundoplication is likely multifactorial. Studies of the motor and sensory function of the stomach after fundoplication have shown significantly reduced postprandial gastric relaxation and significantly accelerated gastric emptying.¹⁷³ The rapid emptying of a meal into the small intestine results in rapid absorption of glucose into the bloodstream, with early hyperglycemia followed by an exaggerated insulin surge and

subsequent hypoglycemia.¹⁷² Increased secretion of the potent insulinotropic hormone, glucagon-like peptide-1 (GLP-1), after a meal may be partly responsible for the postprandial hyperinsulinemia.¹⁷⁴ This is supported by evidence demonstrating that antagonism of the GLP-1 receptor with exendin-(9-39) blunts the insulin response, suggesting that the exaggerated insulin response to a meal is at least in part because of the effects of GLP-1 on the pancreatic beta cell.¹⁷⁵

The diagnosis of hyperinsulinemic hypoglycemia in neonates and children after fundoplication is established based on the glucose and insulin profile after a mixed meal tolerance test or an oral glucose tolerance test.¹⁷⁴ The typical response is characterized by hyperglycemia that can reach up to more than 11.1 mmol/L (200 mg/dL) within the first hour after the meal, followed by hypoglycemia at 90 to 120 minutes. Plasma insulin reaches a peak that is greater than 200 μ U/mL approximately 30 minutes after the meal. A variety of therapies have been used with varying success to treat dumping syndrome, including uncooked cornstarch,¹⁷⁶ pectin,¹⁷⁷ octreotide,¹⁷⁸ acarbose,¹⁷² and dietary manipulations.¹⁷⁹ Many of the patients suffering from postprandial hyperinsulinemic hypoglycemia continue to have severe symptoms, despite these interventions, and require a regimen of continuous enteral feedings to avoid hypoglycemia, but continue to be at high risk of hypoglycemic events if feedings are abruptly stopped. In most cases, the postprandial hyperinsulinemic hypoglycemia improves with time, especially after solids have been introduced to the diet.

Defects in Counterregulatory Response

Hypoglycemia associated with endocrine deficiency is usually caused by glucocorticoid or GH deficiency, more commonly the combination of both. In children with panhypopituitarism, isolated GH deficiency, or a combination of ACTH deficiency and GH deficiency, the incidence of hypoglycemia is as high as 20%. In the newborn period, hypoglycemia may be the presenting feature of hypopituitarism. In males, a micropallus and small testes may provide a clue to a coexistent deficiency of gonadotropin^{180,181} (Fig. 7.12). Newborns with hypopituitarism may also have liver dysfunction resembling cholestatic liver disease, and some have midline malformations, such as



Fig. 7.12 Micropenis and undescended testes in an infant with congenital hypopituitarism. The infant was hypoglycemic at 12 hours of age (glucose, 24 mg/dL). At 72 hours of age, he was jaundiced—and a liver biopsy demonstrated neonatal hepatitis. His endocrine evaluation was positive for hypothyroidism, low cortisol level, undetectable growth hormone level, and an elevated prolactin level (confirming hypothalamic hypopituitarism).

the syndrome of septooptic dysplasia.^{182–184} Clinically, such children may manifest nystagmus, or have mid-face hypoplasia syndromes, including cleft palate.

Although older infants and children with pituitary deficiency present with ketotic hypoglycemia in the neonatal period, the hypoglycemia may mimic hyperinsulinism, because mechanisms of ketogenesis are not fully developed in the immediate newborn period. However, their hypoglycemia is not responsive to diazoxide and only remits with replacement of deficient hormones (including thyroxine, as well as GH and cortisol). Of note, mutations in the transcription factor *Foxa2* cause both hypopituitarism and hyperinsulinism and present in the neonatal period with persistent hypoglycemia.^{76,137}

When adrenal disease is severe, as in congenital adrenal hyperplasia, bilateral adrenal hemorrhage, congenital absence or hypoplasia of the adrenals, or various syndromes of ACTH resistance,^{185–187} hyperpigmentation, disturbances in serum electrolytes with hyponatremia and hyperkalemia, or ambiguous genitalia may provide diagnostic clues. Abnormalities of the ACTH receptor or adrenal hypoplasia may be phenotypically difficult to distinguish from cortisol deficiency of other causes, with the exception of the marked elevations of serum ACTH concentration, noted if the ACTH receptor or adrenal gland is malfunctioning.^{188,189} However, all states with ACTH elevations may be clinically suspected by virtue of the associated hyperpigmentation (see Chapter 14). These cases of isolated adrenal insufficiency very rarely cause hypoglycemia in neonates, whereas in older infants and children, treated congenital adrenal hyperplasia and isolated ACTH deficiency may cause profound stress-induced hypoglycemia. Congenital ACTH deficiency caused by mutation in *TPIT*, which encodes *TBX19*, a transcription factor that is required for the expression of the proopiomelanocortin (*POMC*) gene and for terminal differentiation of the pituitary corticotroph lineage, is the exception, presenting in the neonatal period with severe and persistent hypoglycemia, until hormonal replacement is established.¹⁹⁰ It is important to have in mind that a low GH and cortisol level at the time of hypoglycemia is not diagnostic of a true deficiency¹⁹¹ and provocative testing may be needed to establish the diagnosis.

The mechanism of hypoglycemia in GH deficiency may be the result of decreased lipolysis. The mechanism of hypoglycemia with cortisol deficiency may be reduced liver glycogen reserves plus diminished gluconeogenesis, owing to a failure to supply endogenous gluconeogenic substrate in the form of amino acids from muscle proteolysis. Investigation of a child with hypoglycemia therefore requires exclusion of ACTH, cortisol, or GH deficiency—and if diagnosed, appropriate replacement with cortisol or GH. Cortisol replacement at stress dose concentrations should be the first line of treatment and GH replacement given only if glucocorticoid therapy alone fails to control hypoglycemia. There is evidence that the synthesis and secretion of GH in the newborn requires cortisol, so that apparent GH deficiency resolves with cortisol treatment alone.¹⁹²

Although glucagon deficiency^{193,194} had been described as a cause of hypoglycemia, the authors have retracted their conclusions and now believe these patients had SCHAD hyperinsulinism; thus this disorder is exceedingly rare and may be nonexistent. Epinephrine deficiency or deficiency of its action is also rare but must be a consideration in familial dysautonomia or in children treated with adrenergic receptor blockers, such as beta-blockers.

Defects in Glycogenolysis and Gluconeogenesis

Most of the disorders of glycogenolysis and gluconeogenesis that result in hypoglycemia are usually diagnosed in childhood

and will be discussed in [Chapter 23](#). The exception is GSD I, which impairs the release of glucose from glycogenolysis and gluconeogenesis and presents in the neonatal period, although in some cases, it is diagnosed later.

Glucose 6-Phosphatase Deficiency (GSD Type 1)

Glycogen storage disease type 1 is an autosomal recessive disorder caused by defects in the glucose 6-phosphatase (G6Pase) complex, which catalyzes the terminal steps of both hepatic gluconeogenesis and glycogenolysis, the hydrolysis of glucose 6-phosphate to glucose and inorganic phosphate. Deficiency of G6Pase activity in liver, kidney, and intestine results in the accumulation of glycogen in these organs, fasting hypoglycemia as a result of inadequate glucose production, and other secondary biochemical abnormalities, including hyperlacticacidemia, hyperuricemia, and hyperlipidemia.

G6Pase is a multicomponent complex consisting of a catalytic unit, G6Pase, localized on the luminal side of the endoplasmic reticulum, and a G6P-specific bidirectional translocase (G6PT) that allow the entry of glucose 6-phosphate to the catalytic unit.¹⁹⁵ The gene for the catalytic unit is located on chromosome 17,¹⁹⁶ whereas the gene for the glucose 6-phosphate translocase is located on chromosome 11.¹⁹⁷ Mutations in the catalytic unit cause GSD type 1a,¹⁹⁸ whereas mutations in G6PT cause GSD type 1b.¹⁹⁷ The phenotype for these two subtypes is identical, except that in type 1b, in addition to the liver phenotype, there are recurrent bacterial infections and inflammatory bowel disease associated with neutropenia and neutrophil dysfunction.¹⁹⁹

The estimated incidence of GSD type 1 is 1 in 100,000 live births, with GSD type 1a representing approximately 80% of cases. Clinically, GSD type 1 may present in the neonatal period with severe hypoglycemia, occurring 2 to 2.5 hours after a meal, and tachypnea secondary to respiratory compensation for the metabolic acidemia. However, because lactate and ketones may provide adequate brain substrate to protect central nervous system function (and because in early infancy regular feedings are consistently provided), the diagnosis may be delayed for months until massive hepatomegaly brings the infant to medical attention, although the hepatomegaly may be missed because the liver is soft. After infancy, affected patients may be seen walking with a waddling gait, secondary to their prominent abdomen and muscle weakness. Other consistent features are hyperuricemia, hypophosphatemia, a bleeding diathesis, secondary to impairment of platelet adhesiveness, and growth retardation.

Hypoglycemia may occur anytime these children are exposed to even brief periods of fasting. They are completely dependent on the provision of glucose from exogenous sources, with the exception of the small amount of free glucose—which is released as part of the process of debranching glycogen. Because less than 10% of glycogen consists of branch points, this mechanism provides little protection against hypoglycemia during fasting. Hypoglycemia in the setting of suppressed insulin and increased glucagon promotes glycogenolysis, but the absence of G6Pase commits the glucose-1-phosphate produced by phosphorylase to glycolytic catabolism, resulting in increased lactate production (see [Fig. 7.1](#)). Another consequence of the impaired activity of G6Pase is the shunting of G6P through the pentose phosphate pathway to yield ribose-6-phosphate, which ultimately yields uric acid, resulting in hyperuricemia. Hypertriglyceridemia results from increased triglyceride formation as a major route of disposal of pyruvate from lactate and amino acids, when glucose yield is blocked in G6Pase deficiency.²⁰⁰ Massive accumulation of fat in the liver is responsible for the massive hepatomegaly characteristic of GSD type 1.

Renal disease is a frequent complication of GSD type 1 (with an estimated prevalence of 30%).²⁰¹ Manifestations include proximal renal tubular dysfunction (Fanconi-like syndrome), distal tubular acidification defect, and hypercalciuria. There is an inverse relationship between age and citrate excretion, and the combination of low citrate excretion and hypercalciuria predispose these children to nephrocalcinosis and nephrolithiasis.²⁰² Citrate supplementation may prevent or ameliorate these complications.²⁰³ The widespread prevalence and serious prognosis of kidney involvement is manifested by severe glomerular hyperfiltration and microalbuminuria over time, systemic arterial hypertension, and consequently renal failure.^{204–206} The early implementation of treatment with angiotensin-converting enzyme inhibitors has been shown to delay the progression of renal damage.²⁰¹ The pathologic findings include focal segmental glomerulosclerosis with interstitial fibrosis. The etiology of the renal involvement is unclear, but it correlates negatively with metabolic control. It has been proposed that the dyslipidemia contributes to the kidney injury.²⁰⁷

In addition to the characteristic hepatomegaly, the liver undergoes adenomatous changes. Adenomas are usually first observed in the second and third decades of life but may appear before puberty.^{206,208} The adenomas may undergo malignant degeneration or hemorrhage and are frequently associated with chronic iron-resistant anemia.²⁰⁹ Other complications of GSD1 include osteopenia and growth retardation.

The diagnosis of GSD type 1 is based on the clinical and biochemical characteristics: hypoglycemia after a short period of fasting, hepatomegaly, lactic acidemia, and elevation of uric acid and triglycerides. Glucagon stimulation, 2 to 4 hours after a meal containing carbohydrates, results in increased lactate concentration, but plasma glucose does not rise.²⁰⁹ The diagnosis can be confirmed by mutation analysis.

The goal of treatment of children with GSD I is to completely eliminate hypoglycemia and suppress secondary metabolic decompensation. Continuous nasogastric or intra-gastric feedings during the night have been demonstrated to either reduce or eliminate the metabolic and clinical findings through complete avoidance of hypoglycemia.²¹⁰ However, this approach places children at risk of severe hypoglycemia, if feedings are abruptly stopped. A safer approach is the introduction of oral uncooked cornstarch, which helps prolonged the fasting tolerance,^{211,212} and can be used in older infants, usually older than 6 months of age. The doses of cornstarch used are approximately 1.6 g/kg per dose every 4 hours in infants and 1.7 to 2.5 g/kg per dose every 6 hours in older patients. A typical regimen consists of daytime feedings every 3 to 4 hours that are calculated to provide adequate carbohydrate calories to avoid the need for hepatic glucose output.²⁰⁹ Most of these calories consist of carbohydrates, primarily providing pure glucose as an energy source and avoiding disaccharides containing fructose or galactose. At night, the regimen consists of an intragastric infusion of glucose with or without protein designed to infuse at rates of about 125% calculated hepatic glucose output²⁰⁰ for normal young infants. For older children, a regimen of uncooked cornstarch can be implemented at nighttime.

Meticulous dietary control can lead to a significant clinical and metabolic improvement and prevention of complications. Adjunctive therapies should include careful monitoring of the uric acid level and treatment with allopurinol if the uric acid level is elevated. With increasing awareness of the renal tubular dysfunction, treatment of the hyperfiltration state with an angiotensin-converting enzyme inhibitor should be initiated promptly. Treatment with granulocyte-macrophage colony-stimulating factor to augment neutrophil production has been shown to ameliorate mouth ulcers and the enteritis in type 1b.²¹³

Disorders of Fatty Acid Oxidation: Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency

The inborn errors of metabolism associated with deficiencies of fatty acid oxidation and ketogenesis (Fig. 7.13) are inherited in an autosomal-recessive fashion. All of these disorders may be provoked by fasting and exhibit acute life-threatening events characterized by coma, hypoketotic hypoglycemia, cardiorespiratory collapse, and fatty hepatomegaly; those disorders which impair early steps in oxidation of longer-chain fatty acids may also include chronic hypertrophic or dilated cardiomyopathy and muscle weakness or exercise-induced rhabdomyolysis.²¹⁴ Metabolic defects of fatty acid oxidation may be divided according to the site of the enzymatic defect (see Fig. 7.13)^{214,215}: (1) defects of mitochondrial carnitine cycle for transferring fatty acids into the mitochondrial matrix, (2) defects of the β -oxidation cycle, (3) defects of electron transfer to the electron transport chain, and (4) defects in the pathway of ketone synthesis. The age at presentation is usually beyond the neonatal period, but neonatal presentation may occur in breastfeeding babies who have not begun to receive adequate milk.^{216,217} Because of the life-threatening and the largely treatable nature of these disorders, newborn screening is universally available in all US states and many other countries using filter paper blood spots and tandem-mass spectrometric analysis of acylcarnitine profiles.²¹⁸ This detects most of the fatty acid oxidation disorders, with the exception of β -hydroxy-methylglutaryl-CoA synthase deficiency. This section reviews the diagnosis, clinical features, and treatment of MCAD deficiency; other fatty acid oxidation defects are reviewed in Chapter 23.

MCAD deficiency is the most frequent defect of fatty acid oxidation and is one of the more common inborn errors of metabolism.^{219,220} Neonatal screening in Pennsylvania has shown an incidence of 1 in 15,700 newborns and the disorder is especially common in northern European countries.²²¹

MCAD (encoded by *ACADM* on 1p31) is responsible for the first step in the β -oxidation cycle for medium-chain fatty acyl-CoAs, with a chain length between 4 and 12 carbon atoms. Deficiency of MCAD enzyme activity leads to accumulation of medium-chain fatty acid metabolites (between 6 and 10 carbons) in plasma (octanoylcarnitine-C8, hexanoylcarnitine-C6, decanoylcarnitine-C10, decenoylcarnitine-C10:1; and abnormal acylglycines—hexanoylglycine and suberylglycine). Urinary metabolic abnormalities include increased excretion of dicarboxylic acids. Metabolite profiles are usually diagnostic, but can be confirmed by genetic sequence testing. The most common mutation among Northern Europeans, 985A>G (p.Lys304Glu), is homozygous in 80% of cases presenting clinically, and in 60% of cases identified by newborn screening.

Children with MCAD deficiency may appear normal at birth, although symptoms may manifest before results from newborn screening are available, especially in breastfed neonates.²²² The typical clinical presentation (if not detected by newborn screening) occurs between 3 and 24 months of age and is precipitated in response to prolonged fasting (e.g., at the time of weaning from nighttime feedings or during an intercurrent illnesses). Manifestations can include vomiting, lethargy, coma, and hepatic encephalopathy. Acute decompensations can rapidly progress to coma and death and may resemble sudden infant death syndrome (SIDS) or Reye syndrome.

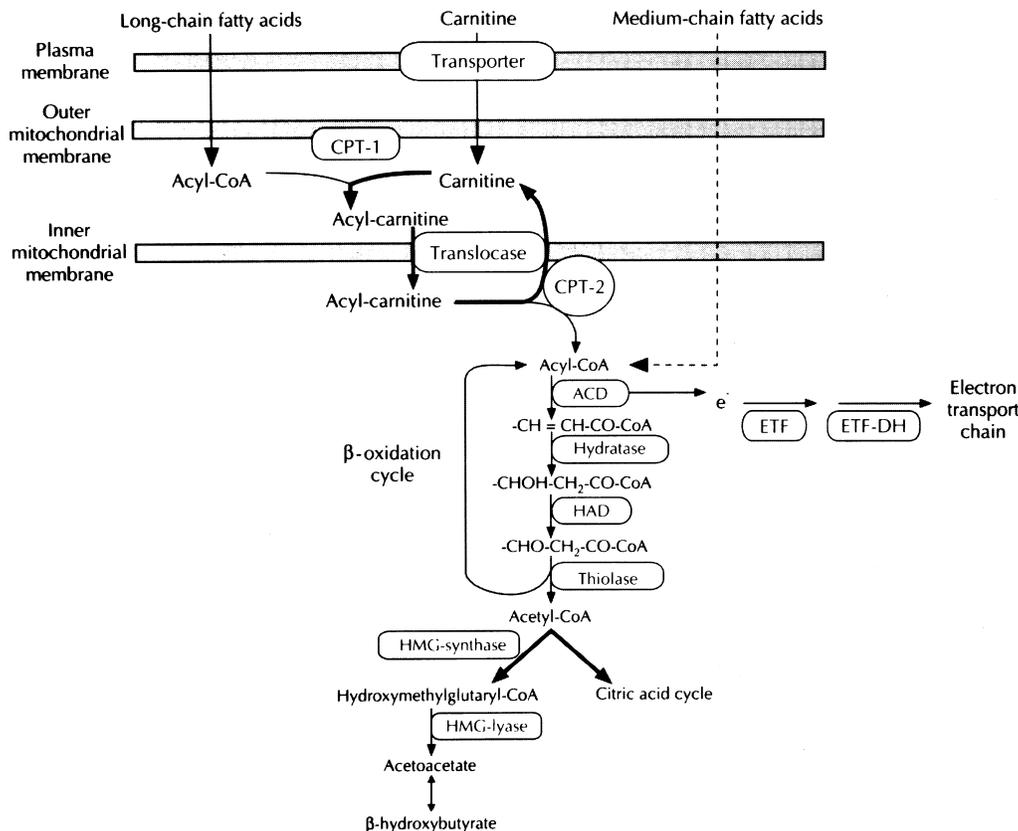


Fig. 7.13 The pathways of mitochondrial fatty acid oxidation and ketone body synthesis. *ACD*, Acyl-CoA dehydrogenase; *CPT-1* and *CPT-2*, carnitine palmitoyltransferase I and II; *ETF* 5, electron-transferring flavoprotein; *ETF-DH*, electron-transferring flavoprotein dehydrogenase; *HAD*, hydroxyacyl-CoA dehydrogenase. (From Stanley, C.A., Hale, D.E. (1994). Genetic disorders of mitochondrial fatty acid oxidation. *Curr Opin Pediatr*, 6, 476.)

In children not previously diagnosed, the mortality rate from the first episode is as high as 18%.²²³

Although there is significant heterogeneity in the presentation of MCAD, the most frequent clinical presentation is one of intermittent hypoketotic hypoglycemia with little or no acidemia, elevation of serum urea, ammonia, and uric acid, liver function abnormalities, and hepatic steatosis. The risk of severe complications, brain damage, and death is very high unless appropriate treatment to reverse the catabolic state is implemented.²²³ The diagnosis of these cases may be confused with Reye syndrome. Although hypoglycemia may be a prominent late feature with MCAD, the phenotype of a defect of a fatty acid oxidation disorder may not manifest if a fasting state is avoided. A high index of suspicion for fatty acid oxidation defects is important because appropriate therapy may result in an interruption and prevention of these potentially life-threatening episodes. Affected patients have also been misdiagnosed with idiopathic SIDS.²²⁴ Decreased plasma carnitine levels and an increase in the ratio of esterified to free carnitine is a frequently associated laboratory finding.²²⁵

Evaluation of suspected MCAD deficiency or other errors in fatty acid oxidation should first include a determination of the profile of plasma acylcarnitines by mass spectrometry and measurement of plasma total, esterified, and free carnitine. Table 7.4 shows the abnormalities of plasma acylcarnitines associated with specific fatty acid oxidation disorders are associated with specific abnormalities of plasma acylcarnitines—octanoylcarnitine in cases with MCAD deficiency.²²¹ Determinations of urinary organic acids with assessment of the presence or absence of dicarboxylic aciduria may also be useful. Other tests which may be useful in patients who require further evaluation include assays of fatty acid oxidation and specific enzyme assays in cultured skin fibroblasts or lymphoblasts. Since the early 1990s, the use of tandem mass spectrometry has made newborn screening possible for most fatty acid oxidation disorders, based on the acylcarnitine profile in blood spots. Presymptomatic identification of these individuals can prevent catastrophic events, such as sudden death. Genetic mutation analysis can be performed for all of these defects, which is particularly useful in MCAD, in which most cases are caused by a single common mutations (Lys304Gly).²²⁶

The primary treatment of disorders of fatty acid oxidation is a devoted avoidance of fasting.²¹⁴ For infants younger than 1 year old, 6 to 8 hours of fasting may be sufficient to precipitate an episode. On the other hand, as children become older,

they are able to tolerate periods of fasting of as long as 10 to 12 hours without decompensation. A high index of suspicion and rapid institution of IV glucose will often reverse an evolving episode. Glucose monitoring is not useful, because the presence of hypoglycemia is usually an event that occurs late in the evolution of an episode of metabolic decompensation. High-fat (e.g., ketogenic) diets should be avoided, although normal amounts of dietary fats are permissible. The use of uncooked cornstarch (as used for the treatment of type 1 glycogen storage disease) is rarely indicated.²²⁰

Defects of Glucose Transporters

GLUT1 Deficiency

GLUT1 deficiency syndrome is an autosomal dominant inborn error of glucose transport characterized by seizures, developmental delay, spasticity, acquired microcephaly, and ataxia. The biochemical hallmark is the finding of hypoglycorrachia (low cerebrospinal fluid glucose concentration), despite normal plasma glucose concentrations.

GLUT1, encoded by *SLC2A1*, is the fundamental vehicle that facilitates glucose entry into the brain. Diagnosis of GLUT1 deficiency is based on the finding of low cerebrospinal fluid glucose, in the absence of hypoglycemia, and identification of *SLC2A1* mutation (located on chromosome 1). The classic phenotype is a severe form of early-onset epileptic encephalopathy in approximately 90% of cases (classic form). A nonepileptic form represents 10% of cases, which includes a broad phenotypic spectrum that may present with paroxysmal nonepileptic manifestations that have been reported to include intermittent ataxia, choreoathetosis, dystonia, and alternating hemiplegia.²²⁷

Treatment efforts have been based on providing alternative brain fuel sources by a ketogenic diet.^{10,228} The ketogenic diet effectively controls the seizures and other paroxysmal activities, but it has less effect on the cognitive function.

GLUT2 Deficiency

Originally described by Fanconi and Bickel as a syndrome characterized by hypoglycemia and ketonuria in the fasting state and hyperglycemia in the postabsorptive state,²²⁹ Fanconi-Bickel syndrome is caused by recessive mutations of the GLUT2 plasma membrane transporter for glucose (encoded by

TABLE 7.4 Fatty Acid Oxidation Disorders With Distinguishing Metabolic Markers

Disorder	Plasma Acylcarnitines	Urinary Acylglycines	Urinary Organic Acids
VLCAD MCAD	Tetradecenoyl- Octanoyl	Hexanoyl-Suberyl- Phenylpropionyl-	
SCAD LCHAD	Butyryl- 3-Hydroxy-palmitoyl-3-Hydroxy- oleoyl-3-Hydroxy-linoleoyl-	Butyryl-	Ethylmalonic 3-Hydroxydicarboxylic
DER ETF and ETF-DH	Dodecadienoyl- Butyryl-Isovaleryl-Glutaryl-	Isovaleryl- Hexanoyl-	Ethylmalonic Glutaric Isovaleric 3-Hydroxy-3-methyl-glutaric
HMG-CoA lyase	Methylglutaryl-		

DER, 2,4-dienoyl-coenzyme A reductase; *ETF*, electron-transferring flavoprotein; *ETF-DH*, ETF dehydrogenase; *HMG-CoA*, 3-hydroxy-3-methylglutaryl-coenzyme A; *LCHAD*, long-chain 3-hydroxyacyl-coenzyme A dehydrogenase; *MCAD*, medium-chain acyl-coenzyme A dehydrogenase; *SCAD*, short-chain acyl-coenzyme A dehydrogenase; *VLCAD*, very-long-chain acyl-coenzyme A dehydrogenase.

(From Stanley, C.A. (1990). Disorders of fatty acid oxidation. In: Fernandes, J., Bremer, E., Saudubray, J-M. (eds.), *Inborn Metabolic Diseases: Diagnosis and Treatment*. Springer-Verlag, New York, pp. 394–410.)

SLC2A2).²³⁰ The defect on GLUT2 results in hepatorenal glycogen accumulation, proximal renal tubular dysfunction, and impaired utilization of glucose and galactose.²³¹ GLUT2 is expressed in hepatocyte, pancreatic β -cells, and the basolateral membranes of intestinal and renal tubular epithelial cells.²³² The clinical manifestations reflect impairment of glucose release from liver and of glucose reabsorption from renal tubular cells. Galactose clearance and conversion to glucose is delayed.

Patients with GLUT2 deficiency usually present at an age of 3 to 10 months. The typical clinical signs are hepatomegaly caused by glycogen accumulation, a severe Fanconi-type renal tubulopathy with disproportionately severe glucosuria, glucose and galactose intolerance, hypophosphatemic rickets, and severely stunted growth.²³³ These patients may present with a combination of fasting hypoglycemia and postprandial hyperglycemia. The hypoglycemia during fasting is explained by altered glucose transport out of the liver, resulting in an increased intracellular glucose concentration that may inhibit glycogen degradation, leading to glycogen storage and hepatomegaly. The hypoglycemia is exacerbated by renal loss of glucose caused by a transport defect for glucose and galactose across the basolateral membranes of the tubular cells. Hyperglycemia (and hypergalactosemia) in the fed state is explained by decreased monosaccharide uptake by the liver and enhanced by an inappropriately low insulin secretion because of impaired glucose-sensing by the pancreatic β -cells.²³⁰

The therapeutic goal for patients with GLUT2 deficiency is to ameliorate the consequences of renal tubulopathy by replacing water, electrolytes, and alkali—and by providing phosphate and vitamin D supplementation. In terms of diet, an adequate caloric intake is recommended to compensate for renal and intestinal glucose loss—given as frequent feedings containing slowly absorbed carbohydrates (such as cornstarch) to avoid fasting hypoglycemia.

TREATMENT

A rational therapeutic approach to the treatment of hypoglycemia relies first on a systematic diagnostic evaluation that results in a specific diagnosis. Before that specific diagnosis being made, and before the implementation of individual therapy plans, the goal of therapy is to preserve central nervous system function and to prevent catabolic states in which intermediary metabolites, such as free fatty acids and their acylcarnitines, ketones, or lactate may cause secondary harm. IV glucose infusion remains the mainstay of emergency therapy, particularly for the child with severe hypoglycemia. It is important to follow an initial glucose bolus with a continuous glucose infusion to prevent further episodes of hypoglycemia, until specific therapies are established. For neonates in the first 24 to 48 hours of life see earlier (Physiology of Perinatal Glucose Homeostasis).

Initial glucose bolus may range from 0.2 to 0.5 g/kg glucose (2–5 mL/kg dextrose 10%) as an IV bolus, followed by an infusion of 8 mg/kg/min (approximately 5 mL/kg/h of dextrose 10%). This may be reduced if plasma glucose is greater than 5 mmol/L (>90 mg/dL), but should be rapidly increased if frequent glucose checks indicate failure to elevate plasma glucose to greater than 3.8 mmol/L (>70 mg/dL). This may be initially done in increments of 1.6 mg/kg/min (1 mL/kg/h of dextrose 10%), but if glucose fails to rise to greater than 3.8 mmol/L (>70 mg/dL) after 2 increments every 15 minutes, then increase by 3.2 mg/kg/h (2 mL/kg/h of dextrose 10%).

Ideally, once a specific diagnosis is made, treatment should maintain normoglycemia on a normal feeding schedule for the patient's age. In addition, allowance should be made for the effects of intercurrent illness on glucose regulation and

the longest fasting interval a patient should undergo routinely, should be defined for both the well state and during intercurrent illness. It is advisable to periodically reassess the efficacy of the treatment for any form of hypoglycemia by a formal fasting study on treatment. All patients should have an emergency letter stating their specific diagnosis, advice on prevention of hypoglycemia and information on acute treatment of a hypoglycemia episode, in addition to contact information for the physician of record for ongoing assistance.

CONCLUSIONS

Since the early reports of neonatal hypoglycemia in the 1950s by McQuarrie⁶³ and Comblath and colleagues,²³⁴ our understanding of the molecular genetics and pathophysiology of the different disorders that cause hypoglycemia in neonates and infants has progressed significantly. Discoveries since the prior edition of this book include the description of new forms of syndromic hyperinsulinism, particularly, Kabuki syndrome and Turner syndrome; a combined phenotype of hyperinsulinism and hypopituitarism caused by inactivating mutations in *FOXA2*; and activation of insulin receptor signaling via mutations in *PI3K*, complementing those in *AKT2* that present with a phenotype of hypoketotic hypoglycemia without hyperinsulinism. With the rapid advancement in the understanding of molecular genetics and genotype-phenotype correlations for the different types of congenital hyperinsulinism, the most common cause of persistent hypoglycemia in neonates, a model of personalized medicine is starting to emerge. The best example is the current approach to children with suspected focal hyperinsulinism—from the genetic analysis to determine the likelihood of focal hyperinsulinism, to the localization of the lesion with noninvasive imaging, using ¹⁸F-fluoro-L-DOPA PET, and the surgical removal of the lesion sparing the normal pancreas and curing the disease. These and future discoveries should result in continued improvement in outcomes.

REFERENCES

1. Cryer PE. Glucose homeostasis and hypoglycemia. In: Kronenberg HLP, Melmed S, Polonsky K, eds. *Williams Textbook of Endocrinology*. 11 ed. Philadelphia: Elsevier; 1970:2003.
2. Cahill Jr GF. Starvation in man. *N Engl J Med*. 1970;282:668–675.
3. Nehlig A. Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia. *Diabetes Metabol*. 1997;23:18–29.
4. Devaskar SU, Mueckler MM. The mammalian glucose transporters. *Pediatr Res*. 1992;31:1–13.
5. Partridge WM, Boado RJ, Farrell CR. Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. *J Biol Chem*. 1990;265:18035–18040.
6. Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE. Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes*. 2004;53:549–559.
7. James DE, Strube M, Mueckler M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature*. 1989;338:83–87.
8. Birnbaum MJ. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell*. 1989;57:305–315.
9. Settergren G, Lindblad BS, Persson B. Cerebral blood flow and exchange of oxygen, glucose ketone bodies, lactate, pyruvate and amino acids in anesthetized children. *Acta Paediatr Scand*. 1980;69:457–465.
10. De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N Engl J Med*. 1991;325:703–709.
11. Fishman RA. The glucose-transporter protein and glucopenic brain injury. *N Engl J Med*. 1991;325:731–732.

12. Pascual JM, Wang D, Lecumberri B, et al. GLUT1 deficiency and other glucose transporter diseases. *Eur J Endocrinol.* 2004;150:627–633.
13. Choi IY, Lee SP, Kim SG, Gruetter R. In vivo measurements of brain glucose transport using the reversible Michaelis-Menten model and simultaneous measurements of cerebral blood flow changes during hypoglycemia. *J Cereb Blood Flow Metab.* 2001;21:653–663.
14. Gerich JE. Lilly lecture 1988. Glucose counterregulation and its impact on diabetes mellitus. *Diabetes.* 1988;37:1608–1617.
15. Cryer PE, Gerich JE. Glucose counterregulation, hypoglycemia, and intensive insulin therapy in diabetes mellitus. *N Engl J Med.* 1985;313:232–241.
16. Menon RK, Sperling MA. Carbohydrate metabolism. *Semin Perinatol.* 1988;12:157–162.
17. Lubchenco LO, Bard H. Incidence of hypoglycemia in newborn infants classified by birth weight and gestational age. *Pediatrics.* 1971;47:831–838.
18. Greenham R. Brain damage by neonatal hypoglycaemia. *Lancet (London, England).* 1989;2:446–447.
19. Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *BMJ (Clinical research ed).* 1988;297:1304–1308.
20. Morriss Jr FH, Makowski EL, Meschia G, Battaglia FC. The glucose/oxygen quotient of the term human fetus. *Biol Neonate.* 1974;25:44–52.
21. Novakovic B, Gordon L, Robinson WP, Desoye G, Saffery R. Glucose as a fetal nutrient: dynamic regulation of several glucose transporter genes by DNA methylation in the human placenta across gestation. *J Nutr Biochem.* 2013;24:282–288.
22. Marconi AM, Paolini C, Buscaglia M, Zerbe G, Battaglia FC, Pardi G. The impact of gestational age and fetal growth on the maternal-fetal glucose concentration difference. *Obstet Gynecol.* 1996;87:937–942.
23. Hauguel S, Desmazieres V, Challier JC. Glucose uptake, utilization, and transfer by the human placenta as functions of maternal glucose concentration. *Pediatr Res.* 1986;20:269–273.
24. Menon RK, Cohen RM, Sperling MA, Cutfield WS, Mimouni F, Khoury JC. Transplacental passage of insulin in pregnant women with insulin-dependent diabetes mellitus. Its role in fetal macrosomia. *N Engl J Med.* 1990;323:309–315.
25. Sayed S, Matschinsky FM, Stanley CA. Hyperinsulinism due to activating mutations of glucokinase. In: Stanley CA, Leon DDD, eds. *Monogenic hyperinsulinism hypoglycemia disorders.* Basel: Karger; 2012:146–157.
26. Sperling MA, DeLamater PV, Phelps D, Fiser RH, Oh W, Fisher DA. Spontaneous and amino acid-stimulated glucagon secretion in the immediate postnatal period. Relation to glucose and insulin. *J Clin Invest.* 1974;53:1159–1166.
27. Hawdon JM, Weddell A, Aynsley-Green A, Ward Platt MP. Hormonal and metabolic response to hypoglycaemia in small for gestational age infants. *Arch Dis Child.* 1993;68:269–273.
28. Desmond MM, Hild JR, Gast JH. The glycemic response of the newborn infant to epinephrine administration: a preliminary report. *J Pediatr.* 1950;37:341–350.
29. Cornblath M, Reisner SH. Blood glucose in the neonate and its clinical significance. *N Engl J Med.* 1965;273:378–381.
30. Stanley CA, Rozance PJ, Thornton PS, et al. Re-evaluating “transitional neonatal hypoglycemia”: mechanism and implications for management. *J Pediatr.* 2015;166:1520–1525.e1.
31. Martens GA, Motte E, Kramer G, et al. Functional characteristics of neonatal rat beta cells with distinct markers. *J Mol Endocrinol.* 2014;52:11–28.
32. Thorrez L, Laudadio I, Van Deun K, et al. Tissue-specific disallowance of housekeeping genes: the other face of cell differentiation. *Genome Res.* 2011;21:95–105.
33. Tan C, Tuch BE, Tu J, Brown SA. Role of NADH shuttles in glucose-induced insulin secretion from fetal beta-cells. *Diabetes.* 2002;51:2989–2996.
34. Blum B, Hrvatin S, Schuetz C, Bonal C, Rezania A, Melton DA. Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat Biotechnol.* 2012;30:261–264.
35. Huang C, Walker EM, Dadi PK, et al. Synaptotagmin 4 regulates Pancreatic beta Cell Maturation by Modulating the Ca²⁺ sensitivity of insulin secretion vesicles. *Dev Cell.* 2018;45:347–361.e5.
36. Melichar V, Drahota Z, Hahn P. Changes in the blood levels of acetoacetate and ketone bodies in newborn infants. *Biol Neonat.* 1965;8:348–352.
37. Anday EK, Stanley CA, Baker L, Delivoria-Papadopoulos M. Plasma ketones in newborn infants: absence of suckling ketosis. *J Pediatr.* 1981;98:628–630.
38. Cahill Jr GF, Veech RL. Ketoacids? Good medicine? *Trans Am Clin Climatol Assoc.* 2003;114:149–161, discussion 62–63.
39. Hawdon JM, Ward Platt MP, Aynsley-Green A. Patterns of metabolic adaptation for preterm and term infants in the first neonatal week. *Arch Dis Child.* 1992;67:357–365.
40. Alkalay AL, Sarnat HB, Flores-Sarnat L, Elashoff JD, Farber SJ, Simmons CF. Population meta-analysis of low plasma glucose thresholds in full-term normal newborns. *Am J Perinatol.* 2006;23:115–119.
41. Cornblath M, Hawdon JM, Williams AF, et al. Controversies regarding definition of neonatal hypoglycemia: suggested operational thresholds. *Pediatrics.* 2000;105:1141–1145.
42. Collins JE, Leonard JV. Hyperinsulinism in asphyxiated and small-for-dates infants with hypoglycaemia. *Lancet (London, England).* 1984;2:311–313.
43. Collins JE, Leonard JV, Teale D, et al. Hyperinsulinaemic hypoglycaemia in small for dates babies. *Arch Dis Child.* 1990;65:1118–1120.
44. Hoe FM, Thornton PS, Wanner LA, Steinkrauss L, Simmons RA, Stanley CA. Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. *J Pediatr.* 2006;148:207–212.
45. Thornton PS, Stanley CA, De Leon DD, et al. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. *J Pediatr.* 2015;167:238–245.
46. Chaussain JL, Georges P, Olive G, Job JC. Glycemic response to 24-hour fast in normal children and children with ketotic hypoglycemia: II. Hormonal and metabolic changes. *J Pediatr.* 1974;85:776–781.
47. FDA executive summary. *Meeting Materials of the Clinical Chemistry and Clinical Toxicology Devices Panel.* 30 March 2018. Administration USFaD, ed; 2018:2018.
48. Gruetter R, Ugurbil K, Seaquist ER. Steady-state cerebral glucose concentrations and transport in the human brain. *J Neurochem.* 1998;70:397–408.
49. Blackman JD, Towle VL, Lewis GF, Spire JP, Polonsky KS. Hypoglycemic thresholds for cognitive dysfunction in humans. *Diabetes.* 1990;39:828–835.
50. Lord K, Radcliffe J, Gallagher PR, Adzick NS, Stanley CA, De Leon DD. High risk of diabetes and neurobehavioral deficits in individuals with surgically treated hyperinsulinism. *J Clin Endocrinol Metab.* 2015;100:4133–4139.
51. Meissner T, Wendel U, Burgard P, Schaetzle S, Mayatepek E. Long-term follow-up of 114 patients with congenital hyperinsulinism. *Eur J Endocrinol.* 2003;149:43–51.
52. Menni F, de Lonlay P, Sevin C, et al. Neurologic outcomes of 90 neonates and infants with persistent hyperinsulinemic hypoglycemia. *Pediatrics.* 2001;107:476–479.
53. Steinkrauss L, Lipman TH, Hendell CD, Gerdes M, Thornton PS, Stanley CA. Effects of hypoglycemia on developmental outcome in children with congenital hyperinsulinism. *J Pediatr Nurs.* 2005;20:109–118.
54. Ludwig A, Enke S, Heindorf J, Empting S, Meissner T, Mohnike K. Formal Neurocognitive Testing in 60 Patients with Congenital Hyperinsulinism. *Horm Res Paediatr.* 2018;89:1–6.
55. Lord K, De Leon DD. Hyperinsulinism in the neonate. *Clin Perinatol.* 2018;45:61–74.
56. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. *J Clin Endocrinol Metab.* 2016;101:815–826.
57. Farquhar JW. The child of the diabetic woman. *Arch Dis Child.* 1959;34:76–96.
58. Stenninger E, Schollin J, Aman J. Early postnatal hypoglycaemia in newborn infants of diabetic mothers. *Acta Paediatr.* 1997;86:1374–1376.
59. Knip M, Lautala P, Leppaluoto J, Akerblom HK, Kouvalainen K. Relation of enteroinsular hormones at birth to macrosomia and

- neonatal hypoglycemia in infants of diabetic mothers. *J Pediatr*. 1983;103:603–611.
60. Kuhl C, Andersen GE, Hertel J, Molsted-Pedersen L. Metabolic events in infants of diabetic mothers during first 24 hours after birth. I. Changes in plasma glucose, insulin and glucagon. *Acta Paediatr Scand*. 1982;71:19–25.
 61. Artal R, Doug N, Wu P, Sperling MA. Circulating catecholamines and glucagon in infants of strictly controlled diabetic mothers. *Biol Neonate*. 1988;53:121–125.
 62. Harris DL, Weston PJ, Harding JE. Incidence of neonatal hypoglycemia in babies identified as at risk. *J Pediatr*. 2012;161:787–791.
 63. McQuarrie I. Idiopathic spontaneously occurring hypoglycemia in infants; clinical significance of problem and treatment. *AMA Am J Dis Child*. 1954;87:399–428.
 64. De Leon DD, Stanley CA. Mechanisms of Disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab*. 2007;3:57–68.
 65. De Vos A, Heimberg H, Quartier E, et al. Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *TJ Clin Invest*. 1995;96:2489–2495.
 66. Henquin JC, Ishiyama N, Nenquin M, Ravier MA, Jonas JC. Signals and pools underlying biphasic insulin secretion. *Diabetes*. 2002;51(Suppl 1):S60–S67.
 67. Thomas PM, Cote GJ, Wohlk N, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science (New York, NY)*. 1995;268:426–429.
 68. Thomas P, Ye Y, Lightner E. Mutation of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet*. 1996;5:1809–1812.
 69. Glaser B, Kesavan P, Heyman M, et al. Familial hyperinsulinism caused by an activating glucokinase mutation. *N Engl J Med*. 1998;338:226–230.
 70. Stanley CA, Lieu YK, Hsu BY, et al. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med*. 1998;338:1352–1357.
 71. Clayton PT, Eaton S, Aynsley-Green A, et al. Hyperinsulinism in short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency reveals the importance of beta-oxidation in insulin secretion. *J Clin Invest*. 2001;108:457–465.
 72. Otonkoski T, Jiao H, Kaminen-Ahola N, et al. Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. *Am J Hum Genet*. 81(3): 467–474.
 73. Gonzalez-Barroso MM, Giurgea I, Bouillaud F, et al. Mutations in UCP2 in congenital hyperinsulinism reveal a role for regulation of insulin secretion. *PLoS One*. 2008;3(12): e3850.
 74. Pearson ER, Boj SF, Steele AM, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med*. 2007;4:e118.
 75. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab*. 2012;97: E2026–E2030.
 76. Vajravelu ME, Chai J, Krock B, et al. Congenital hyperinsulinism and hypopituitarism attributable to a mutation in FOXA2. *J Clin Endocrinol Metab*. 2018;103:1042–1047.
 77. De Leon DD, Stanley CA. Pathophysiology of diffuse ATP-sensitive potassium channel hyperinsulinism. In: De Leon DD, Stanley CA, eds. *Monogenic Hyperinsulinemic Hypoglycemia Disorders*. Basel: Karger; 2012:18–29.
 78. Macmullen CM, Zhou Q, Snider KE, et al. Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the beta-cell sulfonylurea receptor SUR1. *Diabetes*. 2011;60:1797–1804.
 79. Grimberg A, Ferry Jr RJ, Kelly A, et al. Dysregulation of insulin secretion in children with congenital hyperinsulinism due to sulfonylurea receptor mutations. *Diabetes*. 2001;50:322–328.
 80. Shiota C, Larsson O, Shelton KD, et al. Sulfonylurea receptor type 1 knock-out mice have intact feeding-stimulated insulin secretion despite marked impairment in their response to glucose. *J Biol Chem*. 2002;277:37176–37183.
 81. Henquin JC, Nenquin M, Sempoux C, et al. In vitro insulin secretion by pancreatic tissue from infants with diazoxide-resistant congenital hyperinsulinism deviates from model predictions. *J Clin Invest*. 2011;121:3932–3942.
 82. Li C, Ackermann A.M., Boodhansingh K.E., et al. Functional and metabolomic consequences of katp channel inactivation in human islets. *Diabetes*. 66:1901–1913.
 83. Fournier SH, Stanley CA, Kelly A. Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. *J Pediatr*. 2006;149:47–52.
 84. Laje P, Halaby L, Adzick NS, Stanley CA. Necrotizing enterocolitis in neonates receiving octreotide for the management of congenital hyperinsulinism. *Pediatr Diabetes*. 2010;11: 142–147.
 85. McMahon AW, Wharton GT, Thornton P, De Leon DD. Octreotide use and safety in infants with hyperinsulinism. *Pharmacoevidemio Drug Saf*. 2017;26:26–31.
 86. Mazor-Aronovitch K, Landau H, Gillis D. Surgical versus non-surgical treatment of congenital hyperinsulinism. *Pediatr Endocrinol Rev*. 2009;6:424–430.
 87. Vajravelu ME, Congdon M, Mitteer L, et al. Continuous intragastric dextrose: a therapeutic option for refractory hypoglycemia in congenital hyperinsulinism. *Horm Res Paediatr*. 2018;1–7.
 88. de Lonlay-Debeney P, Poggi-Travert F, Fournet JC, et al. Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med*. 1999;340:1169–1175.
 89. de Lonlay P, Fournet JC, Rahier J, et al. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *J Clin Invest*. 1997;100:802–807.
 90. Lord K, Dzata E, Snider KE, Gallagher PR, De Leon DD. Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. *J Clin Endocrinol Metab*. 2013;98: E1786–E1789.
 91. Snider KE, Becker S, Boyajian L, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab*. 2013;98:E355–E363.
 92. Suchi M, MacMullen C, Thornton PS, Ganguly A, Stanley CA, Ruchelli ED. Histopathology of congenital hyperinsulinism: retrospective study with genotype correlations. *Pediatr Dev Pathol*. 2003;6:322–333.
 93. Dubois J, Brunelle F, Touati G, et al. Hyperinsulinism in children: diagnostic value of pancreatic venous sampling correlated with clinical, pathological and surgical outcome in 25 cases. *Pediatr Radiol*. 1995;25:512–516.
 94. Stanley CA, Thornton PS, Ganguly A, et al. Preoperative evaluation of infants with focal or diffuse congenital hyperinsulinism by intravenous acute insulin response tests and selective pancreatic arterial calcium stimulation. *J Clin Endocrinol Metab*. 2004;89:288–296.
 95. Ribeiro MJ, De Lonlay P, Delzescaux T, et al. Characterization of hyperinsulinism in infancy assessed with PET and 18F-fluoro-L-DOPA. *J Nucl Med*. 2005;46:560–566.
 96. Otonkoski T, Nanto-Salonen K, Seppanen M, et al. Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. *Diabetes Metabol*. 2006;55:13–18.
 97. Hardy OT, Hernandez-Pampaloni M, Saffer JR, et al. Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. *J Pediatr*. 2007;150:140–145.
 98. Ericson LE, Hakanson R, Lundquist I. Accumulation of dopamine in mouse pancreatic B-cells following injection of L-DOPA. Localization to secretory granules and inhibition of insulin secretion. *Diabetologia*. 1977;13:117–124.
 99. Borelli MI, Villar MJ, Orezzoli A, Gagliardino JJ. Presence of DOPA decarboxylase and its localisation in adult rat pancreatic islet cells. *Diabetes Metabol*. 1997;23:161–163.
 100. Miki Y, Taki T, Ohura T, Kato H, Yanagisawa M, Hayashi Y. Novel missense mutations in the glutamate dehydrogenase gene in the congenital hyperinsulinism-hyperammonemia syndrome. *J Pediatr*. 2000;136:69–72.
 101. MacMullen C, Fang J, Hsu BY, et al. Hyperinsulinism/hyperammonemia syndrome in children with regulatory mutations in the inhibitory guanosine triphosphate-binding domain of glutamate dehydrogenase. *J Clin Endocrinol Metab*. 2001;86: 1782–1787.

102. Huijmans JG, Duran M, de Klerk JB, Rovers MJ, Scholte HR. Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatr Res.* 2000;106:596–600.
103. Yorifuji T, Muroi J, Uematsu A, Hiramatsu H, Momoi T. Hyperinsulinism-hyperammonemia syndrome caused by mutant glutamate dehydrogenase accompanied by novel enzyme kinetics. *Hum Genet.* 1999;104:476–479.
104. Kelly A, Palladino A, Stanley CA. Congenital hyperinsulinism due to activating mutations of glutamate dehydrogenase: the hyperinsulinism/hyperammonemia syndrome. In: De Leon DD, Stanley CA, eds. *Monogenic hyperinsulinemic hypoglycemia disorders*. Basel: Karger; 2012:100–111.
105. Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr.* 2001;138:383–389.
106. Li C, Matter A, Kelly A, et al. Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem.* 2006;281:15064–15072.
107. Kelly A, Ng D, Ferry Jr RJ, et al. Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab.* 2001;86:3724–3728.
108. Treberg JR, Clow KA, Greene KA, Brosnan ME, Brosnan JT. Systemic activation of glutamate dehydrogenase increases renal ammoniogenesis: implications for the hyperinsulinism/hyperammonemia syndrome. *Am J Physiol Endocrinol Metab.* 2010;298:E1219–E1225.
109. Raizen DM, Brooks-Kayal A, Steinkrauss L, Tennekoon GI, Stanley CA, Kelly A. Central nervous system hyperexcitability associated with glutamate dehydrogenase gain of function mutations. *J Pediatr.* 2005;146:388–394.
110. Kapoor RR, Flanagan SE, Fulton P, et al. Hyperinsulinism-hyperammonemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *Eur J Endocrinol.* 2009;161:731–735.
111. Bahi-Buisson N, Roze E, Dionisi C, et al. Neurological aspects of hyperinsulinism-hyperammonemia syndrome. *Dev Med Child Neurol.* 2008;50:945–949.
112. Molven A, Matre GE, Duran M, et al. Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes.* 2004;53:221–227.
113. Hussain K, Clayton PT, Krywawych S, et al. Hyperinsulinism of infancy associated with a novel splice site mutation in the SCHAD gene. *J Pediatr.* 2005;146:706–708.
114. Li C, Chen P, Palladino A, et al. Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem.* 2010;285:31806–31818.
115. Kapoor RR, James C, Flanagan SE, Ellard S, Eaton S, Hussain K. 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab.* 2009;94:2221–2225.
116. Matschinsky FM. Regulation of pancreatic beta-cell glucokinase: from basics to therapeutics. *Diabetes.* 2002;51(Suppl 3):S394–404.
117. Christesen HB, Jacobsen BB, Odili S, et al. The second activating glucokinase mutation (A456V): implications for glucose homeostasis and diabetes therapy. *Diabetes.* 2002;51:1240–1246.
118. Davis EA, Cuesta-Munoz A, Raoul M, et al. Mutants of glucokinase cause hypoglycemia- and hyperglycemia syndromes and their analysis illuminates fundamental quantitative concepts of glucose homeostasis. *Diabetologia.* 1999;42:1175–1186.
119. Vionnet N, Stoffel M, Takeda J, et al. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature.* 1992;356:721–722.
120. Njolstad PR, Sovik O, Cuesta-Munoz A, et al. Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med.* 2001;344:1588–1592.
121. Gloyn AL, Noordam K, Willemsen MA, et al. Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations. *Diabetes.* 2003;52:2433–2440.
122. Wabitsch M, Lahr G, Van de Bunt M, et al. Heterogeneity in disease severity in a family with a novel G68V GCK activating mutation causing persistent hyperinsulinaemic hypoglycaemia of infancy. *Diabetic Med.* 2007;24:1393–1399.
123. Sayed S, Langdon DR, Odili S, et al. Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. *Diabetes.* 2009;58:1419–1427.
124. Kassem S, Bhandari S, Rodriguez-Bada P, et al. Large islets, beta-cell proliferation, and a glucokinase mutation. *N Engl J Med.* 2010;362:1348–1350.
125. Cuesta-Munoz AL, Huopio H, Otonkoski T, et al. Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. *Diabetes.* 2004;53:2164–2168.
126. Voza A, Parisi G, De Leonardi F, et al. UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation. *Proc Natl Acad Sci U S A.* 2014;111:960–965.
127. Ferrara CT, Boodhansingh KE, Paradise E, et al. Novel hypoglycemia phenotype in congenital hyperinsulinism due to dominant mutations of uncoupling protein 2. *J Clin Endocrinol Metab.* 2017;102:942–949.
128. Ferrer J. A genetic switch in pancreatic beta-cells: implications for differentiation and haploinsufficiency. *Diabetes.* 2002;51:2355–2362.
129. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med.* 2001;345:971–980.
130. Fajans SS, Bell GI. Macrosomia and neonatal hypoglycemia in RW pedigree subjects with a mutation (Q268X) in the gene encoding hepatocyte nuclear factor 4alpha (HNF4A). *Diabetologia.* 2007;50:2600–2601.
131. Kapoor RR, Locke J, Colclough K, et al. Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes.* 2008;57:1659–1663.
132. Flanagan SE, Kapoor RR, Mali G, et al. Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations. *Eur J Endocrinol.* 2010;162:987–992.
133. Tung JY, Boodhansingh K, Stanley CA, De Leon DD. Clinical heterogeneity of hyperinsulinism due to HNF1A and HNF4A mutations. *Pediatr Diabetes.* 2018;19:910–916.
134. Kapoor RR, James CT, Hussain K. HNF4A and hyperinsulinemic hypoglycemia. In: Stanley CA, Leon DDD, eds. *Monogenic hyperinsulinemic hypoglycemia disorders*. Basel: Karger; 2012:182–190.
135. Hamilton AJ, Bingham C, McDonald TJ, et al. The HNF4A R76W mutation causes atypical dominant Fanconi syndrome in addition to a beta cell phenotype. *J Med Genet.* 2014;51:165–169.
136. Improda N, Shah P, Guemes M, et al. Hepatocyte nuclear factor-4 alfa mutation associated with hyperinsulinaemic hypoglycaemia and atypical renal Fanconi syndrome: expanding the clinical phenotype. *Horm Res Paediatr.* 2016;86:337–341.
137. Giri D, Vignola ML, Gualtieri A, et al. Novel FOXA2 mutation causes hyperinsulinism, hypopituitarism with craniofacial and endoderm-derived organ abnormalities. *Hum Mol Genet.* 2017;26:4315–4326.
138. Brioude F, Kalish JM, Mussa A, et al. Expert consensus document: Clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol.* 2018;14:229–249.
139. DeBaun MR, King AA, White N. Hypoglycemia in Beckwith-Wiedemann syndrome. *Semin Perinatol.* 2000;24:164–171.
140. Hussain K, Cosgrove KE, Shepherd RM, et al. Hyperinsulinemic hypoglycemia in Beckwith-Wiedemann syndrome due to defects in the function of pancreatic beta-cell adenosine triphosphate-sensitive potassium channels. *J Clin Endocrinol Metab.* 2005;90:4376–4382.
141. Stefan Y, Bordi C, Grasso S, Orzi L. Beckwith-Wiedemann syndrome: a quantitative, immunohistochemical study of pancreatic islet cell populations. *Diabetologia.* 1985;28:914–919.
142. De Leon DD, Stanley CA. Congenital hypoglycemia disorders: new aspects of etiology, diagnosis, treatment and outcomes: highlights of the proceedings of the congenital hypoglycemia disorders symposium, Philadelphia April 2016. *Pediatr Diabetes.* 2017;18:3–9.
143. Bogershausen N, Gatinois V, Riehrer V, et al. Mutation update for kabuki syndrome genes KMT2D and KDM6A and further delineation of x-linked Kabuki syndrome subtype 2. *Hum Mutat.* 2016;37:847–864.

144. Geneviève D, Amiel J, Le Merrer M, et al. Atypical findings in Kabuki syndrome: Report of 8 patients in a series of 20 and review of the literature. *Am J Med Genet.* 2004;129A:64–68.
145. Subbarayan A, Hussain K. Hypoglycemia in Kabuki syndrome. *Am J Med Genet.* 2013;164:467–471.
146. Toda N, Ihara K, Kojima-Ishii K, et al. Hyperinsulinemic hypoglycemia in Beckwith-Wiedemann, Sotos, and Kabuki syndromes: a nationwide survey in Japan. *Am J Med Genet A.* 2017;173:360–367.
147. Bakalov VK, Cheng C, Zhou J, Bondy CA. X-chromosome gene dosage and the risk of diabetes in Turner syndrome. *J Clin Endocrinol Metab.* 2009;94:3289–3296.
148. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. *J Clin Epidemiol.* 1998;51:147–158.
149. Alkhayyat H, Christesen HB, Steer J, Stewart H, Brusgaard K, Hussain K. Mosaic Turner syndrome and hyperinsulinaemic hypoglycaemia. *J Pediatr Endocrinol Metab.* 2006;19:1451–1457.
150. Pietzner V, Weigel JF, Wand D, Merkschlagler A, Bernhard MK. Low-level hyperinsulinism with hypoglycemic spells in an infant with mosaic Turner syndrome and mild Kabuki-like phenotype: a case report and review of the literature. *J Pediatr Endocrinol Metab.* 2014;27:165–170.
151. Gibson CE, Boodhansingh KE, Li C, et al. Congenital hyperinsulinism in infants with Turner syndrome: possible association with monosomy X and KDM6A haploinsufficiency. *Horm Res Paediatr.* 2018;89:413–422.
152. Glassman MS, Schultz RM, MacGillivray MH. Gonadal dysgenesis and leucine-sensitive hypoglycemia. *J Pediatr.* 1979;94:930–931.
153. Cappella, M., Graziani, V., Pragliola, A., et al. Hyperinsulinemic hypoglycaemia in a Turner syndrome with ring (X). *Case Rep Pediatr.* 2015;2015:561974.
154. Avatapalle HB, Banerjee I, Shah S, et al. Abnormal neurodevelopmental outcomes are common in children with transient congenital hyperinsulinism. *Front Endocrinol (Lausanne).* 2013;4:60.
155. Hawkes CP, Lado JJ, Givler S, De Leon DD. The effect of continuous intravenous glucagon on glucose requirements in infants with congenital hyperinsulinism. *JIMD Rep.* 2019;45:45–50.
156. Herrera A, Vajravelu ME, Givler S, et al. Prevalence of adverse events in children with congenital hyperinsulinism treated with diazoxide. *J Clin Endocrinol Metab.* 2018;103:4365–4372.
157. Dayton PG, Pruitt AW, Faraj BA, Israeli ZH. Metabolism and disposition of diazoxide. A mini-review. *Drug metabolism and disposition: the biological fate of chemicals.* 1975;3:226–229.
158. Banerjee I, Avatapalle B, Petkar A, et al. The association of cardiac ventricular hypertrophy with congenital hyperinsulinism. *Eur J Endocrinol.* 2012;167:619–624.
159. Yorifuji T, Kawakita R, Hosokawa Y, et al. Efficacy and safety of long-term, continuous subcutaneous octreotide infusion for patients with different subtypes of KATP-channel hyperinsulinism. *Clin Endocrinol (Oxford).* 2013;78:891–897.
160. Palladino AA, Stanley CA. A specialized team approach to diagnosis and medical versus surgical treatment of infants with congenital hyperinsulinism. *Semin Pediatr Surg.* 2011;20:32–37.
161. Demirbilek H, Shah P, Arya VB, et al. Long-term follow-up of children with congenital hyperinsulinism on octreotide therapy. *J Clin Endocrinol Metab.* 2014;99:3660–3667.
162. Laje P, Halaby L, Adzick NS, Stanley CA. Necrotizing enterocolitis in neonates receiving octreotide for the management of congenital hyperinsulinism. *Pediatr Diabetes.* 2010;11:142–147.
163. Hawkes CP, Adzick NS, Palladino AA, De Leon DD. Late presentation of fulminant necrotizing enterocolitis in a child with hyperinsulinism on octreotide therapy. *Horm Res Paediatr.* 2016;86:131–136.
164. Le Quan Sang KH, Arnoux JB, Mamoune A, et al. Successful treatment of congenital hyperinsulinism with long-acting release octreotide. *Eur J Endocrinol.* 2012;166:333–339.
165. Modan-Moses D, Koren I, Mazor-Aronovitch K, Pinhas-Hamiel O, Landau H. Treatment of congenital hyperinsulinism with lanreotide acetate (Somatuline Autogel). *J Clin Endocrinol Metab.* 2011;96:2312–2317.
166. Kuhnen P, Marquard J, Ernert A, et al. Long-term lanreotide treatment in six patients with congenital hyperinsulinism. *Horm Res Paediatr.* 2012;78:106–112.
167. Hussain K, Challis B, Rocha N, et al. An activating mutation of AKT2 and human hypoglycemia. *Science (New York, NY).* 2011;334:474.
168. Leiter SM, Parker VER, Welters A, et al. Hypoinsulinaemic, hypoketotic hypoglycaemia due to mosaic genetic activation of PI3-kinase. *Eur J Endocrinol.* 2017;177:175–186.
169. Calabria AC, Gallagher PR, Simmons R, Blinman T, De Leon DD. Postoperative surveillance and detection of postprandial hypoglycemia after fundoplasty in children. *J Pediatr.* 2011;159:597–601.e1.
170. Ralphs DN, Thomson JP, Haynes S, Lawson-Smith C, Hobsley M, Le Quesne LP. The relationship between the rate of gastric emptying and the dumping syndrome. *Br J Surg.* 1978;65:637–641.
171. Rivkees SA, Crawford JD. Hypoglycemia pathogenesis in children with dumping syndrome. *Pediatrics.* 1987;80:937–942.
172. Ng DD, Ferry Jr RJ, Kelly A, Weinzimer SA, Stanley CA, Katz LE. Acarbose treatment of postprandial hypoglycemia in children after Nissen fundoplication. *J Pediatr.* 2001;139:877–879.
173. Vu MK, Straathof JW, v d Schaar PJ, et al. Motor and sensory function of the proximal stomach in reflux disease and after laparoscopic Nissen fundoplication. *Am Assoc Gastroenterol.* 1999;94:1481–1489.
174. Palladino AA, Sayed S, Levitt Katz LE, Gallagher PR, De Leon DD. Increased glucagon-like peptide-1 secretion and postprandial hypoglycemia in children after Nissen fundoplication. *J Clin Endocrinol Metab.* 2009;94:39–44.
175. Calabria AC, Charles L, Givler S, De Leon DD. Postprandial hypoglycemia in children after gastric surgery: clinical characterization and pathophysiology. *Horm Res Paediatr.* 2016;85:140–146.
176. Borovoy J, Furuta L, Nurko S. Benefit of uncooked cornstarch in the management of children with dumping syndrome fed exclusively by gastrostomy. *Am J Gastroenterol.* 1998;93:814–818.
177. Samuk I, Afriat R, Horne T, Bistrizer T, Barr J, Vinograd I. Dumping syndrome following Nissen fundoplication, diagnosis, and treatment. *J Pediatr Gastroenterol Nutr.* 1996;23:235–240.
178. Lehnert H, Beyer J, Weber P, Krause U, Schrezenmeier J. Treatment of severe reactive hypoglycemia with a somatostatin analogue (SMS 201-995). *Arch Int Med.* 1990;150:2401–2402.
179. Khoshoo V, Reifen RM, Gold BD, Sherman PM, Pencharz PB. Nutritional manipulation in the management of dumping syndrome. *Arch Dis Child.* 1991;66:1447–1448.
180. Salisbury DM, Leonard JV, Dezateux CA, Savage MO. Micropenis: an important early sign of congenital hypopituitarism. *Br Med J (Clinical research ed).* 1984;288:621–622.
181. Lovinger RD, Kaplan SL, Grumbach MM. Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus: four cases secondary to hypothalamic hormone deficiencies. *J Pediatr.* 1975;87:1171–1181.
182. Kaufman FR, Costin G, Thomas DW, Sinatra FR, Roe TF, Neufstein HB. Neonatal cholestasis and hypopituitarism. *Arch Dis Child.* 1984;59:787–789.
183. Parks JS. Congenital hypopituitarism. *Clin Perinatol.* 2018;45:75–91.
184. Choo-Kang LR, Sun CC, Counts DR. Cholestasis and hypoglycemia: manifestations of congenital anterior hypopituitarism. *J Clin Endocrinol Metab.* 1996;81:2786–2789.
185. Renier WO, Nabben FA, Hustinx TW, et al. Congenital adrenal hypoplasia, progressive muscular dystrophy, and severe mental retardation, in association with glycerol kinase deficiency, in male sibs. *Clin Genet.* 1983;24:243–251.
186. Toyofuku T, Takashima S, Takeshita K, Nagafuji H. Progressive muscular dystrophy with congenital adrenal hypoplasia: an unusual autopsy case. *Brain Dev.* 1986;8:285–289.
187. Hannah-Shmouni F, Stratakis CA. An overview of inborn errors of metabolism manifesting with primary adrenal insufficiency. *Rev Endocr Metabol Dis.* 2018;19:53–67.
188. Clark AJ, Weber A. Molecular insights into inherited ACTH resistance syndromes. *Trends Endocrinol Metabol.* 1994;5:209–214.
189. Meimaridou E, Hughes CR, Kowalczyk J, Chan LF, Clark AJ, Metherell LA. ACTH resistance: genes and mechanisms. *Endocr Dev.* 2013;24:57–66. 2013.
190. Atasay B, Aycan Z, Evliyaoglu O, et al. Congenital early onset isolated adrenocorticotropin deficiency associated with a TPIT gene mutation. *J Pediatr Endocrinol Metabol.* 2004;17:1017–1020.

191. Kelly A, Tang R, Becker S, Stanley CA. Poor specificity of low growth hormone and cortisol levels during fasting hypoglycemia for the diagnoses of growth hormone deficiency and adrenal insufficiency. *Pediatrics*. 2008;122: e522–528.
192. McEachern R, Drouin J, Metherell L, Huot C, Van Vliet G, Deal C. Severe cortisol deficiency associated with reversible growth hormone deficiency in two infants: what is the link? *J Clin Endocrinol Metab*. 2011;96:2670–2674.
193. Vidnes J, Oyasaeter S. Glucagon deficiency causing severe neonatal hypoglycemia in a patient with normal insulin secretion. *Pediatr Res*. 1977;11:943–949.
194. Kollee LA, Monnens LA, Cecjka V, Wilms RM. Persistent neonatal hypoglycaemia due to glucagon deficiency. *Arch Dis Child*. 1978;53:422–424.
195. Foster JD, Nordlie RC. The biochemistry and molecular biology of the glucose-6-phosphatase system. *Exp Biol Med (Maywood, NJ)*. 2002;227:601–608.
196. Lei KJ, Pan CJ, Shelly LL, Liu JL, Chou JY. Identification of mutations in the gene for glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1a. *J Clin Invest*. 1994;93:1994–1999.
197. Gerin I, Veiga-da-Cunha M, Achouri Y, Collet JF, Van Schaftingen E. Sequence of a putative glucose 6-phosphate translocase, mutated in glycogen storage disease type 1b. *FEBS Lett*. 1997;419:235–238.
198. Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY. Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. *Science (New York, NY)*. 1993;262:580–583.
199. Visser G, Rake JP, Fernandes J, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type 1b: results of the European Study on Glycogen Storage Disease type I. *J Pediatr*. 2000;137:187–191.
200. Stanley CA, Mills JL, Baker L. Intra-gastric feeding in type I glycogen storage disease: factors affecting the control of lactic acidemia. *Pediatr Res*. 1981;15:1504–1508.
201. Melis D, Parenti G, Gatti R, et al. Efficacy of ACE-inhibitor therapy on renal disease in glycogen storage disease type 1: a multicentre retrospective study. *Clin Endocrinol*. 2005;63:19–25.
202. Restaino I, Kaplan BS, Stanley C, Baker L. Nephrolithiasis, hypocitraturia, and a distal renal tubular acidification defect in type 1 glycogen storage disease. *J Pediatr*. 1993;122:392–396.
203. Weinstein DA, Somers MJ, Wolfsdorf JL. Decreased urinary citrate excretion in type 1a glycogen storage disease. *J Pediatr*. 2001;138:378–382.
204. Chen YT, Coleman RA, Scheinman JI, Kolbeck PC, Sidbury JB. Renal disease in type I glycogen storage disease. *N Engl J Med*. 1988;318:7–11.
205. Chen YT, Scheinman JI, Park HK, Coleman RA, Roe CR. Amelioration of proximal renal tubular dysfunction in type I glycogen storage disease with dietary therapy. *N Engl J Med*. 1990;323:590–593.
206. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GP. Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European Study on Glycogen Storage Disease Type I (ESGSD I). *Eur J Pediatr*. 2002;161(Suppl 1): S20–34.
207. Moses SW. Historical highlights and unsolved problems in glycogen storage disease type 1. *Eur J Pediatr*. 2002;161(Suppl 1): S2–S9.
208. Bianchi L. Glycogen storage disease I and hepatocellular tumours. *Eur J Pediatr*. 1993;152(Suppl 1): S63–S70.
209. Wolfsdorf JL, Weinstein DA. Glycogen storage diseases. *Rev Endocr Metabol Dis*. 2003;4:95–102.
210. Greene HL, Slonim AE, O'Neill Jr JA, Burr IM. Continuous nocturnal intra-gastric feeding for management of type 1 glycogen-storage disease. *N Engl J Med*. 1976;294:423–425.
211. Chen YT, Cornblath M, Sidbury JB. Cornstarch therapy in type I glycogen-storage disease. *N Engl J Med*. 1984;310:171–175.
212. Wolfsdorf JL, Keller RJ, Landy H, Crigler Jr JF. Glucose therapy for glycogenesis type 1 in infants: comparison of intermittent uncooked cornstarch and continuous overnight glucose feedings. *J Pediatr*. 1990;117:384–391.
213. Roe TF, Coates TD, Thomas DW, Miller JH, Gilsanz V. Brief report: treatment of chronic inflammatory bowel disease in glycogen storage disease type 1b with colony-stimulating factors. *N Engl J Med*. 1992;326:1666–1669.
214. Hale DE, Bennett MJ. Fatty acid oxidation disorders: a new class of metabolic diseases. *J Pediatr*. 1992;121:1–11.
215. Stanley CA, Bennett MJ. Disorders of mitochondrial fatty acid beta-oxidation. In: Robert M, Kliegman BFS, Joseph W, St. Geme III, Schor Nina F, Richard E, Behrman, eds. *Nelson Textbook of Pediatrics*. 19th ed. Philadelphia: Elsevier Saunders; 2011: 456–462.
216. Wilcken B, Hammond J, Silink M. Morbidity and mortality in medium chain acyl coenzyme A dehydrogenase deficiency. *Arch Dis Child*. 1994;70:410–412.
217. Ahrens-Nicklas RC, Pyle LC, Ficocioglu C. Morbidity and mortality among exclusively breastfed neonates with medium-chain acyl-CoA dehydrogenase deficiency. *Genet Med*. 2016;18: 1315–1319.
218. Rinaldo P, Matern D. Disorders of fatty acid transport and mitochondrial oxidation: challenges and dilemmas of metabolic evaluation. *Genet Med*. 2000;2:338–344.
219. Stanley CA, Hale DE, Coates PM, et al. Medium-chain acyl-CoA dehydrogenase deficiency in children with non-ketotic hypoglycemia and low carnitine levels. *Pediatr Res*. 1983;17: 877–884.
220. Matern D., Rinaldo P. Medium-chain acyl-coenzyme a dehydrogenase deficiency. In: Pagon, R.A., Bird, T.D., Dolan, C.R., et al., eds. *GeneReviews*. University of Washington, Seattle, WA; 2000.
221. Chace DH, Kalas TA, Naylor EW. The application of tandem mass spectrometry to neonatal screening for inherited disorders of intermediary metabolism. *Annu Rev Genomics Hum Genet*. 2002;3:17–45.
222. Ensenauer R, Winters JL, Parton PA, et al. Genotypic differences of MCAD deficiency in the Asian population: novel genotype and clinical symptoms preceding newborn screening notification. *Genet Med*. 2005;7:339–343.
223. Iafolla AK, Thompson Jr RJ, Roe CR. Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children. *J Pediatr*. 1994;124:409–415.
224. Ding JH, Roe CR, Iafolla AK, Chen YT. Medium-chain acyl-coenzyme A dehydrogenase deficiency and sudden infant death. *N Engl J Med*. 1991;325:61–62.
225. Stanley CA. Carnitine deficiency disorders in children. *Ann N Y Acad Sci*. 2004;1033:42–51.
226. Rhead WJ. Newborn screening for medium-chain acyl-CoA dehydrogenase deficiency: a global perspective. *J Inher Metab Dis*. 2006;29:370–377.
227. Leen WG, Klepper J, Verbeek MM, et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain*. 2010;133:655–670.
228. Klepper J, Voit T. Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain—a review. *Eur J Pediatr*. 2002;161:295–304.
229. Santer R, Schneppenheim R, Suter D, Schaub J, Steinmann B. Fanconi-Bickel syndrome—the original patient and his natural history, historical steps leading to the primary defect, and a review of the literature. *Eur J Pediatr*. 1998;157:783–797.
230. Santer R, Schneppenheim R, Dombrowski A, Gotze H, Steinmann B, Schaub J. Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. *Nat Genet*. 1997;17:324–326.
231. Manz F, Bickel H, Brodehl J, et al. Fanconi-Bickel syndrome. *Pediatr Nephrol (Berlin, Germany)*. 1987;1:509–518.
232. Mueckler M, Kruse M, Strube M, Riggs AC, Chiu KC, Permutt MA. A mutation in the Glut2 glucose transporter gene of a diabetic patient abolishes transport activity. *J Biol Chem*. 1994;269:17765–17767.
233. Santer R, Steinmann B, Schaub J. Fanconi-Bickel syndrome—a congenital defect of facilitative glucose transport. *Curr Mol Med*. 2002;2:213–227.
234. Cornblath M, Odell GB, Levin EY. Symptomatic neonatal hypoglycemia associated with toxemia of pregnancy. *J Pediatr*. 1959;55:545–562.

CHAPTER OUTLINE

INTRODUCTION	202
EMBRYOLOGY, PHYSIOLOGY, AND PHYSIOPATHOLOGY	202
Development of the Thyrotrophic Axis	202
Maternofetal Transfers and Their Clinical Consequences	203
Maturation of Thyroid Hormone Synthesis and Secretion	204
Maturation of Thyroid Hormone Metabolism	206
Thyroid Hormone Cell Membrane Transporters and Receptors	207
Perinatal Changes in Thyroid Function	207
CONGENITAL HYPOTHYROIDISM	208
Newborn Screening	208
Thyroid Dysgenesis	208
Thyroid Dyshormonogenesis	210
Transient Neonatal Hyperthyrotropinemia and Hypothyroidism	212
Consumptive Hypothyroidism	212
Defects in Thyroid Hormone Signaling Pathways	212
Central Hypothyroidism	213
Evaluation of Infants With a Positive Newborn Screening Test	213
Treatment of Congenital Hypothyroidism	214
CONGENITAL HYPERTHYROIDISM	214
Graves Disease	214
Nonautoimmune Hyperthyroidism	215
DISORDERS OF SERUM THYROID HORMONE TRANSPORT	215
References	215

INTRODUCTION

The thyroid axis is probably the best example of the physiological interactions between the mother, the fetus, and their environment. The discovery of the crucial role of maternal iodine intake for the normal development of the fetus also led to the first successful intervention in preventive medicine.¹ Two centuries ago, the idea to use iodine to treat endemic goiter, which predominantly affected women, encountered a lot of resistance because it was believed that iodine was toxic for humans. The demonstration that 10% of the insoluble fraction of the thyroid was iodine and that it could be used to treat myxedema and goiter was followed by the first systematic clinical trial to assess the efficacy of iodized salt in preventing goiter. Iodized salt at the population level was next introduced and resulted in a marked decrease in the prevalence of goiter in children and, even more importantly, in the disappearance of “endemic cretinism.” However, pregnant women require double the iodine intake of nonpregnant women to maintain normal thyroid hormone concentrations, so they and their fetuses

are most vulnerable to decreases in iodine intake at the population level. The iodine deficiency that resurfaces in adolescent girls in certain geographic areas is therefore worrisome and illustrates the need for continuous surveillance of intake of this essential nutrient.^{2,3} With this caveat, endemic goiter and cretinism have all but disappeared in the industrialized world and the most severe cases of congenital hypothyroidism (CH) are caused by developmental defects of the thyroid and, albeit much less frequently, to defective pituitary control of thyroid function. These are the focus of this chapter.

EMBRYOLOGY, PHYSIOLOGY, AND PHYSIOPATHOLOGY

Development of the Thyrotrophic Axis

The Pituitary

As for all organs, anatomic development of the hypothalamic-pituitary-thyroid system occurs during the first trimester of gestation. By 3 weeks of gestation, a series of homeodomain proteins or transcription factors begin to drive the differentiation of the human embryonic forebrain and hypothalamus. Immunoreactive thyrotropin-releasing hormone (TRH) becomes detectable in human embryonic hypothalamus by 8 to 9 weeks of postconceptional age and is also produced by the fetal gut and pancreas.

Anatomically, the pituitary gland develops from two ectodermal anlagen: a neural component from the floor of the primitive forebrain, and Rathke’s pouch from the primitive oral cavity. The latter is visible by 5 weeks, evolving to a morphologically mature pituitary gland by 14 to 15 weeks. The pituitary-portal blood vessels are present by this time and mature further through 30 to 35 weeks. A wide spectrum of congenital malformations collectively named *midline defects*, including holoprosencephaly and septooptic dysplasia, may be associated with central hypothyroidism and other anterior pituitary deficiencies.⁴ The molecular mechanisms underlying these malformations have been identified in some cases.⁵ Within the pituitary itself, PROP-1 and PIT-1 are terminal factors in the differentiation cascade of pituitary cells and PIT-1 or PROP-1 deficiency results in profound defects in growth hormone, prolactin, and thyroid-stimulating hormone (TSH) secretion, as well as age-dependent pituitary hypoplasia.⁶

The Thyroid

The human thyroid gland also develops from two endodermal anlagen: the median anlage derives from the primitive pharyngeal floor and the two paired lateral anlagen from the fourth pharyngeal pouches. A branching morphogenesis program is essential for the thyroid to attain a normal size.⁷ The long-held belief that the lateral anlagen were the only source of calcitonin-producing cells has been challenged by the observation that sublingual thyroids, which are derived exclusively from the median anlage, contain calcitonin messenger ribonucleic acid (mRNA) and protein.⁸ Thyroid follicular cells can differentiate within the lateral anlagen, as illustrated by histological observations, as well as by patients in whom the only thyroid tissue is a lateral ectopy. Both the median and lateral structures are visible by day 16 to 17 of gestation; by 50 days, they have fused and the

thyroid gland has migrated to its definitive location in the anterior neck. The thyroglossal duct, from the foramen cecum to the final location of the thyroid, may persist and is constituted of degenerated thyroid follicular cells. Within the thyroid gland, iodine concentration, TSH receptors, thyroglobulin (TG), and thyroperoxidase (TPO) mRNA and protein can be demonstrated by 70 days.⁹

Thyroid embryogenesis depends on the expression of a programmed sequence of transcription factors, including thyroid transcription factors-1 and -2 (TTF-1 or *TiTF-1*, now designated as NKX2 homeobox 1 -NKX2.1-; and TTF-2, now Forkhead box E1 -FOXE1-) and paired box gene 8 (PAX8). In newborn mice, biallelic inactivation of *Nkx2.1* results in the absence of both pituitary and thyroid glands, with complete absence of both thyroid follicular cells and of calcitonin-producing C-cells, whereas that of *Pax8* results in a small thyroid gland composed almost exclusively of C-cells. FoxE-1 null mouse embryos have either an absent thyroid or an ectopic sublingual gland, but all newborn pups have athyreosis in addition to cleft palate. Mutations in the homologous genes, however, account for at most 2% of cases of thyroid dysgenesis in humans. Therefore genes extrinsic to the thyroid may be involved in the control of its migration.¹⁰

Interactions Between Cardiovascular and Thyroid Development

The coordination of the development of the brain and of its vasculature suggests that brain and vessels may follow the same extrinsic cues and/or that there are reciprocal interactions between the two.¹¹ By analogy, the lack of epithelial to mesenchymal transition during thyroid migration suggests it is not an active process but rather involves movement of the surrounding tissues and vessels.^{12,13} Further evidence that the development of the heart and vessels influences thyroid migration stems from the observation that a patient with cooccurring sublingual thyroid and congenital heart disease had a deletion of *NETRIN1* and that *ntn1a* zebrafish morphants had abnormal thyroid morphogenesis, resulting from a lack of proper guidance by the dysplastic vasculature.¹⁴

Maternofetal Transfers and Their Clinical Consequences (Fig. 8.1)

Iodine is an essential component of thyroid hormones. In this chapter, the term iodine will refer to both iodine itself (I^2) and iodide (I^-). The human placenta expresses the sodium-iodine symporter throughout gestation,¹⁵ which explains why the mother's iodine status is reflected in the fetus. If the mother's iodine intake is suboptimal, the fetal thyroid cannot constitute appropriate stores of iodine and fetal hypothyroidism will ensue. Worldwide, inadequate maternal iodine intake leading to late consequences remains a major public health problem, although an increasing number of emerging countries appear to have achieved iodine sufficiency.¹⁶ Prevention of intellectual disability in the offspring by supplying the mother with adequate iodine is a public health intervention with undisputed benefits.¹⁷

In contrast with iodine, thyroxine (T4) was for a long time thought not to cross the placenta in substantial amounts. However, several findings clearly indicate that maternal T4 must cross the placenta in physiologically relevant amounts. First, T4 is detectable in human embryonic tissues before the onset of fetal thyroid function and must therefore be of maternal origin. Second, the transfer of T4 from mother to fetus must continue later in gestation because the concentration in cord blood from neonates with complete absence of thyroid function is 30% to 50% of that of normal neonates.¹⁸ Third, birth weight and neonatal TSH levels are lower in infants born to a mother

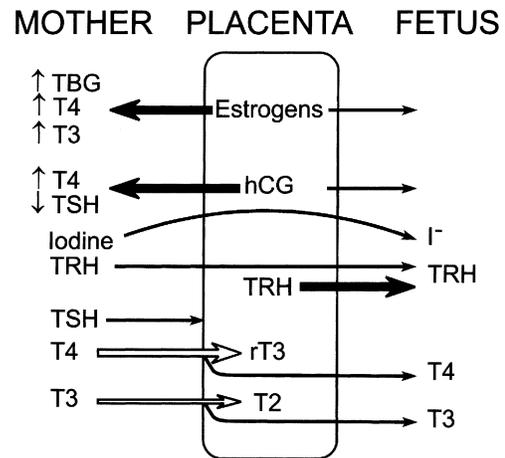


Fig. 8.1 The placental role in thyroid metabolism during human pregnancy. The placenta produces estrogens and a human chorionic gonadotropin (hCG), which increase maternal thyroxine-binding globulin (TBG) levels and stimulate maternal thyroid hormone production, respectively. Both activities tend to increase maternal thyroxine (T4) and triiodothyronine (T3) concentrations and inhibit maternal thyroid-stimulating hormone (TSH) secretion. Iodine and thyrotropin-releasing hormone (TRH) readily cross the placenta. In addition, the placenta synthesizes TRH. The placenta is impermeable to TSH and only partially permeable to T4 and T3. Placental type 3 iodothyronine monodeiodinase enzymes degrade T4 to reverse T3 and T3 to 3,3'-T2. The placenta is also permeable to the thiourea drugs used to treat maternal Graves disease.

who has chronically higher T4 concentrations because of a mutation inactivating the thyroid hormone receptor who have not inherited the maternal mutation than in those who have.¹⁹ These data indicate that maternal T4 crosses the placenta in physiologically relevant amounts throughout gestation and that, in excess, has a direct toxic effect on the fetus.¹⁹

However, low T4 concentrations in pregnant women do not appear to be causally related to a lower IQ in the children: two randomized controlled trials have shown a similar developmental outcome in the offspring of T4-treated versus placebo-treated hypothyroxinemic pregnant women.^{20,21} Moreover, in two case series of women with severe hypothyroidism diagnosed during pregnancy, but corrected by the third trimester, the intellectual outcome of the offspring was normal.^{22,23} Thus universal screening for thyroid dysfunction in the mother during pregnancy remains hotly debated. On the other hand, women with known hypothyroidism require closer monitoring during pregnancy, as 85% of women who are already receiving T4 therapy require a 30% to 50% increase in dose during pregnancy, because of the estradiol-induced increase in serum T4-binding globulin.²⁴

The transplacental transfer of T4 is not always sufficient to prevent the development of fetal goiter if the fetus has severe thyroid dyshormonogenesis (Fig. 8.2). Fetal goiters may be large enough to interfere with the flow of amniotic fluid into the oropharynx, causing progressive hydramnios and eventual lung hypoplasia. In such cases, levothyroxine injected into the amniotic fluid is swallowed by the fetus, leading to a decrease in the size of the fetal thyroid and in the degree of hydramnios.²⁵ The injection of thyroxine into the umbilical vein, which carries an even higher risk of triggering premature labor or fetal loss than amniocentesis,²⁶ should be restricted to fetuses with a goiter that continues to increase in spite of repeated intraamniotic injections. Invasive and potentially risky procedures should not be undertaken to protect the brain of affected fetuses:^{27,28} indeed, the fetal brain is, to a large extent, protected from the deleterious effect of hypothyroidism

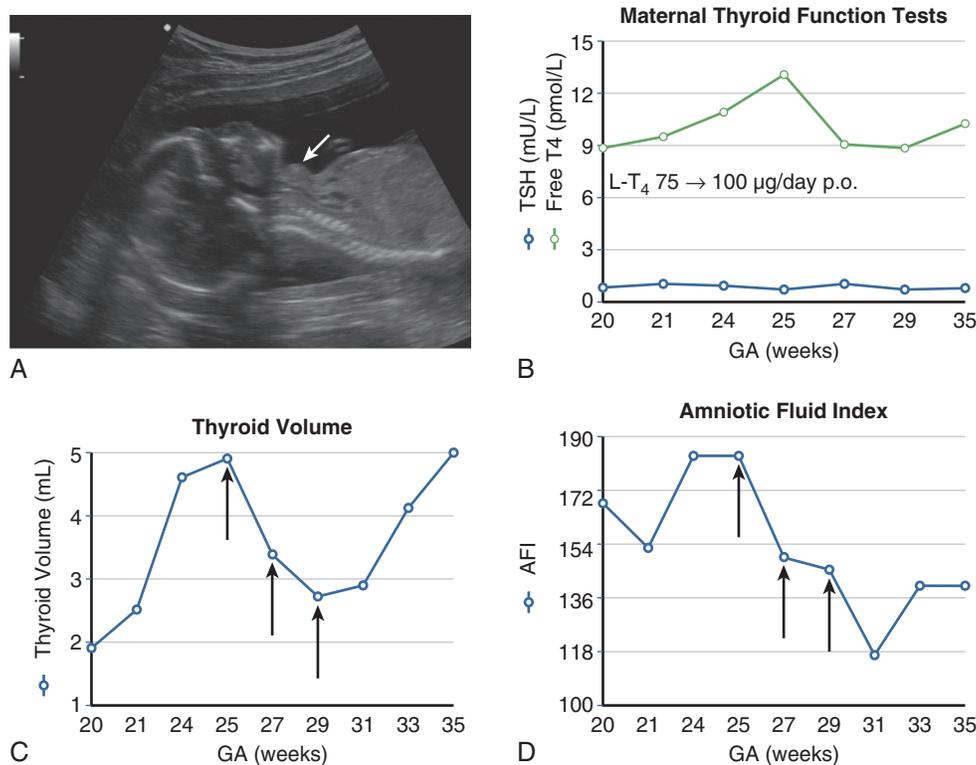


Fig. 8.2 A, Fetal goiter discovered at a routine ultrasound at 19 weeks (white arrow). The mother was euthyroid and had no autoantibodies. Cordocentesis showed a serum thyroid-stimulating hormone (TSH) of 90 mU/L. B, Initial management was through oral treatment of the mother with increasing doses of levothyroxine; this resulted in increased maternal free thyroxine (T4) concentrations, but presumably not enough to cross the placenta in sufficient amounts to prevent the progression of the fetal goiter (C) and the development of hydramnios (D). C, Effect of three intraamniotic injections of 200 μ g of levothyroxine (black arrows) on fetal thyroid size. D, Effect of three intraamniotic injections of 200 μ g of levothyroxine (black arrows) on amniotic fluid index. At birth, cord serum TSH was 224 mU/L and thyroglobulin was low at 3.55 μ g/L; thyroglobulin deficiency was confirmed by molecular genetic analyses. Treatment with 50 μ g of levothyroxine orally from day 1 has allowed this child, now aged 9 years, to have normal intellectual development. GA, Gestational age. (From Stoppa-Vaucher et al. (2010). *J Pediatr*, 156, 1026–1029.)

through upregulation of brain type 2 deiodinase, which converts the prohormone T4 into its biologically active derivative, triiodothyronine (T3). This likely accounts for the observation that even in CH with delayed bone maturation at diagnosis (indicating a prenatal onset), the intellectual outcome is within normal limits if continuous and adequate treatment is instituted shortly after birth.^{29,30} Thus the in utero treatment of fetal hypothyroidism should only be considered for goiter causing progressive hydramnios. Although the identification of a goiter by prenatal ultrasound may be increasing,³¹ it remains rare and even direct examination at birth often fails to detect goiters that are obvious on imaging. Goiters can also be detected in fetuses borne by women with Graves disease (see section on congenital hyperthyroidism).

Although a pro-TRH molecule is produced by the placenta, TRH concentrations in the maternal circulation are very low and thus have little effect on fetal thyroid function. However, TRH, a tripeptide, crosses the placenta readily and, when injected into the mother, increases thyroid hormone concentrations in the fetus. Because thyroid hormones stimulate fetal lung maturation, maternal TRH treatment to decrease neonatal respiratory distress syndrome has been attempted, but with negative results.³²

Immunoglobulin(Ig) of the IgG type cross the placenta, hence transient fetal/neonatal hyperthyroidism from transplacental transfer of TSH-receptor activating antibodies can occur in women with past or present Graves disease. On the other hand, when pregnant women are overtreated with antithyroid drugs, which also cross the placenta readily, their fetus may develop hypothyroidism; however, only one case of CH out

of about 30,000 births is attributable to this in the Québec database (unpublished observations). In addition, the antithyroid medication has a short half-life relative to that of immunoglobulin, so that a baby with apparently normal thyroid function tests at birth may develop hyperthyroidism within several days of birth, as the effects of antithyroid medication wanes, allowing the effects of thyroid-stimulating immunoglobulin (TSI) to become apparent. Lastly, transient neonatal hypothyroidism from maternofetal transfer of TSH-receptor blocking antibodies can also occur but only accounts for 2% of cases of neonatal hypothyroidism identified by screening,³³ and a specific screening strategy is not required for babies born to women with Hashimoto thyroiditis.

Thyroid function tests are often ordered clinically in newborns whose mothers have a history of thyroid disease and, if abnormal, require special consideration of optimal approach to therapy. For example, hypothyroidism at birth caused by treatment of maternal Graves disease with antithyroid medication may only require observation, in the expectation that the effects of the drugs will dissipate over a few days; hyperthyroidism may follow, albeit exceptionally. For hypothyroidism or hyperthyroidism resulting from maternal blocking or stimulating antibodies, respectively, treatment will be required as these effects may last several months.³⁴

Maturation of Thyroid Hormone Synthesis and Secretion (Figs. 8.3 and 8.4)

Maturation of thyroid function in the fetus reflects changes at the level of the hypothalamus, pituitary, and thyroid. Serum

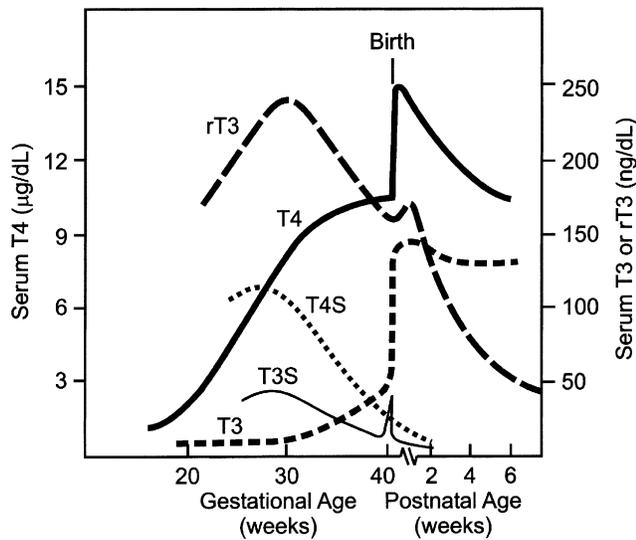
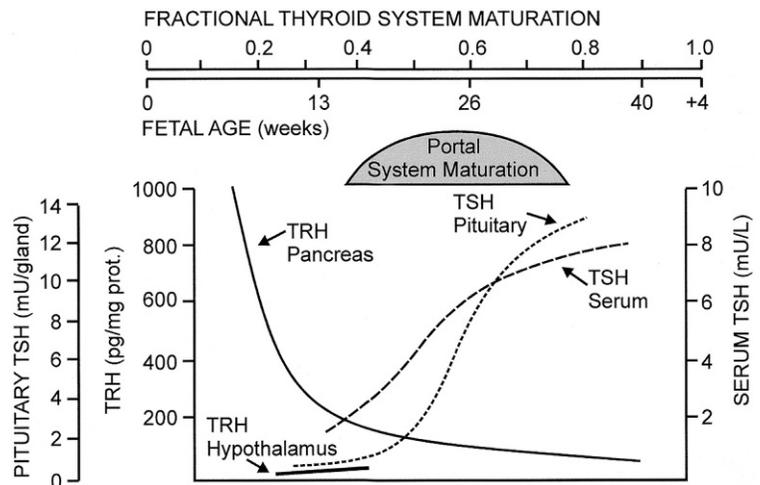


Fig. 8.3 The pattern of change in fetal and neonatal thyroid function parameters during pregnancy and extrauterine adaptation. Fetal serum thyroxine (T4) concentrations begin to increase at midgestation and increase progressively thereafter to term. This increase is caused largely by the increase in thyroxine-binding globulin concentration, but free T4 concentrations (not shown) also increase progressively between 20 and 40 weeks. T4 in the fetus is metabolized predominantly to inactive reverse triiodothyronine (rT3) and sulfated analogs (T4S, T3S). Monodeiodination of T4 to active triiodothyronine (T3) increases at about 30 weeks to levels approximating 50 ng/dL at term. The thyroid-stimulating hormone (TSH) surge (not shown), which peaks at 25 to 30 minutes after extrauterine exposure, stimulates thyroidal T4 and T3 secretion. Neonatal T4 and T3 peak at 2 to 3 days. Serum T3 concentrations remain at higher postnatal levels because of the increased T4-to-T3 conversion mediated by increased type 1 iodothyronine deiodinase activity in newborn tissues.

TRH concentrations are relatively high in the human fetus, because it is produced at both hypothalamic and extrahypothalamic sites and because the TRH-degrading activity of fetal blood is low. Fetal serum TSH increases from a low level at 18 to 20 weeks to a peak value of approximately 7 to 10 mU/L at term. After delivery, in response to exposure to the relatively colder extrauterine environment, there is an acute release of TSH with mean serum levels peaking at 30 minutes

Fig. 8.4 Changes in fetal thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) levels in pancreas, hypothalamus, serum, and pituitary during human gestation. Hypothalamic TRH concentrations increase progressively after midgestation, but the pattern of change has not been documented in the human fetus. (From Fisher, D. A., Polk, D.H. (1994). Development of the fetal thyroid system. In: Thorburn, G.D., Harding, R. (eds.), *Textbook of Fetal Physiology*. Oxford, UK: Oxford University Press, 359–368. With permission.)



at concentrations of approximately 70 mU/L. A rapid decline follows within 24 hours, and levels return more gradually to 5 to 10 IU/L within the first week of life. The increase in serum T4 levels immediately after birth is TSH dependent. T3 rises as a result of both TSH-stimulated thyroidal production and peripheral conversion of T4 to T3. For newborn screening purposes, the percentage of samples with TSH greater than 15 mU/L, which is 9% in the first 24 hours, drops to 0.3%, as early as the second day,³⁵ so any sample obtained after 24 hours can be used.

Only free thyroid hormones enter cells, and hormones bound to serum thyroxine-binding globulin (TBG) and other transport proteins are not available to tissues. In addition, T4 is a prohormone and it is T3 that is biologically active within the cell, so that deiodination of T4 is essential for tissue euthyroidism. Both serum transport proteins and intracellular deiodination change during development. As previously mentioned, the fetal thyroid gland is capable of iodine concentration and iodothyronine synthesis as early as 70 days of gestation, a reflection of a sharp increase in the expression of the sodium-iodine symporter and of the appearance of a follicular architecture.³⁶ Starting at 18 to 20 weeks, TBG and total T4 concentrations in fetal serum increase steadily until the final weeks of pregnancy.

The study of free T4 (fT4) concentrations in fetal/neonatal blood has been hampered by the relative inadequacy of the commercially available immunoassay systems for measurements in these samples. The fetal serum T3 concentration remains low until 30 weeks because of two factors: first, the low activities of type I iodothyronine monodeiodinase result in relatively low rates of T4 to T3 conversion in fetal tissues; second, type III monodeiodinase in placenta and selected fetal tissues degrades T3 to T2. After 30 weeks, serum T3 increases slowly until birth. This prenatal increase in serum T3 is caused by progressive maturation of liver type I deiodinase activity increasing hepatic conversion of T4 to T3, and to decreased placental T3 degradation. Postnatally, T3 and T4 serum concentrations increase 2- to 6-fold within the first few hours, peaking on the second day of life. These levels then gradually decline to levels characteristic of infancy over the first 4 to 5 weeks of life. The deiodinase enzymes are described in greater detail later.

In the human, the fetal thyroid gland grows and its production increases under the influence of the increasing serum TSH level during the second half of gestation, as evidenced by the severely atrophic and hypofunctional glands observed in newborns with biallelic mutations that inactivate either the beta-subunit of TSH³⁷ or the TSH receptor.³⁸ On the other hand, the maturation of the negative feedback control

system appears to occur earlier than previously thought because an elevated TSH in serum obtained at cordocentesis can be seen in fetuses with primary hypothyroidism as early as 18 weeks.³⁹ Thyroid function in the premature infant is characterized by low circulating concentrations of T4 and fT4, a normal or low concentration of TSH, and a normal or prolonged TSH response to TRH, suggesting a degree of relative hypothalamic (tertiary) hypothyroidism (see Fig. 8.3).

In summary, fetal thyroid hormone secretion results from increasing hypothalamic TRH secretion and increasing thyroid follicular cell sensitivity to TSH and is regulated by increasing pituitary sensitivity to thyroid hormone inhibition of TSH release. The marked cold-stimulated TRH-TSH surge at birth is followed by a marked increase in T4 secretion and fT4 concentration with a new equilibrium reached by 1 to 2 months. During infancy and childhood, there is a progressive decrease in T4 secretion rate (based on a micrograms/kg per day) correlating with a decreasing metabolic rate.

Maturation of Thyroid Hormone Metabolism (Fig. 8.5)

The thyroid gland is the sole source of T4. Most of the circulating T3 after birth is derived from conversion of T4 to T3 via monodeiodination in peripheral tissues. Deiodination of the iodothyronines is the major route of metabolism, and monodeiodination may occur either at the outer (phenolic) ring or the inner (tyrosyl) ring of the iodothyronine molecule. Outer ring monodeiodination of T4 produces T3, the form of thyroid hormone with the greatest affinity for the thyroid nuclear receptor. Inner ring monodeiodination of T4 produces reverse T3 (rT3), an inactive metabolite. In adults, between 70% and 90% of circulating T3 is derived from peripheral conversion of T4 and 10% to 30% from direct glandular secretion. Nearly all circulating rT3 derives from peripheral conversion, with only 2% to 3% coming directly from the thyroid gland. T3 and rT3 are progressively metabolized to diiodo, monoiodo, and noniodinated forms of thyroxine, none of which possesses biologic activity.

Two types of outer ring iodothyronine monodeiodinases have been described.⁴⁰ Type I deiodinase (predominantly expressed in liver, kidney, and thyroid) is a high-K_m enzyme inhibited by propylthiouracil and stimulated by thyroid hormone. Type II deiodinase (predominantly located in brain,

pituitary, placenta, skeletal muscle, heart, thyroid, and brown adipose tissue) is a low-K_m enzyme insensitive to propylthiouracil and inhibited by thyroid hormone. Type I and II deiodinases contribute to circulating T3 production, whereas type II acts to increase local tissue levels of T3 as well. An inner ring deiodinase (type III deiodinase) has been characterized in most fetal tissues, including placenta. This enzyme system catalyzes the conversion of T4 to rT3 and T3 to diiodothyronine. All three deiodinase enzymes are selenoproteins.

Deiodination is developmentally and thyroid-state regulated. In the human fetal brain, type II deiodinase activity in the cortex increases between 13 and 20 weeks' gestation and by about 50% over the last third of gestation. There is a general inverse correlation of type II and type III activities. Both of these deiodinase species are thyroid hormone responsive.

Fetal thyroid hormone metabolism is characterized by a predominance of type III enzyme activity (particularly in liver, kidney, and placenta), accounting for the increased circulating concentrations of rT3 observed in the fetus. However, the persistence of high circulating rT3 concentrations for several weeks in the newborn indicate that type III deiodinase activity expressed in nonplacental tissues is important. The mixture of type II and type III deiodinase activities in the placenta provides for the conversion of T4 to T3 and of T4 and T3 to rT3 and T2, respectively.

Sulfated iodothyronines are the major thyroid hormone metabolites circulating in the fetus. Sulfokinase enzymes are present early in fetal life, and sulfation of the phenolic hydroxyl group of the iodothyronine molecule may be a normal prerequisite step for monodeiodination. The sulfated iodothyronines are preferred substrates for the type I deiodinase, and concentrations are high in fetal serum in part because of low type I deiodinase activity. However, increased production of sulfated metabolites is also involved. There is evidence that T3S has biologic activity, that is, it suppresses TSH *in vivo*, suggesting that it can be desulfated by one or more tissue sulfatase enzymes. The low production rates and low levels of T3 metabolites and the high ratio of inactive to active metabolites suggest that fetal thyroid hormone metabolism is largely oriented to inactivating T4, presumably to avoid tissue thermogenesis and to potentiate the anabolic state of the rapidly growing fetus. This is mediated by early activation of type III monodeiodinase, inactivation of type I monodeiodinase, and augmented iodothyronine sulfation.

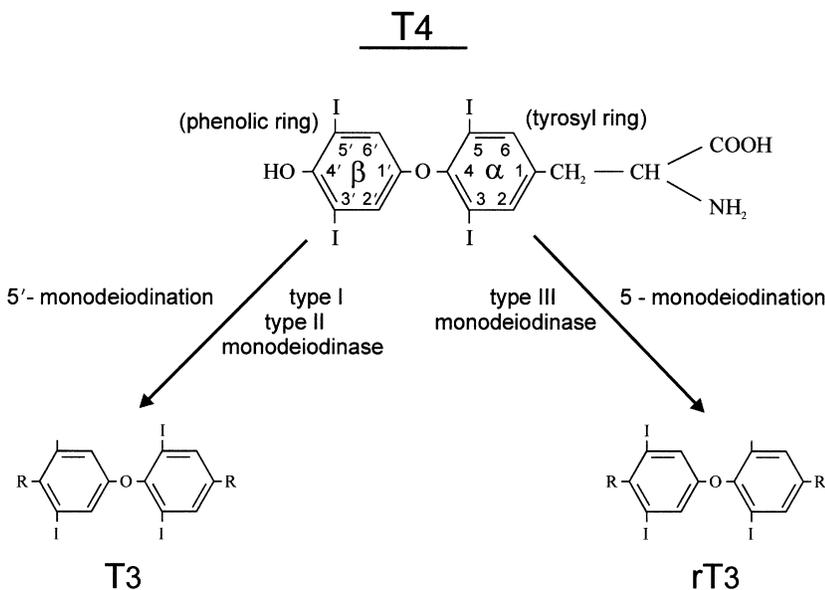


Fig. 8.5 The deiodination of thyroxine by types 1, 2, and 3 iodothyronine monodeiodinase enzymes. The type 1 enzyme is also capable of inner ring monodeiodination, particularly of the sulfated conjugates (not shown).

The developmental expression of type II deiodinase in brain and other tissues provides for local T3 supply to specific tissues (particularly in the event of T4 deficiency) and helps guarantee provision of T3 during gestation, when brain development is thyroid hormone dependent.

Thyroid Hormone Cell Membrane Transporters and Receptors

All thyroid-sensitive cell populations express iodothyronine membrane transporters, which are required for hormone entry into the cell. These belong to families of integrin, organic anion, amino acid, and monocarboxylate solute carriers. The importance of these transporters is highlighted by the role of mutations inactivating human monocarboxylate transporter 8 (MCT8) in an X-linked syndrome of severe psychomotor retardation (previously called the *Allan-Herndon-Dudley syndrome*) combined with mild abnormalities of thyroid function, characterized by high T3, low T4, and normal or high TSH.^{41,42} MCT8 is thought to play a role in the entry of T3 into neurons, after deiodination of T4 to T3 in neighboring astrocytes. In addition, MCT8 is involved in the transfer of T3 across the blood-brain barrier. Lastly, the abnormalities in thyroid hormone levels and TSH are also caused by the effect of MCT8 on deiodination.

Thyroid hormone effects are mediated predominantly via nuclear thyroid hormone receptors (TRs), which bind to deoxyribonucleic acid (DNA) to regulate gene transcription. Two mammalian genes code for TR, *TRalpha* and *TRbeta*, and alternative mRNA splicing leads to the production of four major thyroid hormone-binding transcripts: TRalpha1, TRalpha2, TRbeta1, and TRbeta2. The TRs exist as monomers, homodimers, and heterodimers with other nuclear receptor family members, such as the retinoid X receptors. TRalpha1 is the predominant subtype in bone, gastrointestinal tract, heart, and brain. TRbeta1 is expressed in liver, kidney, heart, lung, brain, cochlea, and pituitary. TRbeta2 is expressed in pituitary, retina, and cochlea. The receptors function redundantly, as indicated by knockout studies in mice, but predominant effects of one or another TR have been described.

In humans, the specific roles of TRalpha and TRbeta are illustrated by the phenotypes observed in patients with inactivating mutations in the corresponding genes. The syndrome of thyroid hormone resistance initially described in 1967 was later found to be caused by mutations inactivating TRbeta that occur either de novo or are inherited in an autosomal dominant fashion; however, in some patients with thyroid hormone resistance, TRbeta is normal and the molecular defect remains elusive. Mutations in TRalpha have also been described, occurring de novo in one patient and transmitted from father to daughter in one pedigree.⁴³

Thyroid hormone-programmed development of fetal tissues requires the interaction of local tissue monodeiodinase I and II, TRs, thyroid receptor coactivators, and thyroid-responsive genes. In most responsive tissues, the timing of maturation events is controlled by the TRs acting as a molecular switch. In the absence of T3, the unliganded receptor recruits corepressors, thereby repressing gene transcription. Local tissue maturation events are stimulated by the coincident availability of T3, liganded T3 receptor, T3-mediated receptor exchange of corepressors with coactivators, and activation of responsive gene transcription.⁴⁴

In the human fetus, low levels of TR binding have been detected in brain tissue at 10 weeks' gestational age—and liver, heart, and lung TR binding is observed at 16 to 18 weeks. TR levels in human fetal cerebral cortex and cerebellum increase markedly during the second and third trimesters. Information is limited regarding the timing of appearance of thyroid hormone tissue effects in the human fetus.

The birth length of the athyreotic human neonate is within normal limits: the linear growth of the human fetus is programmed independently of thyroid hormones by a complex interplay of genetic, nutritional, and hormonal factors, as well as by mechanical uterine constraint. However, 50% to 60% of athyreotic newborns manifest delayed epiphyseal maturation and have large fontanelles. In addition, neonates with severe CH may have large fontanelles, macroglossia, umbilical hernia, prolonged jaundice, and feeding difficulties. However, the classic clinical manifestations of CH appear progressively only during the early months of life. These include myxedema, a slow linear growth, and delayed attainment of psychomotor milestones, which can be mitigated by early diagnosis through neonatal screening programs with institution of thyroid hormone replacement. The normal IQ of athyreotic infants treated early through newborn screening³⁰ seems attributable to T4 of maternal origin coupled with upregulation of type II monodeiodinase in fetal brain tissue in the face of low fetal serum T4.

Postnatal thermogenesis is mediated via the brown adipose tissue prominent in subscapular and perirenal areas in the mammalian fetus and neonate. Heat production in brown adipose tissue is stimulated by catecholamines via beta-adrenergic receptors and is thyroid hormone dependent. The uncoupling protein thermogenin unique to brown adipose tissue is located on the inner mitochondrial membrane and uncouples phosphorylation by dissipating the proton gradient created by the mitochondrial respiratory chain. The type II monodeiodinase in brown adipose tissue mediates local T4 to T3 conversion. Full thermogenin expression in brown adipose tissue requires both catecholamine and T3 stimulation.⁴⁵ Brown adipose tissue matures progressively in the fetus but remains thermoneutral until stimulated by catecholamines in the perinatal period. Brown adipose tissue thermogenesis is immature in small premature infants, and brown adipose tissue mass decreases in the neonatal period in full-term infants, as the capacity for nonshivering thermogenesis develops in other tissues. Uncoupling protein-2 is found in many tissues but does not appear to be regulated by beta-adrenergic agonists or thyroid hormone. Uncoupling protein-3 is expressed in muscle and white adipose tissue, as well as brown adipose tissue. Muscle uncoupling protein-3 is regulated by beta3-adrenergic stimulation and thyroid hormone and presumably contributes to nonshivering thermogenesis in humans. mRNA levels for uncoupling protein-3 are also regulated by dexamethasone, leptin, and starvation, but the regulation differs in brown adipose tissue and muscle.

The critical role of thyroid hormones in central nervous system (CNS) maturation has long been recognized. Nervous system development involves neurogenesis, gliogenesis, neural cell migration, neuronal differentiation, dendritic and axonal growth, synaptogenesis, myelination, and neurotransmitter synthesis. Thyroid hormones have been shown to stimulate several developmentally regulated nervous tissue genes, but the role of these factors in the CNS developmental program remains undefined. Available evidence suggests that deficiency or excess of thyroid hormones alters the timing or synchronization of the CNS developmental program, presumably by initiating critical gene actions or other genetic CNS maturation events.⁴⁶

Perinatal Changes in Thyroid Function

After delivery, the neonate must rapidly convert from the fetal state of predominant thyroid hormone inactivation to a state of relative thyroidal hyperactivity. During the first hours after birth, there is an abrupt increase in circulating T4 and T3 levels. This is caused by the abrupt increase in hypothalamic TRH and pituitary TSH secretion stimulating increased thyroid hormone

TABLE 8.1 Estimated Birth Prevalence of Various Types of Permanent Thyroid Disorders in Iodine-Sufficient Populations, Based on Universal Newborn Screening With Either Thyroid-Stimulating Hormone or Thyroxine

Type of Permanent Congenital Disorder	Estimated Birth Prevalence
Thyroid Dysgenesis:	
– Ectopy	1:5,000
– Athyreosis	1:15,000
Thyroid Dyshormonogenesis	1:30,000
Hypothalamic-Pituitary Hypothyroidism	1:16,000
Thyroid Hormone Resistance	1:40,000

From Deladoey, J., Ruel, J., Giguere, Y., Van Vliet, G. (2011). Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. *J Clin Endocrinol Metab*, 96(8), 2422–2429; Van Tijn, D.A., de Vijlder, J.J., Verbeeten, B., Jr., Verkerk, P.H., Vulmsa, T. (2005). Neonatal detection of congenital hypothyroidism of central origin. *J Clin Endocrinol Metab*, 90(6), 3350–3359; Lafranchi, S.H., Snyder, D.B., Sesser, D.E., Skeels, M.R., Singh, N., Brent, G.A., et al. (2003). Follow-up of newborns with elevated screening T4 concentrations. *J Pediatr*, 143(3), 296–301.

orthotopic glands. In about 10%, there is a dumbbell-shaped image,⁶¹ suggesting that some cells have initiated migration and others not. The molecular mechanisms regulating migration of the embryonic thyroid are largely unknown. Ectopic thyroids are fully differentiated and have a normal follicular architecture and the associated CH likely reflects a reduced number of cells (lack of lateral lobes) and limited TSH-induced cell growth.⁶² The severity of CH is variable but is stable over time,⁶³ suggesting normal postnatal survival of the ectopic cells. The 25% of newborns with dysgenetic CH who have no visible uptake of tracer on scintiscan may have true athyreosis, in which cases serum TG is very low. When there is no visible tissue but serum TG is detectable, it seems appropriate to use the term *apparent athyreosis*, which may result from complete inactivation of the TSH receptor, either from the transplacental passage of blocking immunoglobulins (in which case CH is transient)⁶⁴ or from biallelic inactivating mutations of the

TSH receptor gene (in which case CH is permanent).³⁸ Orthotopic thyroid hypoplasia accounts for a smaller percentage of cases of overt CH. Lastly, congenital absence of a thyroid lobe, most often the left, is found in up to one in 500 normal children⁶⁵ but may be seen in association with permanent or transient CH.⁶⁶

A genetic susceptibility to thyroid dysgenesis was suggested by the observation that about 1% of affected patients have an affected first-degree relative,⁶⁷ giving a 40-fold increase in relative risk over the population prevalence of one in 4000 for dysgenetic CH. On the other hand, discordance between monozygotic twins is the rule⁵⁵ and the three to one female predominance of thyroid ectopy⁴⁷ are not compatible with standard Mendelian inheritance. To reconcile these contradictory epidemiological findings, a two-hit model has been proposed, in which thyroid dysgenesis requires the association of a germline susceptibility variant with a postzygotic event occurring at the somatic level, in developing thyroid tissue.⁶⁸ Because ectopic thyroids seldom need to be surgically removed, most of the work of the past 20 years has focused on leukocyte DNA, but disease-causing mutations have been found in a small percentage of patients with dysgenetic CH and in only a handful of those with the most common phenotype, thyroid ectopy. Clinically, a DNA diagnosis only needs to be sought if there are suggestive extrathyroidal manifestations or a family history of dominant or recessive transmission of dysgenetic CH (Table 8.2). In addition, generally mild CH, mostly associated with left hemithyroid, appears to occur more frequently in Di George⁶⁹ and Williams⁷⁰ syndrome than in the general population. In Di George syndrome, the deleted region on chromosome 22q11 generally encompasses *TBX1*, a gene involved in vessel development, further emphasizing the link between the development of the thyroid and of the cervical vasculature.⁷¹

Thyroid-Stimulating Hormone Receptor Mutations

The molecular cloning of the G protein-coupled TSH receptor⁷² was followed by the discovery of mono- or biallelic inactivating mutations in the *thyroid-stimulating hormone receptor* gene.⁷³ Monoallelic mutations account for 10% to 20% of persistent, mild, nonautoimmune hyperthyrotropinemia,⁷⁴

TABLE 8.2 Dysgenetic Congenital Hypothyroidism Associated With TSHR and Transcription Factor Mutations: Phenotypes (Thyroid and Other) and Mode of Inheritance

Gene	Transmission	Thyroid Phenotype	Other Features
<i>TSHR</i>	AR	From apparent athyreosis to normally appearing gland	None
<i>NKX2.1</i>	De novo or AD	From apparent athyreosis to normal gland, usually mild ↑ TSH	– RDS – Developmental delay/Hypotonia – Ataxia/Choreoathetosis
<i>FOXE1</i>	AR	True athyreosis	– Cleft palate – Choanal atresia – Kinky hair – Bifid epiglottis
<i>Pax8</i>	AD or de novo	From apparent athyreosis to normally appearing gland	Cysts within thyroid remnants
<i>GLIS3</i>	AR	From apparent athyreosis to normally appearing gland	– Congenital glaucoma – Deafness – Liver, kidney and pancreas abnormalities
<i>NETRIN</i>	de novo	Ectopy	Ventricular septal defect
<i>JAG1</i>	de novo	Ectopy, apparent athyreosis, orthotopic hypoplasia	Alagille syndrome
<i>BOR</i>	AR	Ectopy, athyreosis	None
<i>TUBB1</i>	AR	Ectopy, orthotopic hypoplasia, hemithyroid	Macroplatelets

AD, Autosomal dominant; AR, autosomal recessive; CH, congenital hypothyroidism; RDS, respiratory distress syndrome; TSH, thyroid-stimulating hormone.

which does not require treatment.⁷⁵ The phenotypic spectrum of biallelic mutations ranges from hyperthyrotropinemia with normal thyroid hormones and anatomy⁷³ to apparent athyreosis.^{38,76} There is no associated extrathyroidal phenotype.

Thyroid Transcription Factor Mutations

The thyroid transcription factors *NKX2.1* and *PAX8* are necessary and sufficient for the differentiation of stem cells into follicular cells,⁷⁷ whereas *FOXE1* may be involved in their migration.⁷⁸ Given their expression in extrathyroidal tissues, mutations mostly lead to syndromic phenotypes.

NKX2.1

The thyroid phenotype of patient bearing monoallelic inactivation of *NKX2.1* is typically restricted to mild hyperthyrotropinemia and it is the lung (lethal neonatal hypoplasia or recurrent infections of the lungs) and brain (hypotonia followed by choreoathetosis) manifestations of the “brain-lung-thyroid” syndrome that led to the diagnosis. These mutations may occur de novo or be transmitted in an autosomal-dominant fashion.⁷⁹

FOXE1

Only four homozygous mutations in *FOXE1* have been reported in athyreotic children with cleft palate and spiky hair, with or without bifid epiglottis and choanal atresia (Bamforth–Lazarus syndrome). Three mutations led to loss of function, and one led to gain of function.⁸⁰

Paired Box Gene 8

PAX8 is involved in thyroid and kidney development. The phenotypic spectrum of de novo or autosomal-dominantly inherited mutations varies widely both among individuals and within families carrying the same mutations, and ranges from normal thyroid morphology to hypoplasia, ectopy or athyreosis, with or without renal manifestations.⁸¹

GLIS3

Biallelic inactivation of GLI-similar3 (*GLIS3*), which is essential for TSH/TSHR-mediated proliferation of thyroid follicular cells and hormone biosynthesis, has been reported in a handful of cases. The patients present with a multisystem disorder dominated by athyreosis and neonatal diabetes, but also with glaucoma, sensorineural deafness, craniosynostosis, cardiac, liver, kidney, and exocrine pancreatic failure.⁸²

Hematopoietically Expressed Homeobox Protein

Thyroid follicular cells are characterized by the coexpression of *PAX8*, *NKX2.1*, *FOXE1*, and hematopoietically expressed homeobox protein (*HHEX*). However, the only patient with a monoallelic *HHEX* variant also had biallelic *TPO* mutations and a goiter, so the role of *HHEX* in thyroid development remains unclear.⁸³

NKX2.5

Because thyroid dysgenesis may be associated with defects in heart septation, monoallelic variants in *NK2* homeobox-5 (*NKX2.5*), a gene primarily known for its role in inherited heart conduction defects, have been investigated in patients with CH. However, the variants found in the probands were also carried by euthyroid parents with normal thyroid anatomy and, therefore, *NKX2.5* variants are not sufficient to cause thyroid dysgenesis.⁸⁴

NETRIN

In a single dysmorphic patient with thyroid ectopy, a ventricular and atrial septal defect, a patent ductus and a complex chromosome rearrangement, copy number variant analysis revealed a deletion of *NETRIN1* (*NTN1*), a gene encoding a laminin-related secreted protein that acts as an axon guidance molecule during neural development.¹⁴

JAG1

Alagille syndrome type 1 is a rare syndrome with liver and cardiovascular involvement generally caused by a heterozygous mutation in *JAG1*, a gene encoding a Notch pathway ligand or the receptor *NOTCH2*. Among 21 young patients with this syndrome, three were found to have severe CH caused by ectopy, apparent athyreosis, or orthotopic hypoplasia.⁸⁵

BOREALIN and *TUBULIN*

Mono- or biallelic mutations in these genes have been found in three families each with different forms of CH, including ectopy. In addition to CH, patients with *TUBB1* mutations have macroplatelets.^{86,87}

Thyroid Dyshormonogenesis

General Features

Functionally, the thyroid is an iodine pump that organifies this nutrient to make thyroid hormone. Iodine availability is the rate-limiting step for thyroid hormone synthesis, and thus a recycling mechanism exists to conserve it maximally. TSH is the main regulator of hormone biosynthesis: binding of the TSH receptor on the follicular cell surface triggers cyclic adenosine monophosphate (cAMP) activation, which in turn stimulates iodine transport across the cell membrane. Iodine is trapped in the follicular cell, diffuses to its apex, and is transported into the colloid. Next, oxidation and incorporation into tyrosine residues within the TG molecules takes place. TG is then endocytosed and fuses with intracellular phagolysosomes. Proteolysis leads to the release of iodotyrosines (monoiodotyrosine [MIT] and diiodotyrosine [DIT]) and iodothyronines (T4 and T3). Lastly, deiodination of MIT and DIT by dehalogenase leads to intracellular iodine recycling, while T4 and T3 are released into the circulation.⁸⁸ TG that escapes from the gland via the lymphatic system can be detected in blood and can be used as a marker for the presence of thyroid tissue. A schematic representation of thyroid hormone biosynthesis is shown in Fig. 8.7. Any defect in biosynthesis will, because of increased TSH secretion, result in a goiter. By analogy with the adrenal, thyroid dyshormonogenesis could therefore be conceptualized as “congenital thyroid hyperplasia.”

Dyshormonogenesis accounts for about 10% of CH,⁴⁷ except in consanguineous populations. In contrast to the mostly sporadic nature of dysgenesis, thyroid dyshormonogenesis follows a classically Mendelian, autosomal-recessive pattern. Decreased iodine trapping, defective organization of trapped iodine, abnormalities of TG structure, and deficiency of iodotyrosine recycling have all been described. *TPO* mutations appear to be most common in Caucasians⁸⁹ and *DUOX2* in Asians.⁹⁰

The goiter of patients with dyshormonogenetic CH can be seen as early as in utero²⁵ or at birth, but in many newborns, thyroid enlargement goes unnoticed, or onset of goiter occurs later. The diagnosis of the specific type of dyshormonogenesis does not affect initial treatment or counseling: a 25% recurrence risk for siblings of affected subjects can be given

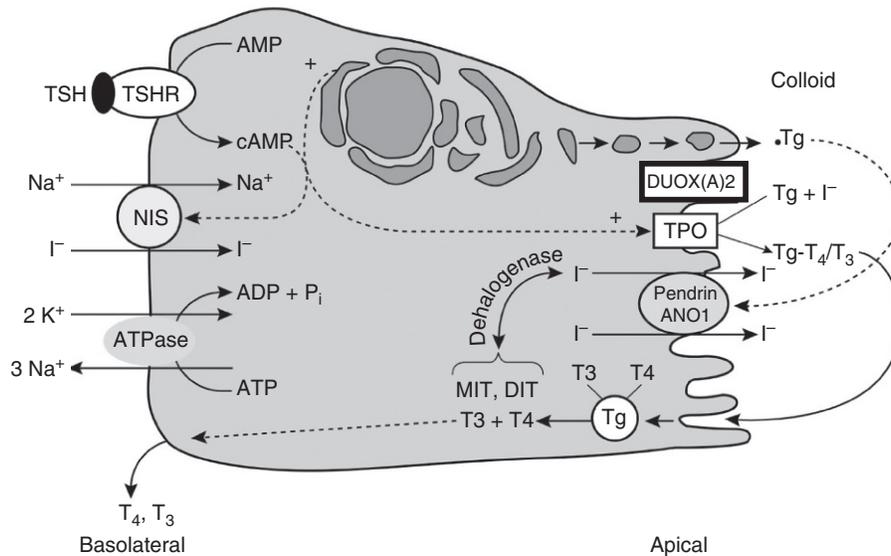


Fig. 8.7 Schematic representation of a thyroid follicular cell indicating all the important steps in thyroid hormone biosynthesis. *ANO1*, Anoctamin-1.

empirically. In cases with transient CH, *DUOX2*, or *DUOX(A)2*, mutations are likely.⁹⁰ Otherwise, a molecular diagnosis should only be sought when the inheritance pattern is other than autosomal recessive.⁹¹

Sodium-Iodine Symporter Defects

Mutations in the *SLC5A5* gene encoding the sodium-iodine symporter (NIS) impair the first step of thyroid hormone biosynthesis, whereby iodine is transported across the plasma membrane into the follicular cell cytosol. NIS is also present in several other tissues, including the salivary glands, gastric mucosa, mammary glands, ciliary body, choroid plexus, and placenta. Although these tissues can concentrate iodine (and thus be visible on iodine scintigraphy), the capacity to organify inorganic iodine is limited to the thyroid gland. Based on the degree of residual NIS activity, patients may present as neonates, infants, or during childhood. Diagnosis is based on detection of a goiter on physical or ultrasound examination with absent or reduced iodine uptake on scintigraphy. Infants may present after normal newborn screening and therefore risk neurodevelopmental delay.⁹²

Pendred Syndrome

Biallelic loss-of-function mutations in the *SLC26A4* gene encoding for the anion exchanger protein pendrin lead to a partial iodide organification defect caused by impaired iodide efflux. Pendrin is also expressed in the inner ear where it is involved in normal anion transport, and in the kidney where it functions as a chloride/bicarbonate exchanger. Clinical features of Pendred syndrome (PDS) include congenital bilateral neurosensory deafness and goiter, but no renal abnormalities. The thyroid phenotype is usually mild: the development of goiter and mild hypothyroidism depends on nutritional iodine intake and shows variable onset within the same families.⁹³ It is therefore seldom identified by newborn screening.⁹⁴ Anoctamin-1 is another apical iodide channel⁹⁵ but its role in human disease has not yet been proven.

Thyroperoxidase Defects

The heme protein TPO is membrane bound at the apex of follicular cells where it catalyzes the iodination of TG-bound

tyrosyl residues to form the iodotyrosines MIT and DIT, and the coupling of these in association with thyroid (nicotinamide adenine dinucleotide phosphate) oxidases. Over 100 mutations have been described.⁹⁶ Up to 17% of patients with monoallelic mutations have TPO deficiency and monoallelic expression of the mutant allele in the thyroid has been shown in three siblings born to a mutation-carrying but unaffected father.⁹⁷ Overall, no clear genotype-phenotype correlations have been established, and phenotypes may vary even within the same families. TPO deficiency manifests as goitrous CH with elevated levels of serum TG and high iodine uptake on scintigraphy, although a family with thyroid hypoplasia has recently been reported.⁹⁸

DUOX2 Defects

DUOX2, like TPO, is located at the apical membrane of thyroid follicular cells and its H_2O_2 -generating role is the rate-limiting factor for TG iodination during thyroid hormone biosynthesis. Originally, it was thought that biallelic mutations inactivating *DUOX2* or its maturation factor *DUOXA2* cause permanent CH, whereas monoallelic mutations cause transient CH.⁹⁰ However, there are many exceptions to this rule and the phenotype of *DUOX2* deficiency ranges from life-threatening goiter in utero to life-long euthyroidism.^{99–101} Compensation by *DUOX1* has been postulated to explain these findings.

Thyroglobulin Defects

Mutations in the *TG* gene affect the organification step of thyroid hormone biosynthesis through a variety of structural changes in the TG glycoprotein (abnormal folding, defective transport, lack of access to tyrosine residues, or defective iodotyrosine coupling). Clinically, the result is goitrous CH as early as in utero or at birth, with low or undetectable serum TG levels.¹⁰²

Dehalogenase Defects

The *dehalogenase* (*DEHAL1*) or *IYD* gene encodes iodotyrosine deiodinase, the enzyme responsible for iodine recycling in the follicular cell to avoid the leaking of iodine in the bloodstream and subsequent loss in the urine. In the few individuals described to date, the thyroid phenotype is variable and, as

in PDS and *DUOX2* deficiency, seems dependent on iodine intake and likely other yet unidentified modifiers. The biochemical hallmark of this condition is an increase in urinary MIT and DIT concentrations.¹⁰³

Transient Neonatal Hyperthyrotropinemia and Hypothyroidism

Transient CH is a temporary deficiency of thyroid hormone that occurs postnatally, with eventual restoration of normal thyroid function over time, typically within weeks to months. This may be primary hypothyroidism (elevated TSH, low T4) or isolated hyperthyrotropinemia (elevated TSH, normal T4). Identification and treatment of transient CH is likely the reason for the apparent increase in prevalence of congenital hypothyroidism because screening was implemented.^{47,104,105} The reported proportion of those diagnosed with CH who have transient dysfunction is widely variable, likely reflecting different screening algorithms and cutoffs, as well as the lack of a standardized definition and evaluation strategy for transient CH.

Iodine Deficiency

Worldwide, iodine deficiency likely remains the most common cause of transient CH. Even in industrialized countries, maternal iodine intake should be regularly monitored. The National Health and Nutrition Examination Survey (NHANES) demonstrated a decline in iodine nutrition from NHANES I to III with an apparent stabilization thereafter, but evidence of persistent mild deficiency exists.³ The American Thyroid Association recommends that women take a dietary supplement containing 150 µg of iodine daily during preconception, pregnancy, and lactation, but adherence to this recommendation has been poor.³ Premature newborns are at higher risk of iodine deficiency caused by inadequate time for accruing adequate iodine stores within the gland.

Iodine Excess

Excessive iodine exposure can also cause hypothyroidism caused by the Wolff-Chaikoff effect. Prenatal exposure may occur through maternal use of amiodarone, iodine antiseptics, or exposure to iodinated contrast agents. Postnatally, a systematic review found that neonates exposed to iodinated contrast media are at risk of abnormal thyroid function and development of hypothyroidism and that the risk might be higher in premature infants.¹⁰⁶ Exposure can occur through the use of iodinated skin preparations, as well as contrast media for cardiac catheterization, urinary and gut imaging, or treatment with amiodarone. This has led to many nurseries discontinuing the use of iodine containing antiseptics for skin preparation. Hypothyroidism may not be detected at newborn screening, as low T4 and high TSH levels generally appear 2 to 3 days after exposure. Some nurseries have recommendations for thyroid function testing after exposure to iodine. The duration of effect of iodine excess on thyroid function is variable.

Transfer of Drugs or Antibodies From Mother to Fetus

Maternal use of antithyroid drugs at the time of delivery can result in transient hypothyroidism in the neonate with return of normal thyroid hormone metabolism typically within 2 to 5 days.¹⁰⁷ Thyrotropin receptor blocking antibodies (TRABs) block the TSH receptor in the thyroid gland of the newborn, resulting in transient hypothyroidism. This resolves within 3 to 6 months because the antibodies of maternal origin disappear. This should be a diagnostic consideration in babies born to women with known autoimmune thyroid disease (Graves

disease or autoimmune thyroiditis) and families with a previous sibling detected with CH. Radionuclide uptake might be blocked partially or completely.

Diagnosis and Management of Transient Congenital Hypothyroidism

In newborns, permanent CH can only be diagnosed in those with either true athyreosis or ectopic thyroid on scintiscan. Those with an orthotopic gland of normal or even increased size may have transient CH. Practice guidelines¹⁰⁸ recommend treating all those with presumptive CH for a period of 3 years during the critical period of thyroid hormone-sensitive neurocognitive development, followed by a controlled withdrawal. During these 3 years, a low T4 requirement is suggestive of transience of CH, but there is overlap with permanent CH.¹⁰⁹

Transient Hypothyroxinemia of Prematurity

Transient hypothyroxinemia of prematurity (THOP; low T4 with normal TSH) is observed in up to 50% of infants born before 28 weeks. Multiple observational studies have shown a correlation between neuropsychological development and neonatal T4 concentrations, but a long-term randomized, placebo-controlled trial did not show evidence of benefit from thyroxine administration.¹¹⁰ On the other hand, premature infants have low intrathyroidal iodine stores and the T4-development correlation may be in fact a reflection of iodine deficiency. A multiple arm-trial comparing various treatment regimens including T4, iodine, and placebo has been ongoing for more than a decade, but definitive results on neurodevelopment have not been reported.¹¹¹

Likely because of their low intrathyroidal iodine stores, fetuses and premature neonates are prone to hypothyroidism from acute iodine overload (the Wolff-Chaikoff effect). This was initially reported in areas of borderline low iodine intake but is now known to occur elsewhere as well.¹¹² Thus iodine-containing disinfectants should be replaced with chlorhexidine in obstetrical and perinatal care and the use of iodine-containing contrast agents minimized, even in full-term infants.¹¹³ Infusion of dopamine, a potent suppressor of TSH secretion, is frequently (as high as 36%) used in infants born before 29 weeks of gestation.¹¹⁴ Both iodine overload and dopamine use contribute to the observations of a “delayed TSH rise” in premature infants and have led many programs to routinely obtain a second screening specimen. However, this problem is transient and the limited evidence does not suggest a benefit from treatment⁵⁶ so the routine second screen may not be justified.¹¹⁵

Consumptive Hypothyroidism

Hemangiomas may produce sufficient amounts of type 3 deiodinase to cause severe hypothyroidism.¹¹⁶ Hypothyroidism develops with the rapid growth of the hemangioma in the first weeks or months of life. It may be the presenting feature of the hemangioma, which may not be visible if located in the liver.¹¹⁷ Thus in severe early-onset hypothyroidism requiring high doses of thyroxine, an abdominal ultrasound is indicated. Consumptive hypothyroidism can be confirmed by the finding of low T4 and high reverse T3 in serum. The hypothyroidism resolves with spontaneous involution or with medical or surgical treatment of the hemangioma.

Defects in Thyroid Hormone Signaling Pathways

The first cases of thyroid hormone resistance had a mutation in the thyroid hormone receptor beta gene. Since then, mutations

in the thyroid hormone receptor alpha have also been described and other mechanisms of impaired sensitivity to thyroid hormone have been identified, including impaired conversion of T4 to T3 and defective transport of T4 and T3 into the cell. All these congenital defects are now collectively named “defects in thyroid hormone signaling pathways.”¹¹⁸ Although rare, thyroid hormone resistance may be detected through an increased TSH at newborn screening.¹¹⁹

Defects in Thyroid Hormone Metabolism

T4 is converted to biologically active T3 in the cell cytoplasm through deiodination by the selenoenzymes, DIO1 and DIO2, mainly in the liver and the thyroid itself. No disease has been reported in humans from mutations in DIO1 or DIO2, presumably because, through compensation, both DIOs would need to be affected for symptoms to develop. Consistent with this concept, biallelic inactivation in *SBP2*, which encodes a selenoprotein that affects both deiodinases, results in a phenotype of mild thyroid dysfunction (high-normal T4, low-normal T3, normal TSH). The clinical manifestations range from mild slowing of growth¹²⁰ to a severe multisystem disorder including hearing impairment, vertigo, muscle weakness, infertility, and mild developmental delay.¹²¹

Defects in Thyroid Hormone Transport Into Cells

T4 and T3 are actively transported into cells, where they bind to the intracellular thyroid hormone receptor. The expression of these transporters is tissue dependent. MTC8 plays an important role in the transport of T3 from glial cells to neurons. Individuals with an MTC8 defect have thyroid hormone deprivation in the brain during embryonic and early postnatal life resulting in varying degrees of psychomotor delay, typically severe and presenting during infancy or early childhood (known as the *X-linked Allan-Herndon-Dudley syndrome*). These patients have characteristically high serum T3 and low rT3 concentrations, low T4, and normal or slightly elevated TSH levels. The high T3 may cause symptoms of hyperthyroidism in peripheral tissues that are not dependent on MTC8, resulting in tachycardia, increased metabolic rate, and sleep disturbances.^{122,123} DITPA (diiodothyropropionic acid) treatment almost completely normalizes thyroid function tests and improves weight gain, but should probably be started very early—perhaps even in utero—to improve neurocognitive function.¹²⁴

Defects in Thyroid Hormone Receptors

The active hormone T3 binds with equal affinity to both TR- α and TR- β receptors with variable expression ratios in different tissues and individuals. For example, the pituitary thyrotrophs express primarily TR- β , whereas other brain areas express only TR- α and the heart expresses both. As a result, the clinical phenotype of hormone resistance depends on whether it is TR- α or TR- β that is mutated. In addition, the variable phenotypes of family members with the same mutations point to genetic or nongenetic modifiers.¹²⁵

The most common resistance to thyroid hormone (RTH) results from de novo or dominantly inherited mutations that inactivate the TR- β gene (*RTH-beta*), with more than 3000 patients reported and an estimated incidence of 1:40,000 as identified through a primary T4 screening program.¹²⁶ Many with RTH will be clinically euthyroid, but goiter is common. Hyperthyroid symptoms may occur in the tissues expressing predominantly TR- α , such as the heart (tachycardia) and some brain regions (hyperactivity) and increased metabolic rate. Hypothyroid symptoms predominate in TR- β -expressing tissues and may include growth failure, delayed bone maturation,

learning disabilities, and sensorineural deafness.¹²⁵ Treatment is usually not required.¹²⁷

RTH caused by inactivating mutations of TR- α , also de novo or dominantly inherited, have also been identified. Patients present clinically with developmental delay, constipation, and short legs and biochemically with low fT₄, high fT₃, and normal TSH.¹²⁸

Central Hypothyroidism

Central CH is an uncommon condition resulting from inadequate stimulation of the thyroid by TSH. Prevalence varies by ascertainment method but may be as high as one in 16,000 by biochemical screening.⁵² The majority of patients will have hypothalamic and/or pituitary pathology with multiple pituitary hormone deficiencies, with or without structural anomalies of the pituitary and additional syndromic features. Magnetic resonance imaging (MRI) generally shows a “classical triad” of ectopic posterior pituitary, thin or absent pituitary stalk, and hypoplastic anterior pituitary. A small minority of these individuals will have mutations in known transcription factors, such as *POU1F1*, *PROP1*, *HESX1*, *LHX3*, and *LHX4*. The exceptional cases of central hypothyroidism that are initially isolated can be caused by mutations in TSH β ,^{37,129} *TRHR*,^{130–132} *IGSF1*,^{133,134} and *TBL1X*,¹³⁵ the first two inherited in an autosomal recessive manner and the last two in an X-linked manner. The prolactin response to TRH is absent in patients with *TRHR* mutations¹³⁰ and present in those with TSH β mutations.³⁷ Prolactin levels may be low in patients with *IGSF1* mutations. X-linked loss-of-function mutations in *IGSF1* cause syndromic central hypothyroidism with testicular enlargement, and variable prolactin deficiency, although the macroorchidism is not present at birth.¹³³ Only central CH caused by TSH β mutations is severe enough at birth to cause intellectual disability; immunoreactive TSH may be slightly elevated³⁷ but not above screening cutoffs, so primary TSH screening will not identify it¹³⁶; given its rarity, population-based screening is not warranted, but a strong clinical suspicion of CH should prompt a measurement of serum fT₄ even if TSH is normal. Overall, primary T₄ screening was not superior to clinical ascertainment in the Indiana program, which therefore switched to a primary TSH screening strategy.⁴

Evaluation of Infants With a Positive Newborn Screening Test

Any positive CH newborn screening result should be acted on immediately to confirm the diagnosis and to promptly initiate treatment. Beyond acting on the screening result, it is important for the individual, as well as for public health purposes, to establish the etiology of CH and to document outcomes.

Thyroid autoimmunity in women of childbearing age is common but only accounts for 1% of cases of CH. The mother should be queried about an unusually high¹³⁷ or low iodine intake during pregnancy and the obstetrical and neonatal records should be scrutinized for the possibility of acute iodine overload.¹¹² Consanguinity increases the likelihood of dyschromonogenesis. Unexplained postmaturity and macrosomia are common in CH.¹³⁸ Unusually large fontanelles and macroglossia seen in CH can be identified immediately after birth¹³⁹ and hypotonia, umbilical hernia, and prolonged jaundice soon afterward. The neck of newborns is short and detecting a goiter is exceptional, even with the neck hyperextended. The general physical examination should focus on dysmorphic features and cardiac auscultation.

Confirmatory thyroid function tests including serum TSH and fT₄ should be obtained on any newborn with a positive

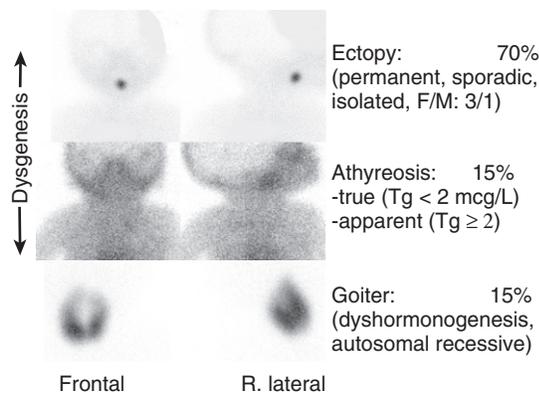


Fig. 8.8 The three most commonly seen etiologies of congenital hypothyroidism as evidenced by nuclear medicine scintigraphy using sodium pertechnetate.

screening result. However, treatment initiation should never be delayed while awaiting test results, especially if TSH on the screening sample is more than 40 mU/L.¹⁰⁸ TSH correlates with CH severity, but only pretreatment fT4 predicts neurocognitive sequelae, with lower values conferring greater risk.^{140,141} If radionuclide imaging reveals no uptake, TG will distinguish apparent from true athyreosis. In the former case, antibodies to, or inactivating mutations in, the TSHR will allow to predict the transience or permanence of the hypothyroidism.

Radionuclide scanning is the only imaging modality that will unequivocally detect ectopic glands, which account for two-thirds of cases of overt CH (Fig. 8.8). With 1 mCi of the readily available and cheap sodium pertechnetate, this etiology is established in 20 minutes and parents can be told that there is a 99% probability that their child's CH is sporadic and will be permanent. In contrast, imaging with ¹²³I, a less available and more expensive isotope, takes hours and only adds information about organification in cases of goiter, which does not alter management. Babies should be fed after the injection of technetium to avoid tracer accumulation in their salivary glands. Advantages of performing the scan at diagnosis include easier administration of the test in newborns who usually fall asleep following an oral feed (vs. a toddler who may not cooperate with the test). Ideally, scintiscanning should be performed before therapy is begun, although it should never delay initiation of hormone replacement. After starting treatment, the time window for scintiscanning depends on CH severity but is a few days, as long as TSH is more than 20 to 30 mU/L on the day of imaging.

Neck ultrasound has been extensively used by clinicians reluctant to use radionuclide imaging but often yields equivocal, false positive or false negative results.¹⁴² Absent knee epiphyses on x-ray in a term newborn documents a prenatal origin of CH, which should lead to an even greater attention to neurocognitive development.

Some clinicians opt to forego imaging altogether as it may be perceived as cumbersome to organize and does not alter immediate management. However, the inability to provide the parents with an etiological diagnosis of permanent CH likely contributes to the observation that up to 40% of the parents of the 1 in 2360 children, labeled as having CH by newborn screening in the United States, stop renewing their child's thyroxine prescriptions by age 4 years.¹⁴³

Treatment of Congenital Hypothyroidism

Thyroid hormone replacement in the form of levothyroxine should be commenced promptly after diagnosis, and at the

latest by 2 weeks of life, as delayed treatment initiation correlates with lower IQ. The goal of treatment is to restore normal thyroid function as quickly as possible and maintain it thereafter. The only randomized controlled trial of different starting doses showed that 50 mcg per day (~15 mcg/kg/d) resulted in the best neurodevelopment at 5 years.¹⁴⁴ Long-term observational studies^{145–147} support this approach, which is endorsed by professional society guidelines, although tiered dosing recommendations based on biochemical severity and imaging have been proposed.¹⁴⁸ Local T4 to T3 conversion is the major source of thyroid hormone in the brain and, consequently, the addition of T3 does not provide further benefit.¹⁴⁹ Thyroxine is well absorbed by the gut but, in situations, such as short bowel syndrome, intravenous thyroxine followed by very doses of oral thyroxine may be needed.¹⁵⁰

As clinical signs and symptoms are not sufficiently reliable to assess thyroid hormone status, thyroid function needs to be monitored closely following initiation of therapy, with progressively decreasing follow-up intervals over time. The first clinical and biochemical follow-up is recommended within about 2 weeks of initiating therapy. In general, subsequent visits are at least every 3 months for the first year of life, every 6 months until 3 years, and yearly until growth is completed.

Both undertreatment and overtreatment should be avoided, but the former is more closely linked to adverse outcomes at school age¹⁵¹ and in adulthood.¹⁵² The goal is to maintain TSH within the age-appropriate reference range and fT4 within the upper half of the normal range. Two weeks after starting at a dose of 15 mcg/kg/d, mean serum fT4 is 56 pmol/L (4.3 ng/dL)¹⁴⁴ but it should be kept in mind that mean serum fT4 of normal newborns at 1 to 4 days of age is 48 pmol/L (3.7 ng/dL).¹⁵³ Serum T3 is generally normal. Likely, a higher T4 suppression threshold for TSH exists in infants, that only resets later in childhood.¹⁵⁴ Especially early on, TSH is the main treatment target and, provided the TSH is not persistently suppressed or a child is clinically hyperthyroid, the dose does not need to be reduced.

Clinical monitoring during follow-up visits should focus on growth, developmental milestones, school progression, and behavior. If treated adequately, length and weight curves are within the normal range for family, and by age 3 years, complete catch-up growth, including normalization of the bone age is expected.²⁹ Head circumference tends to be slightly above average,^{155,156} likely reflecting an immaturity of the skeleton rather than true pathology. With appropriate treatment, children with CH usually have normal neurocognitive development, although the most severely affected may have hearing impairment.¹⁵⁷ A low socioeducational background of the family interacts with the biological effects of CH.¹⁴¹ Thus CH severity, treatment adequacy, and social determinants should all be considered in the management plan and educational interventions. Appropriate genetic counseling should be provided in families with a genetic defect of thyroid formation or function.

CONGENITAL HYPERTHYROIDISM

Graves Disease

In spite of the large number of women of childbearing age with past or present Graves disease, estimated at 0.1% to 2.5%,¹⁵⁸ the transplacental passage of immunoglobulins that stimulate the TSH receptor (TSIs) leading to symptomatic Graves disease occurs in only 1 in 50,000 to 1 in 100,000 fetuses or neonates. The reason why only 1 in about 1000 at-risk fetuses or newborns develop symptomatic hyperthyroidism is unknown. However, fetal/neonatal hyperthyroidism can be severe and, when it occurs, should be managed in centers with the necessary expertise in high-risk pregnancies.

Ideally, all women with Graves disease should have serum TSIs measured in early pregnancy. This applies to those currently treated for hyperthyroidism, as well as to those who are now euthyroid, spontaneously or on replacement after ablative therapy.¹⁵⁸ A high TSI titer in maternal serum makes hyperthyroidism more likely in the fetus or newborn. Unfortunately, this information is often not available at first evaluation¹⁵⁹ and clinicians are confronted at first with the classical signs heralding fetal hyperthyroidism, such as tachycardia and intrauterine growth retardation. In the hands of highly skilled radiologists, a fetal goiter may be the first sign, and its function may be predicted from its ultrasound characteristics, but whether this will be generalizable remains unproven.¹⁶⁰ The hyper- or hypothyroid nature of a goiter in a fetus can generally be guessed on the basis of maternal TSI concentrations or antithyroid drug dose being used, so that cordocentesis, which carries a 1% to 2% risk of fetal loss,²⁶ is usually not indicated.

Traditionally, the mainstay of treatment of hyperthyroidism during pregnancy has been the administration of propylthiouracil (PTU) because of the risk of methimazole (MMZ)-induced embryopathy.¹⁶¹ However, because of the greater liver toxicity of PTU,¹⁶² it has been suggested that MMZ should be preferred after organogenesis is complete but the practicality of switching from one drug to another has been questioned,¹⁶³ and recent data suggest PTU may be teratogenic as well.¹⁶⁴ Whichever drug is used, maternal antithyroid treatment should normalize fetal heart rate within 2 weeks. The antithyroid drug dose can usually be decreased progressively.

Graves disease in the newborn is manifested by irritability, tachycardia, hypertension, poor weight gain, thyroid enlargement, and exophthalmos. Thrombocytopenia, hepatosplenomegaly, jaundice, hypoprothrombinemia, and cardiac failure may occur. The diagnosis is confirmed by an undetectable serum TSH, high levels of serum T4, fT4, and T3. In contrast, on blood drawn on the second day of life in normal children, fT4 is high but TSH is not suppressed. In some neonates, the onset of symptoms and signs may be delayed as long as 8 to 9 days, if they were born to a mother treated with antithyroid drugs, and also reflecting the switch from an inactivating to an activating conversion of T4 to active T3 by liver and other tissues after birth. Neonatal Graves disease resolves spontaneously as maternal TSIs are cleared from the infant's circulation. The usual clinical course of neonatal Graves disease extends from 3 to 12 weeks.

Various biochemical parameters on serum collected from cord or in the first few postnatal days have been proposed to identify at-risk newborns who will develop clinically significant hyperthyroidism.³⁴ All at-risk newborns should, at a minimum, be carefully monitored for postnatal weight gain. Those who develop symptomatic hyperthyroidism should be admitted and their heart rate monitored. Treatment includes propranolol (1–2 mg per kilogram per day, divided in 4 doses) and methimazole in a dose of 0.5 to 1 mg/kg daily in divided doses at 8-hour intervals. Iodine is also often used, because it rapidly inhibits hormone release: Lugol's solution (5% iodine and 10% potassium iodide; 126 mg of iodine per milliliter) is given orally in a dose of one drop (about 8 mg) 3 times daily. A therapeutic response should be observed within 24 to 36 hours. If a satisfactory response is not observed, the dose of antithyroid drug and iodine can be increased by 50%. Glucocorticoids in high doses diminish T4 to T3 conversion and may therefore be helpful. In severe cases, sedatives and digitalization may be necessary.

Nonautoimmune Hyperthyroidism

Mutations that result in increased constitutive activity of the TSH receptor give rise to hyperthyroidism. Mutations can be present in the germline and be inherited in an autosomal

dominant manner or occur de novo or, much more rarely than in adults, at the somatic level, leading to the development of an autonomous adenoma. In all cases, persistent congenital non-autoimmune hyperthyroidism ensues, which is most severe in cases with a de novo germline mutation.¹⁶⁵ Premature delivery and low birth weight in these infants (as in unaffected children of mothers with thyroid hormone resistance),¹⁹ suggest that an increase in thyroid hormone levels in the fetus stunts growth and shortens gestation independent of autoimmunity. Hyperthyroidism is generally associated with a goiter, although this is not always present at diagnosis. Acceleration of bone maturation occurs very early and rapid linear growth, relative thinness, and microcephaly may be observed.¹⁶⁶

In contrast to neonatal Graves disease, nonautoimmune hyperthyroidism is life-long and thyroidectomy, the only curative approach, should be preferred in infants with a hyperfunctioning nodule. In those with a germline mutation and, consequently, a diffuse goiter, surgery is also often required.

DISORDERS OF SERUM THYROID HORMONE TRANSPORT

Several genetic abnormalities of the iodothyronine-binding serum proteins have been described and all are manifest at birth. These include complete TBG deficiency, partial TBG deficiency, TBG excess, transthyretin (prealbumin) variants, and familial dysalbuminemic hyperthyroxinemia. Total T4 is low or high but fT4 is normal and the patients are therefore euthyroid. Nowadays, these abnormalities are only picked up by screening programs that measure total T4, in which an elevated total T4 is at least 10-fold more likely caused by TBG excess than caused by Graves disease.¹²⁶ In serum, fT4, instead of total T4, is now almost universally measured but, depending on the assay method, fT4 can also be spuriously elevated in familial dysalbuminemic hyperthyroxinemia. The key difference between all of these conditions and true hypo- or hyperthyroidism is that serum TSH is normal.¹⁶⁷

REFERENCES

- Zimmermann MB. Research on iodine deficiency and goiter in the 19th and early 20th centuries. *J Nutr.* 2008;138(11):2060–2063.
- Vanderpump MP. Commentary: iodine deficiency as a new challenge for industrialized countries: a UK perspective. *Int J Epidemiol.* 2012;41(3):601–604.
- Perrine CG, Herrick KA, Gupta PM, Caldwell KL. Iodine Status of Pregnant Women and Women of Reproductive Age in the United States. *Thyroid.* 2019;29(1):153–154.
- Nebesio TD, McKenna MP, Nabhan ZM, Eugster EA. Newborn screening results in children with central hypothyroidism. *J Pediatr.* 2010;156(6):990–993.
- McCabe MJ, Alatzoglou KS, Dattani MT. Septo-optic dysplasia and other midline defects: the role of transcription factors: HESX1 and beyond. *Best Pract Res Clin Endocrinol Metab.* 2011;25(1):115–224.
- Pfaffle R, Klammt J. Pituitary transcription factors in the aetiology of combined pituitary hormone deficiency. *Best Pract Res Clin Endocrinol Metab.* 2011;25(1):43–60.
- Liang S, Johansson E, Barila G, Altschuler DL, Fagman H, Nilsson M. A branching morphogenesis program governs embryonic growth of the thyroid gland. *Development.* 2018;145(2).
- Vandernoot I, Sartelet H, Abu-Khudir R, Chanoine JP, Deladoey J. Evidence for calcitonin-producing cells in human lingual thyroids. *J Clin Endocrinol Metab.* 2012;97(3):951–956.
- De Felice M, Di Lauro R. Minireview: Intrinsic and extrinsic factors in thyroid gland development: an update. *Endocrinology.* 2011;152(8):2948–2956.
- Van Vliet G, Deladoey J. Sublingual thyroid ectopy: similarities and differences with Kallmann syndrome. *F1000prime Rep.* 2015;7:20.

11. Vasudevan A, Bhide PG. Angiogenesis in the embryonic CNS: a new twist on an old tale. *Cell Adh Migr*. 2008;2(3):167–169.
12. Fagman H, Grande M, Edsbacke J, Semb H, Nilsson M. Expression of classical cadherins in thyroid development: maintenance of an epithelial phenotype throughout organogenesis. *Endocrinology*. 2003;144(8):3618–3624.
13. Fagman H, Andersson L, Nilsson M. The developing mouse thyroid: embryonic vessel contacts and parenchymal growth pattern during specification, budding, migration, and lobulation. *Dev Dyn*. 2006;235(2):444–455.
14. Opitz R, Hitz MP, Vandernoot I, Trubiroha A, Abu-Khudir R, Samuels M, et al. Functional zebrafish studies based on human genotyping point to netrin-1 as a link between aberrant cardiovascular development and thyroid dysgenesis. *Endocrinology*. 2014. en20141628.
15. Di Cosmo C, Fanelli G, Tonacchera M, Ferrarini E, Dimida A, Agretti P, et al. The sodium-iodide symporter expression in placental tissue at different gestational age: an immunohistochemical study. *Clin Endocrinol (Oxf)*. 2006;65(4):544–548.
16. von Oettingen JE, Brathwaite TD, Carpenter C, Bonnell R, He X, Braverman LE, et al. Population survey of iodine deficiency and environmental disruptors of thyroid function in young children in Haiti. *J Clin Endocrinol Metab*. 2017;102(2):644–651.
17. Cao XY, Jiang XM, Dou ZH, Rakeman MA, Zhang ML, O'Donnell K, et al. Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. *N Engl J Med*. 1994;331(26):1739–1744.
18. Vulsmat T, Gons MH, de Vijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect of thyroid agenesis. *N Engl J Med*. 1989;321:13–16.
19. Anselmo J, Cao D, Karrison T, Weiss RE, Refetoff S. Fetal loss associated with excess thyroid hormone exposure. *JAMA*. 2004;292(6):691–695.
20. Lazarus JH, Bestwick JP, Channon S, Paradise R, Maina A, Rees R, et al. Antenatal thyroid screening and childhood cognitive function. *N Engl J Med*. 2012;366(6):493–501.
21. Casey BM, Thom EA, Peaceman AM, Varner MW, Sorokin Y, Hirtz DG, et al. Treatment of subclinical hypothyroidism or hypothyroxinemia in pregnancy. *N Engl J Med*. 2017;376(9):815–825.
22. Momotani N, Iwama S, Momotani K. Neurodevelopment in children born to hypothyroid mothers restored to normal thyroxine (t4) concentration by late pregnancy in Japan: no apparent influence of maternal t4 deficiency. *J Clin Endocrinol Metab*. 2012;97(4):1104–1108.
23. Downing SD, Halpern L, Carswell J, Brown RS. Severe early maternal hypothyroidism corrected prior to the third trimester associated with normal cognitive outcome in the offspring. *Thyroid*. 2012;22(60):625–630.
24. Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, Larsen PR. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med*. 2004;351(3):241–249.
25. Stoppa-Vaucher S, Francoeur D, Grignon A, Alos N, Pohlenz J, Hermanns P, et al. Non-immune goiter and hypothyroidism in a 19-week fetus: a plea for conservative treatment. *J Pediatr*. 2010;156(6):1026–1029.
26. Wilson RD, Gagnon A, Audibert F, Campagnolo C, Carroll J. Prenatal diagnosis procedures and techniques to obtain a diagnostic fetal specimen or tissue: maternal and fetal risks and benefits. *J Obstet Gynaecol Canada*. 2015;37(7):656–668.
27. Blumenfeld YJ, Davis A, Milan K, Chueh J, Hudgins L, Barth RA, et al. Conservatively managed fetal goiter: an alternative to in utero therapy. *Fetal Diagn Ther*. 2013;34(3):184–187.
28. Vasudevan P, Powell C, Nicholas AK, Scudamore I, Greening J, Park SM, et al. Intrauterine death following intraamniotic triiodothyronine and thyroxine therapy for fetal goitrous hypothyroidism associated with polyhydramnios and caused by a thyroglobulin mutation. *Endocrinol Diabetes Metab Case Rep*. 2017. pii 17-0040.
29. Simoneau-Roy J, Marti S, Deal C, Huot C, Robaey P, Van Vliet G. Cognition and behavior at school entry in children with congenital hypothyroidism treated early with high-dose levothyroxine. *J Pediatr*. 2004;144(6):747–752.
30. Grosse SD, Van Vliet G. Prevention of intellectual disability through screening for congenital hypothyroidism: how much and at what level? *Arch Dis Child*. 2011;96(4):374–379.
31. Stewart CJ, Constantatos S, Joolay Y, Muller L. In utero treatment of fetal goitrous hypothyroidism in a euthyroid mother: a case report. *J Clin Ultrasound*. 2012;40(9):603–606.
32. Ballard RA, Ballard PL, Cnaan A, Pinto-Martin J, Davis DJ, Padbury JF, et al. Antenatal thyrotropin-releasing hormone to prevent lung disease in preterm infants. North American Thyrotropin-Releasing Hormone Study Group. *N Engl J Med*. 1998;338(8):493–498.
33. Brown RS, Bellisario RL, Botero D, Fournier L, Abrams CA, Cowger ML, et al. Incidence of transient congenital hypothyroidism due to maternal thyrotropin receptor-blocking antibodies in over one million babies. *J Clin Endocrinol Metab*. 1996;81(3):1147–1151.
34. Banige M, Polak M, Luton D. Prediction of neonatal hyperthyroidism. *J Pediatr*. 2018;197: 249–254.e1.
35. Dussault J, ed. Early hospital discharge-impact on newborn screening. Council of Regional Networks for Genetics Services; 1995.
36. Szinnai G, Lacroix L, Carre A, Guimiot F, Talbot M, Martinovic J, et al. Sodium/iodide symporter (NIS) gene expression is the limiting step for the onset of thyroid function in the human fetus. *J Clin Endocrinol Metab*. 2007;92(1):70–76.
37. Heinrichs C, Parma J, Scherberg NH, Delange F, Van Vliet G, Duprez L, et al. Congenital central isolated hypothyroidism caused by a homozygous mutation in the TSH-beta subunit gene. *Thyroid*. 2000;10(5):387–391.
38. Gagne N, Parma J, Deal C, Vassart G, Van Vliet G. Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities? *J Clin Endocrinol Metab*. 1998;83(5):1771–1775.
39. Ribault V, Castanet M, Bertrand AM, Guibourdenche J, Vuillard E, Luton D, et al. Experience with intraamniotic thyroxine treatment in nonimmune fetal goitrous hypothyroidism in 12 cases. *J Clin Endocrinol Metab*. 2009;94(10):3731–3739.
40. Bianco AC. Minireview: cracking the metabolic code for thyroid hormone signaling. *Endocrinology*. 2011;152(9):3306–3311.
41. Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol*. 2011;25(1):1–14.
42. Fu J, Refetoff S, Dumitrescu AM. Inherited defects of thyroid hormone-cell-membrane transport: review of recent findings. *Curr Opin Endocrinol Diabetes Obes*. 2013;20(5):434–440.
43. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. *Biochim Biophys Acta*. 2013;1830(7):3987–4003.
44. Flamant F, Gauthier K. Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. *Biochim Biophys Acta*. 2012;1830(7):3900–3907.
45. Silva JE. Physiological importance and control of non-shivering facultative thermogenesis. *Front Biosci (ScholEd)*. 2011;3:352–371.
46. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. *Eur J Endocrinol*. 2004;151(Suppl 3):U25–U37.
47. Deladoey J, Ruel J, Giguere Y, Van Vliet G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Quebec. *J Clin Endocrinol Metab*. 2011;96(8):2422–2429.
48. Lain S, Trumpff C, Grosse SD, Olivieri A, Van Vliet G. Are lower TSH cutoffs in neonatal screening for congenital hypothyroidism warranted? *Eur J Endocrinol*. 2017;177(5):D1–D12.
49. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics*. 1963;32:338–343.
50. Dussault JH, Laberge C. Thyroxine (T4) determination by radioimmunological method in dried blood eluate: new diagnostic method of neonatal hypothyroidism? *Union Med Can*. 1973;102(10):2062–2064.
51. Delange F, Camus M, Winkler M, Dodion J, Ermans AM. Serum thyrotrophin determination on day 5 of life as screening

- procedure for congenital hypothyroidism. *Arch Dis Child*. 1977;52(2):89–96.
52. Van Tijn DA, de Vijlder JJ, Verbeeten Jr B, Verkerk PH, Vulmsa T. Neonatal detection of congenital hypothyroidism of central origin. *J Clin Endocrinol Metab*. 2005;90(6):3350–3359.
 53. Price DA, Ehrlich RM, Walfish PG. Congenital hypothyroidism. Clinical and laboratory characteristics in infants detected by neonatal screening. *Arch Dis Child*. 1981;56(11):845–851.
 54. Azam A, Cutfield W, Mouat F, Hofman PL, Jefferies C, Webster D, et al. Missed congenital hypothyroidism in an identical twin. *J Paediatr Child Health*. 2012;48(10):936–938.
 55. Perry R, Heinrichs C, Bourdoux P, Khoury K, Szots F, Dussault JH, et al. Discordance of monozygotic twins for thyroid dysgenesis: implications for screening and for molecular pathophysiology. *J Clin Endocrinol Metab*. 2002;87(9):4072–4077.
 56. Woo HC, Lizarida A, Tucker R, Mitchell ML, Vohr B, Oh W, et al. Congenital hypothyroidism with a delayed thyroid-stimulating hormone elevation in very premature infants: incidence and growth and developmental outcomes. *J Pediatr*. 2011;158(4):538–542.
 57. Olney RS, Grosse SD, Vogt Jr RF. Prevalence of congenital hypothyroidism—current trends and future directions: workshop summary. *Pediatrics*. 2010;125(Suppl 2):S31–S36.
 58. Stoppa-Vaucher S, Van Vliet G, Deladoey J. Variation by ethnicity in the prevalence of congenital hypothyroidism due to thyroid dysgenesis. *Thyroid*. 2011;21(1):13–18.
 59. Ford G, LaFranchi SH. Screening for congenital hypothyroidism: a worldwide view of strategies. *Best Pract Res Clin Endocrinol Metab*. 2014;28(2):175–187.
 60. Devos H, Rodd C, Gagne N, Laframboise R, Van Vliet G. A search for the possible molecular mechanisms of thyroid dysgenesis: sex ratios and associated malformations. *J Clin Endocrinol Metab*. 1999;84(7):2502–2506.
 61. Wildi-Runge S, Stoppa-Vaucher S, Lambert R, Turpin S, Van Vliet G, Deladoey J. A high prevalence of dual thyroid ectopy in congenital hypothyroidism: evidence for insufficient signaling gradients during embryonic thyroid migration or for the polyclonal nature of the thyroid gland? *J Clin Endocrinol Metab*. 2012;97(6):E978–E981.
 62. Stoppa-Vaucher S, Lapointe A, Turpin S, Rydlewski C, Vassart G, Deladoey J. Ectopic thyroid gland causing dysphonia: imaging and molecular studies. *J Clin Endocrinol Metab*. 2010;95(10):4509–4510.
 63. Leger J, Czernichow P. Secretion of hormones by ectopic thyroid glands after prolonged thyroxine therapy. *J Pediatr*. 1990;116(1):111–114.
 64. Connors MH, Styne DM. Transient neonatal 'athyreosis' resulting from thyrotropin-binding inhibitory immunoglobulins. *Pediatrics*. 1986;78(2):287–290.
 65. Shabana W, Delange F, Freson M, Osteaux M, De Schepper J. Prevalence of thyroid hemiagenesis: ultrasound screening in normal children. *Eur J Pediatr*. 2000;159(6):456–458.
 66. McLean R, Howard N, Murray IP. Thyroid dysgenesis in monozygotic twins: variants identified by scintigraphy. *Eur J Nucl Med*. 1985;10(7–8):346–348.
 67. Castanet M, Polak M, Bonaiti-Pellie C, Lyonnet S, Czernichow P, Leger J. Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. *J Clin Endocrinol Metab*. 2001;86(5):2009–2014.
 68. Deladoey J, Vassart G, Van Vliet G. Possible non-mendelian mechanisms of thyroid dysgenesis. *Endocr Dev*. 2007;10:29–42.
 69. de Almeida JR, James AL, Papsin BC, Weksburg R, Clark H, Blaser S. Thyroid gland and carotid artery anomalies in 22q11.2 deletion syndromes. *Laryngoscope*. 2009;119(8):1495–1500.
 70. Cammareri V, Vignati G, Nocera G, Beck-Peccoz P, Persani L. Thyroid hemiagenesis and elevated thyrotropin levels in a child with Williams syndrome. *Am J Med Genet*. 1999;85(5):491–494.
 71. Fagman H, Liao J, Westerlund J, Andersson L, Morrow BE, Nilsson M. The 22q11 deletion syndrome candidate gene *Tbx1* determines thyroid size and positioning. *Hum Mol Genet*. 2007;16(3):276–285.
 72. Parmentier M, Libert F, Maenhaut C, Lefort A, Gerard C, Perret J, et al. Molecular cloning of the thyrotropin receptor. *Science*. 1989;246(4937):1620–1622.
 73. Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, Refetoff S. Brief report: resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med*. 1995;332(3):155–160.
 74. Calebiro D, Gelmini G, Cordella D, Bonomi M, Winkler F, Biebermann H, et al. Frequent TSH receptor genetic alterations with variable signaling impairment in a large series of children with nonautoimmune isolated hyperthyrotropinemia. *J Clin Endocrinol Metab*. 2012;97(1):E156–E160.
 75. Tenenbaum-Rakover Y, Almashanu S, Hess O, Admoni O, Hag-Dahood Mahameed A, Schwartz N, et al. Long-term outcome of loss-of-function mutations in thyrotropin receptor gene. *Thyroid*. 2015;25(3):292–299.
 76. Abramowicz MJ, Duprez L, Parma J, Vassart G, Heinrichs C. Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clin Invest*. 1997;99(12):3018–3024.
 77. Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, et al. Generation of functional thyroid from embryonic stem cells. *Nature*. 2012;491(7422):66–71.
 78. De Felice M, Ovitt C, Biffali E, Rodriguez-Mallon A, Arra C, Anastassiadis K, et al. A mouse model for hereditary thyroid dysgenesis and cleft palate. *Nat Genet*. 1998;19(4):395–398.
 79. Carre A, Szinnai G, Castanet M, Sura-Trueba S, Tron E, Broutin-L'Hermite I, et al. Five new *TTF1/NKX2.1* mutations in brain-lung-thyroid syndrome: rescue by *PAX8* synergism in one case. *Hum Mol Genet*. 2009;18(12):2266–2276.
 80. Carre A, Hamza RT, Kariyawasam D, Guillot L, Teissier R, Tron E, et al. A novel *FOXE1* mutation (R73S) in Bamforth-Lazarus syndrome causing increased thyroidal gene expression. *Thyroid*. 2014;24(4):649–654.
 81. Ramos HE, Carre A, Chevrier L, Szinnai G, Tron E, Cerqueira TL, et al. Extreme phenotypic variability of thyroid dysgenesis in six new cases of congenital hypothyroidism due to *PAX8* gene loss-of-function mutations. *Eur J Endocrinol*. 2014;171(4):499–507.
 82. Dimitri P, Warner JT, Minton JA, Patch AM, Ellard S, Hattersley AT, et al. Novel *GLIS3* mutations demonstrate an extended multisystem phenotype. *Eur J Endocrinol*. 2011;164(3):437–443.
 83. Sun F, Zhang JX, Yang CY, Gao GQ, Zhu WB, Han B, et al. The genetic characteristics of congenital hypothyroidism in China by comprehensive screening of 21 candidate genes. *Eur J Endocrinol*. 2018;178(6):623–633.
 84. van Engelen K, Mommersteeg MT, Baars MJ, Lam J, Ilgun A, van Trotsenburg AS, et al. The ambiguous role of *NKX2-5* mutations in thyroid dysgenesis. *PLoSOne*. 2012;7(12):e52685.
 85. de Filippis T, Marelli F, Nebbia G, Porazzi P, Corbetta S, Fugazzola L, et al. *JAG1* loss-of-function variations as a novel predisposing event in the pathogenesis of congenital thyroid defects. *J Clin Endocrinol Metab*. 2016;101(3):861–870.
 86. Carre A, Stoupa A, Kariyawasam D, Gueriouz M, Ramond C, Monus T, et al. Mutations in *BOREALIN* cause thyroid dysgenesis. *Hum Mol Genet*. 2017;26(3):599–610.
 87. Stoupa A, Adam F, Kariyawasam D, Strassel C, Gawade S, Szinnai G, et al. *TUBB1* mutations cause thyroid dysgenesis associated with abnormal platelet physiology. *EMBO Mol Med*. 2018;10(12):e9569.
 88. Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dysmorphogenesis. *Curr Opin Pediatr*. 2011;23(4):421–428.
 89. Avbelj M, Tahirovic H, Debeljak M, Kusekova M, Toromanovic A, Krznisnik C, et al. High prevalence of thyroid peroxidase gene mutations in patients with thyroid dysmorphogenesis. *Eur J Endocrinol*. 2007;156(5):511–519.
 90. Muzza M, Fugazzola L. Disorders of H2O2 generation. *Best Pract Res Clin Endocrinol Metab*. 2017;31(2):225–240.
 91. Deladoey J, Pfar N, Vuissoz JM, Parma J, Vassart G, Biesterfeld S, et al. Pseudodominant inheritance of goitrous congenital hypothyroidism caused by *TPO* mutations: molecular and in silico studies. *J Clin Endocrinol Metab*. 2008;93(2):627–633.
 92. Szinnai G, Kosugi S, Derrien C, Lucidarme N, David V, Czernichow P, et al. Extending the clinical heterogeneity of iodide transport defect (ITD): a novel mutation R124H of the sodium/iodide symporter gene and review of genotype-phenotype correlations in ITD. *J Clin Endocrinol Metab*. 2006;91(4):1199–1204.

93. Bizhanova A, Kopp P. Genetics and phenomics of Pendred syndrome. *Mol Cell Endocrinol*. 2010;322(1-2):83–90.
94. Gaudino R, Garel C, Czernichow P, Leger J. Proportion of various types of thyroid disorders among newborns with congenital hypothyroidism and normally located gland: a regional cohort study. *Clin Endocrinol (Oxf)*. 2005;62(4):444–448.
95. Twyffels L, Strickaert A, Virreira M, Massart C, Van Sande J, Wauquier C, et al. Anoctamin-1/TMEM16A is the major apical iodide channel of the thyrocyte. *Am J Physiol Cell Physiol*. 2014;307(12):C1102–C1112.
96. Targovnik HM, Citterio CE, Rivolta CM. Iodide handling disorders (NIS, TPO, TG, IYD). *Best Pract Res Clin Endocrinol Metab*. 2017;31(2):195–212.
97. Fugazzola L, Cerutti N, Mannavola D, Vannucchi G, Fallini C, Persani L, et al. Monoallelic expression of mutant thyroid peroxidase allele causing total iodide organification defect. *J Clin Endocrinol Metab*. 2003;88(7):3264–3271.
98. Stoupa A, Chaabane R, Gueriouz M, Raynaud-Ravni C, Nitschke P, Bole-Feysot C, et al. Thyroid hypoplasia in congenital hypothyroidism associated with thyroid peroxidase mutations. *Thyroid*. 2018;28(7):941–944.
99. Sugisawa C, Higuchi S, Takagi M, Hasegawa Y, Taniyama M, Abe K, et al. Homozygous DUOXA2 mutation (p.Tyr138*) in a girl with congenital hypothyroidism and her apparently unaffected brother: Case report and review of the literature. *Endocrine J*. 2017;64(8):807–812.
100. Tanase-Nakao K, Miyata I, Terauchi A, Saito M, Wada S, Hasegawa T, et al. Fetal goitrous hypothyroidism and polyhydramnios in a patient with compound heterozygous DUOXA2 mutations. *Horm Res Paediatr*. 2018;1–6.
101. Dufort GLVS, Eugène D, De Deken X, Seebauer B, Heinimann K, Lévesque S, et al. The wide spectrum of DUOX2 deficiency: from life-threatening compressive goiter in infancy to lifelong euthyroidism. *Thyroid*. 2019;29(7):1018–1022.
102. Targovnik HM, Citterio CE, Rivolta CM. Thyroglobulin gene mutations in congenital hypothyroidism. *Horm Res Paediatr*. 2011;75(5):311–321.
103. Moreno JC, Klootwijk W, van TH, Pinto G, D'Alessandro M, Leger A, et al. Mutations in the iodotyrosine deiodinase gene and hypothyroidism. *N Engl J Med*. 2008;358(17):1811–1818.
104. Krude H, Blankenstein O. Treating patients not numbers: the benefit and burden of lowering TSH newborn screening cut-offs. *Arch Dis Child*. 2011;96(2):121–122.
105. Parks JS, Lin M, Grosse SD, Hinton CF, Drummond-Borg M, Borgfeld L, et al. The impact of transient hypothyroidism on the increasing rate of congenital hypothyroidism in the United States. *Pediatrics*. 2010;125(Suppl 2):S54–S63.
106. Ahmet A, Lawson ML, Babyn P, Tricco AC. Hypothyroidism in neonates post-iodinated contrast media: a systematic review. *Acta Paediatr*. 2009;98(10):1568–1574.
107. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid*. 2017;27(3):315–389.
108. Leger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G, et al. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. *Horm Res Paediatr*. 2014;81(2):80–103.
109. Saba C, Guilmin-Crepon S, Zenaty D, Martinierie L, Paulsen A, Simon D, et al. Early determinants of thyroid function outcomes in children with congenital hypothyroidism and a normally located thyroid gland: a regional cohort study. *Thyroid*. 2018;28(8):959–967.
110. van Wassenae AG, Westera J, Houtzager BA, Kok JH. Ten-year follow-up of children born at <30 weeks' gestational age supplemented with thyroxine in the neonatal period in a randomized, controlled trial. *Pediatrics*. 2005;116(5):e613–e618.
111. La Gamma EF, van Wassenae AG, Ares S, Golombek SG, Kok JH, Quero J, et al. Phase 1 trial of 4 thyroid hormone regimens for transient hypothyroxinemia in neonates of <28 weeks' gestation. *Pediatrics*. 2009;124(2):e258–e268.
112. Khashu M, Chessex P, Chanoine JP. Iodine overload and severe hypothyroidism in a premature neonate. *J Pediatr Surg*. 2005;40(2):E1–E4.
113. Thaker VV, Leung AM, Braverman LE, Brown RS, Levine B. Iodine-induced hypothyroidism in full-term infants with congenital heart disease: more common than currently appreciated? *J Clin Endocrinol Metab*. 2014;99(10):3521–3526.
114. Wong J, Shah PS, Yoon EW, Yee W, Lee S, Dow K. Inotrope use among extremely preterm infants in Canadian neonatal intensive care units: variation and outcomes. *Am J Perinatol*. 2015;32(1):9–14.
115. Vincent MA, Rodd C, Dussault JH, Van Vliet G. Very low birth weight newborns do not need repeat screening for congenital hypothyroidism. *J Pediatr*. 2002;140(3):311–314.
116. Huang SA, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, et al. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N Engl J Med*. 2000;343(3):185–189.
117. Konrad D, Ellis G, Perlman K. Spontaneous regression of severe acquired infantile hypothyroidism associated with multiple liver hemangiomas. *Pediatrics*. 2003;112(6):1424–1426.
118. Flamant F, Cheng SY, Hollenberg AN, Moeller LC, Samarut J, Wondisford FE, et al. Thyroid hormone signaling pathways: time for a more precise nomenclature. *Endocrinology*. 2017;158(7):2052–2057.
119. Weiss RE, Balzano S, Scherberg NH, Refetoff S. Neonatal detection of generalized resistance to thyroid hormone. *JAMA*. 1990;264(17):2245–2250.
120. Dumitrescu AM, Liao XH, Abdullah MS, Lado-Abeal J, Majed FA, Moeller LC, et al. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. *Nat Genet*. 2005;37(11):1247–1252.
121. Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, et al. Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. *J Clin Invest*. 2010;120(12):4220–4235.
122. Dumitrescu AM, Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet*. 2004;74(1):168–175.
123. Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet*. 2004;364(9443):1435–1437.
124. Ramos HE, Morandini M, Carre A, Tron E, Floch C, Mandelbrot L, et al. Pregnancy in women heterozygous for MCT8 mutations: risk of maternal hypothyroxinemia and fetal care. *Eur J Endocrinol*. 2011;164(2):309–314.
125. Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. *Endocr Rev*. 1993;14(3):348–399.
126. Lafranchi SH, Snyder DB, Sesser DE, Skeels MR, Singh N, Brent GA, et al. Follow-up of newborns with elevated screening T4 concentrations. *J Pediatr*. 2003;143(3):296–301.
127. Weiss RE, Refetoff S. Treatment of resistance to thyroid hormone—primum non nocere. *J Clin Endocrinol Metab*. 1999;84(2):401–404.
128. van Gucht ALM, Moran C, Meima ME, Visser WE, Chatterjee K, Visser TJ, et al. Resistance to thyroid hormone due to heterozygous mutations in thyroid hormone receptor alpha. *Curr Top Dev Biol*. 2017;125:337–355.
129. Deladoey J, Vuissoz JM, Domene HM, Malik N, Gruneiro-Papendieck L, Chiesa A, et al. Congenital secondary hypothyroidism due to a mutation C105Vfs114X thyrotropin-beta mutation: genetic study of five unrelated families from Switzerland and Argentina. *Thyroid*. 2003;13(6):553–559.
130. Collu R, Tang J, Castagne J, Lagace G, Masson N, Huot C, et al. A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. *J Clin Endocrinol Metab*. 1997;82(5):1561–1565.
131. Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, et al. A family with complete resistance to thyrotropin-releasing hormone. *N Engl J Med*. 2009;360(7):731–734.
132. Garcia M, Gonzalez de Buitrago J, Jimenez-Roses M, Pardo L, Hinkle PM, Moreno JC. Central hypothyroidism due to a TRHR mutation causing impaired ligand affinity and transactivation of Gq. *J Clin Endocrinol Metab*. 2017;102(7):2433–2442.

133. Bernard DJ, Brule E, Smith CL, Joustra SD, Wit JM. From consternation to revelation: discovery of a role for IGSF1 in pituitary control of thyroid function. *J Endocr Soc.* 2018;2(3):220–231.
134. Sun Y, Bak B, Schoenmakers N, van Trotsenburg AS, Oostdijk W, Voshol P, et al. Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement. *Nat Genet.* 2012;44(12):1375–1381.
135. Garcia M, Barreda-Bonis AC, Jimenez P, Rabanal I, Ortiz A, Vallespin E, et al. Central hypothyroidism and novel clinical phenotypes in hemizygous truncation of TBL1X. *J Endocr Soc.* 2019;3(1):119–128.
136. Brumm H, Pfeufer A, Biebermann H, Schnabel D, Deiss D, Gruters A. Congenital central hypothyroidism due to homozygous thyrotropin beta 313 Delta T mutation is caused by a Founder effect. *J Clin Endocrinol Metab.* 2002;87(10):4811–4816.
137. Connelly KJ, Boston BA, Pearce EN, Sesser D, Snyder D, Braverman LE, et al. Congenital hypothyroidism caused by excess prenatal maternal iodine ingestion. *J Pediatr.* 2012;161(4):760–762.
138. Van Vliet G, Larroque B, Bubuteishvili L, Supernant K, Leger J. Sex-specific impact of congenital hypothyroidism due to thyroid dysgenesis on skeletal maturation in term newborns. *J Clin Endocrinol Metab.* 2003;88(5):2009–2013.
139. Smith DW, Popich G. Large fontanels in congenital hypothyroidism: a potential clue toward earlier recognition. *J Pediatr.* 1972;80(5):753–756.
140. Kempers MJ, van der Sluijs V, Nijhuis-van der Sanden RW, Lanting CI, Kooistra L, Wiedijk BM, et al. Neonatal screening for congenital hypothyroidism in the Netherlands: cognitive and motor outcome at 10 years of age. *J Clin Endocrinol Metab.* 2007;92(3):919–924.
141. Dimitropoulos A, Molinari L, Etter K, Torresani T, Lang-Muritano M, Jenni OG, et al. Children with congenital hypothyroidism: long-term intellectual outcome after early high-dose treatment. *Pediatr Res.* 2009;65(2):242–248.
142. Deladoey J, Van Vliet G. The changing epidemiology of congenital hypothyroidism: fact or artifact? *Exp Rev Endocrinol Metab.* 2014;9(4):387–395.
143. Kemper AR, Ouyang L, Grosse SD. Discontinuation of thyroid hormone treatment among children in the United States with congenital hypothyroidism: findings from health insurance claims data. *BMC Pediatr.* 2010;10: 9.
144. Selva KA, Harper A, Downs A, Blasco PA, Lafranchi SH. Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. *J Pediatr.* 2005;147(6):775–780.
145. Albert BB, Heather N, Derraik JG, Cutfield WS, Wouldes T, Tregurtha S, et al. Neurodevelopmental and body composition outcomes in children with congenital hypothyroidism treated with high-dose initial replacement and close monitoring. *J Clin Endocrinol Metab.* 2013;98(9):3663–3670.
146. Uyttendaele M, Lambert S, Tenoutasse S, Boros E, Ziereisen F, Van Vliet G, et al. Congenital hypothyroidism: long-term experience with early and high levothyroxine dosage. *Horm Res Paediatr.* 2016;85(3):188–197.
147. Aleksander PE, Bruckner-Spieler M, Stoehr AM, Lankes E, Kuhn P, Schnabel D, et al. Mean high-dose l-thyroxine treatment is efficient and safe to achieve a normal IQ in young adult patients with congenital hypothyroidism. *J Clin Endocrinol Metab.* 2018;103(4):1459–1469.
148. Mathai S, Cutfield WS, Gunn AJ, Webster D, Jefferies C, Robinson E, et al. A novel therapeutic paradigm to treat congenital hypothyroidism. *Clin Endocrinol (Oxf).* 2008;69(1):142–147.
149. Cassio A, Cacciari E, Cicognani A, Damiani G, Missiroli G, Corbelli E, et al. Treatment for congenital hypothyroidism: thyroxine alone or thyroxine plus triiodothyronine? *Pediatrics.* 2003;111(5 Pt 1):1055–1160.
150. Schomig CS, Robinson ME, von Oettingen JE. Treatment of congenital hypothyroidism in a newborn with malabsorption after subtotal ileum resection. *Endocrinol Diabetes Metab Case Rep.* 2018. pii EDM170156.
151. Leger J, Larroque B, Norton J. Influence of severity of congenital hypothyroidism and adequacy of treatment on school achievement in young adolescents: a population-based cohort study. *Acta Paediatr.* 2001;90(11):1249–1256.
152. Leger J. Congenital hypothyroidism: a clinical update of long-term outcome in young adults. *Eur J Endocrinol.* 2015;172(2):R67–R77.
153. Nelson JC, Clark SJ, Borut DL, Tomei RT, Carlton EI. Age-related changes in serum free thyroxine during childhood and adolescence. *J Pediatr.* 1993;123(6):899–905.
154. Perlsteyn M, Deladoey J, Van Vliet G. Similar age-dependent levothyroxine requirements of schoolchildren with congenital or acquired hypothyroidism. *Eur J Pediatr.* 2016;175(6):869–872.
155. Bucher H, Prader A, Illig R. Head circumference, height, bone age and weight in 103 children with congenital hypothyroidism before and during thyroid hormone replacement. *Helv Paediatr Acta.* 1985;40(4):305–316.
156. Dubuis JM, Glorieux J, Richer F, Deal CL, Dussault JH, Van Vliet G. Outcome of severe congenital hypothyroidism: closing the developmental gap with early high dose levothyroxine treatment. *J Clin Endocrinol Metab.* 1996;81(1):222–227.
157. Lichtenberger-Geslin L, Dos SS, Hassani Y, Ecosse E, Van Den Abbeele T, Leger J. Factors associated with hearing impairment in patients with congenital hypothyroidism treated since the neonatal period: a national population-based study. *J Clin Endocrinol Metab.* 2013;98(9):3644–3652.
158. van der Kaay DC, Wasserman JD, Palmert MR. Management of neonates born to mothers with Graves' disease. *Pediatrics.* 2016;137(4).
159. Weissenfels PC, Woelfle J, Korsch E, Joergens M, Gohlke B. Inconsistencies in the management of neonates born to mothers with "thyroid diseases" *Eur J Pediatr.* 2018;177(11):1711–1718.
160. Huel C, Guibourdenche J, Vuillard E, Ouahba J, Piketty M, Oury JF, et al. Use of ultrasound to distinguish between fetal hyperthyroidism and hypothyroidism on discovery of a goiter. *Ultrasound Obstet Gynecol.* 2009;33(4):412–420.
161. Clementi M, Di GE, Cassina M, Leoncini E, Botto LD, Mastroiacovo P. Treatment of hyperthyroidism in pregnancy and birth defects. *J Clin Endocrinol Metab.* 2010;95(11):E337–E341.
162. Rivkees SA, Szarfman A. Dissimilar hepatotoxicity profiles of propylthiouracil and methimazole in children. *J Clin Endocrinol Metab.* 2010;95(7):3260–3267.
163. Glinoeir D, Cooper DS. The propylthiouracil dilemma. *Curr Opin Endocrinol Diabetes Obes.* 2012;19(5):402–407.
164. Benavides VC, Mallela MK, Booth CJ, Wendler CC, Rivkees SA. Propylthiouracil is teratogenic in murine embryos. *PLoSOne.* 2012;7(4). e35213.
165. Ferraz C, Paschke R. Inheritable and sporadic non-autoimmune hyperthyroidism. *Best Pract Res Clin Endocrinol Metab.* 2017;31(2):265–275.
166. Kopp P, Van Sande J, Parma J, Duprez L, Gerber H, Joss E, et al. Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med.* 1995;332(3):150–154.
167. Desai PR, Lipscomb AP, Slater J. Familial dysalbuminaemic hyperthyroxinaemia, a thyroid trap. *Arch Dis Child.* 2004;89(12):1161–1162.

9

Disorders of Mineral Metabolism: Normal Homeostasis

Allen W. Root

CHAPTER OUTLINE

CALCIUM	220
PHOSPHATE	230
Phosphatonins	231
MAGNESIUM	232
ALKALINE PHOSPHATASE	233
PARATHYROID HORMONE; PARATHYROID HORMONE-RELATED PROTEIN; PTH/PTHrP RECEPTORS	234
Parathyroid Hormone	234
Parathyroid Hormone-Related Protein	236
Parathyroid Hormone/Parathyroid Hormone-Related Protein Receptors	237
CALCITONIN	238
VITAMIN D	239
Synthesis and Biological Activity of Vitamin D	239
Vitamin D Receptor	242
SKELETON: CARTILAGE AND BONE	245
Chondrogenesis and Skeletal Formation	245
Osteoblastogenesis	248
Osteoclastogenesis	252
Bone Extracellular Matrix	256
Bone Formation and Mineralization	259
Assessment of Bone Quality, Mass, and Strength	265
References	272

Calcium (Ca), phosphorus (as phosphate [HPO_4^{2-}]), and magnesium (Mg) are essential nutrients that are indispensable for the structural integrity of the body and for the function of each of its cells.^{1–3} The genetic and physiological mechanisms that regulate normal mineral homeostasis and bone development, composition, and strength from the prenatal period through adolescence are complex. Lists some of the many genes that direct these processes. Figs. 9.1 and 9.2 schematically depict the factors that regulate calcium and phosphate homeostasis, respectively.

CALCIUM

Calcium is present in extracellular fluids, cell cytoplasm, and bone and is essential for the function of every cell in the body. Together, calcium and phosphate form the hydroxyapatite crystal [$\text{Ca}_{10}(\text{PO}_4)_{10}(\text{OH})_2$] of bone; hydroxyapatite accounts for 65% of bone weight and provides its mechanical and weight-bearing strength and also serves as a reservoir for calcium that may be quickly needed for homeostatic and functional purposes. Although 99% of total body calcium is present in the slowly exchangeable, deeply deposited skeletal crystal, it is the rapidly exchangeable 1% of body calcium in recently accumulated surface bone and in the vascular, extracellular, and intracellular (soft tissues) spaces with which it is in equilibrium

that modulates gene expression, intercellular communication and intracellular signal transduction, neural transmission, cell-to-cell adhesion, clotting, striated, smooth and cardiac muscle contraction, cardiac rhythmicity, enzyme action, synthesis and secretion of endocrine and exocrine factors, fertilization, and cellular proliferation and apoptosis.^{4,5} Approximately 50% of total serum calcium is bound to albumin and globulin; 5% is complexed/chelated to citrate, phosphate, lactate, bicarbonate, and sulfate; and 45% is present as biologically active and closely regulated ionized extracellular calcium (Ca^{2+}). Serum total and ionized calcium concentrations are related to levels of albumin, creatinine, parathyroid hormone (PTH), phosphate, and serum pH. For every 1 g/dL decline in the serum concentration of albumin below 4 g/dL, the total serum calcium level declines by 0.8 mg/dL. The measured Ca^{2+} level is dependent upon the serum pH (normal adult range 1.1–1.3 mmol/L at pH 7.4); increase in alkalinity (higher pH) raises calcium binding to albumin, thus decreasing the plasma Ca^{2+} value, whereas acidic changes (lower pH) decrease binding, thereby increasing the Ca^{2+} concentration. The relationship between pH and Ca^{2+} is best described by an inversely S-shaped third-degree function. The serum concentration of Ca^{2+} is maintained within narrow limits by an integrated system involving the plasma membrane Ca^{2+} sensing receptor (CaSR); PTH and its receptor (PTH/PTH-related protein [PTHrP]-1R); the thyroidal parafollicular C cell product calcitonin and its receptor; and the vitamin D hormone system acting upon the intestinal tract, bone, and kidney. Thus absorption of calcium in the small intestine is enhanced by 1,25-dihydroxyvitamin D3 (calcitriol); the proximal renal tubular reabsorbs calcium filtered through the renal glomerulus; PTH and calcitriol mobilize calcium from the hydroxyapatite apatite crystal, and calcitonin secreted by the parafollicular C cells of the thyroid gland suppresses PTH secretion (see Fig. 9.1).⁶

The intracellular concentration of cytosolic free Ca^{2+} (approximately 100 nM) is 10,000-fold less than that in serum and extracellular fluid (ECF), a gradient maintained by exchange of Ca^{2+} across the cell's plasma membrane and across the membranes of intracellular structures. Within the cell, Ca^{2+} is primarily (99%) stored within the endoplasmic reticulum (sarcoplasmic reticulum in muscle cells) and mitochondria, as well as within endosomes, lysosomes, the Golgi apparatus and secretory granules and bound to the interior of the cell plasma membrane from which sites it can be released by chemical signals, for example, inositol-1,4,5-trisphosphate (IP_3).⁷ Inositol-1,4,5-trisphosphate acts upon IP_3 receptors (encoded by *ITPR1*—see Table 9.1) located in the membrane of the endoplasmic reticulum to effect rapid egress of Ca^{2+} from storage, thereby quickly increasing intracellular Ca^{2+} levels.⁸ Ca^{2+} serves as a “second messenger” signal transducer that controls many cellular activities, including gene transcription, cell division and growth, cell movement, and secretion of synthesized products. Ca^{2+} enters the cell through transmembrane protein “pores,” such as voltage-gated and store-operated calcium channels. Voltage-gated Ca^{2+} channels are active in cells that are electrically excitable, such as cardiac, skeletal, and smooth muscle cells, neurons, gastric mucosa, and pancreatic β cells and that “open” in response to depolarization of the plasma membrane, permitting rapid influx of Ca^{2+} into the cell

Abbreviations

1,25(OH) ₂ D ₃	1,25-Dihydroxyvitamin D ₃ (calcitriol)	LRP	Low-density lipoprotein receptor-related protein
24R,25(OH) ₂ D ₃	24,25-Dihydroxyvitamin D ₃	μFE	Microfinite element
25OH ₂ D ₃	25-Hydroxyvitamin D ₃ (calcidiol)	MAPK	Mitogen-activated protein kinase
AA	Amino acids	MARRS-BP	Membrane-associated rapid response steroid-binding protein
Acan	Aggrecan	MATN3	Matrilin 3
ADHR	Autosomal dominant hypophosphatemic rickets	M-CSF	Macrophage colony-stimulating factor
AF	Activating function	MEPE	Matrix extracellular phosphoglycoprotein
AKT	see PKB	Mg ²⁺	Magnesium
AMP	Adenosine monophosphate	MITF	Microphthalmia-associated transcription factor
APC	Adenomatous polyposis coli	MMP	Matrix metalloproteinase
ARHR	Autosomal recessive hypophosphatemic rickets	MRI	Magnetic resonance imaging
ASARM	Acidic serine aspartate-rich MEPE-associated motif	Na ⁺	Sodium
ATP	Adenosine triphosphate	NAD	Nicotinamide adenine dinucleotide
ATPase	Adenosine triphosphatase	NADPH	Nicotinamide adenine dinucleotide phosphate
BAP	Bone alkaline phosphatase	NF-κB	Nuclear factor-κB
BMAD	Bone mineral apparent density (~volumetric BMD)	NFATC1	Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1
BMC	Bone mineral content	NHERF	Sodium-hydrogen exchanger regulatory factor
BMD	Bone mineral density	NPT2	Sodium/phosphate transporter 2
BMP	Bone morphogenetic protein	NTX	Amino-terminal cross-link telopeptide of collagen type I
BMU	Basic multicellular unit	OMIM	Online Mendelian Inheritance in Man
BRU	Bone remodeling unit	OPG	Osteoprotegerin
BTT	Bone transmission time	OSCAR	Osteoclast-associated receptor
Ca ²⁺	Calcium—ionized	OSTM1	Osteopetrosis-associated transmembrane protein 1
CaMK	Ca ²⁺ /calmodulin-dependent protein kinase	P3H1	Prolyl 3-hydroxylase-1
CART	Cocaine amphetamine-regulated transcript	PHEX	Phosphate-regulating gene with homologies to endopeptidases on the X chromosome
CaSR	Calcium sensing receptor	PICP	Procollagen type I carboxyl-terminal propeptide
CGRP	Calcitonin gene-related peptide	PINP	Procollagen type I amino-terminal propeptide
Cl ⁻	Chloride	PIIINP	Procollagen type III amino-terminal propeptide
CNP	Natriuretic hormone type C	PI3K	Phosphoinositide-3-kinase
COMP	Cartilage oligomeric protein	PKA	Protein kinase A
CREB	Cyclic AMP-responsive element binding protein	PKB	Protein kinase B (AKT)
CTX	Carboxyl-terminal cross-link telopeptide α1 chain of type I collagen (see ICTP)	PLC	Phospholipase C
DAG	1,2-Diacylglycerol	PO ₄	Phosphate (HPO ₄ ²⁻)
DBP	Vitamin D binding protein	PPARγ	Peroxisome proliferator activator receptor gamma
DMP	Dentin matrix protein	PTH	Parathyroid hormone
DNA	Deoxyribonucleic acid	PTHrP	PTH-related protein
DPD	Deoxyripyridinoline	PTH1R	PTH/PTHrP-1 receptor (PTH/PTHrP-1R)
DRIP	Vitamin D interacting protein	PYD	Pyridinoline
DXA	Dual energy x-ray absorptiometry	QCT	Quantitative computed tomography
ECF	Extracellular fluid	QUS	Quantitative ultrasonography
EGF	Epidermal growth factor	RANK	Receptor activator of NF-κB
ER	Estrogen receptor	RANKL	RANK-ligand
ERK	Extracellular signal-regulated kinase	RDA	Recommended dietary allowance
FGF	Fibroblast growth factor	RNA	Ribonucleic acid
FGFR	Fibroblast growth factor receptor	RXR	Retinoid X receptor
FOS	FBJ osteosarcoma oncogene	SIBLING	Small integrin-binding ligand interacting N-glycoprotein (proteins)
FRP4	Frizzled related protein-4	SOS	Speed of sound
FRS	FGF receptor substrate	SOX	SRY-Box
GABA	Gamma (γ)-amino butyric acid	SPP1	Secreted phosphoprotein 1 (osteopontin)
GDNF	Glial cell line-derived neurotrophic factor	Src	SRC oncogene
GDP	Guanosine diphosphate	SRC	Steroid receptor coactivator
G _{ix}	Alpha subunit of inhibitory GTP-binding protein	STAT	Signal transduction and transcription
G _{sx}	Alpha subunit of stimulatory GTP-binding protein	SYK	Spleen tyrosine kinase
G _{qx}	Alpha subunit of a stimulatory GTP-binding protein	TALH	Thick ascending loop of Henle
GH	Growth hormone	TCF/LEF	T-cell factor/Lymphoid enhancer binding factor
Gla protein	Matrix gamma carboxyglutamic acid	TGF	Transforming growth factor
GMP	Guanosine monophosphate	TIO	Tumor induced osteomalacia
GPCR	G-protein-coupled receptor	TIP	Tuberoinfundibular protein (hypothalamic)
GRB2	GRB2-associated binding protein 2	TNF	Tumor necrosis factor
GRK	G-protein-coupled receptor kinase	TNSALP	Tissue nonspecific alkaline phosphatase
GSK3	Glycogen synthase kinase 3	TR	Thyroid (hormone) receptor
GTP	Guanosine triphosphate	TRAF	TNF receptor associated factor
H ⁺	Hydrogen ion (proton)	TRANCE	TNF-related activation induced cytokine
HNRPD	Heterogeneous nuclear ribonucleoprotein D	TRAP	Tartrate-resistant acid phosphatase
HRpQCT	High-resolution peripheral quantitative computed tomography	TREM2	Triggering receptor expressed on myeloid cells-2
HSP	Heat shock protein	TRP	Transient receptor potential (channel)
ICTP	Carboxyl-terminal cross-link telopeptide of collagen type I generated by matrix metalloproteinase (see CTX)	VDIR	Vitamin D-interacting repressor
IGF	Insulin-like growth factor (somatomedin)	VDR	Vitamin D receptor
IHH	Indian hedgehog	VDRE	Vitamin D response element
IL	Interleukin	VEGF	Vascular endothelial growth factor
IP ₃	Inositol-1,4,5-trisphosphate	WIF	WNT inhibitory factor
ITAM	Immunoreceptor tyrosine-based motif	WINAC	WSTF including nucleosome assembly complex
K ⁺	Potassium	WSTF	Williams syndrome transcription factor
		XHR	X-linked hypophosphatemic rickets

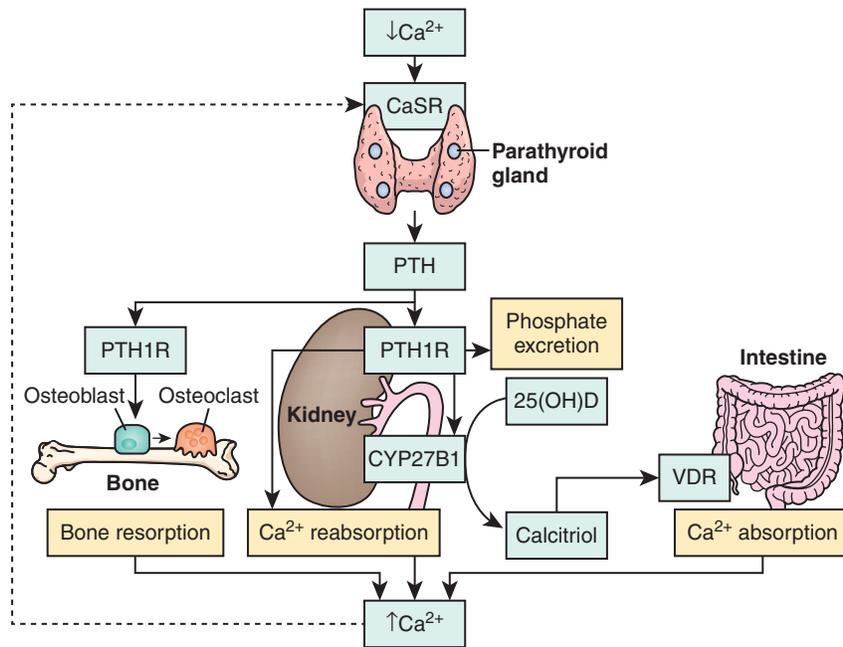


Fig. 9.1 Regulation of calcium homeostasis. Calcium (Ca^{2+}) is absorbed from the intestinal tract, kidney tubule, and bone in response to calcitriol [$1,25(\text{OH})_2\text{D}_3$] and/or parathyroid hormone (PTH). The Ca^{2+} -sensing receptor (CaSR) modulates Ca^{2+} -mediated activity of the parathyroid glands and the renal tubules. Both hypocalcemia and hypophosphatemia enhance renal tubular generation of calcitriol that increases intestinal absorption of calcium and phosphate. PTH increases renal tubular reabsorption of calcium while inhibiting renal tubular reabsorption of phosphate. Increasing serum concentrations of calcium inhibit secretion of PTH. Fibroblast growth factor-23 (FGF23), a phosphatonin secreted by osteoblasts and osteocytes, inhibits renal tubular reabsorption of phosphate and synthesis of calcitriol. Calcitonin, a product of the thyroid gland C cells, inhibits resorption of calcium from bone. (Please see Fig. 9.2 and text for further details.) (From Mannstadt, M., Bilezikian, J.P., Thakker, R.V., et al. (2017). Hypoparathyroidism. *Nat Rev Dis Primers*, 3, 17055. With permission.)

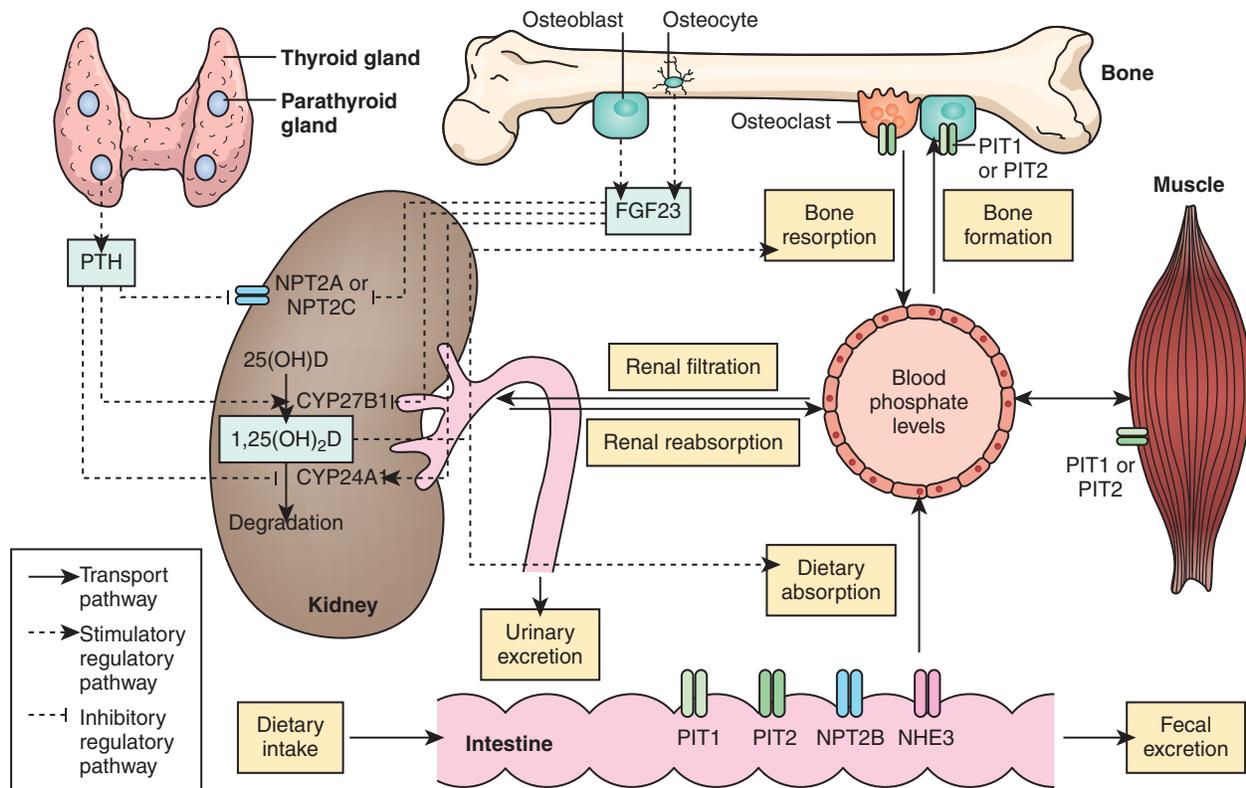


Fig. 9.2 Regulation of phosphate homeostasis. Ingested phosphate is absorbed from the gastrointestinal tract by passive paracellular mechanisms and through active phosphate cotransporters (NP2B, NHE3, PIT1, PIT2). Serum concentrations of phosphate are depressed by parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23), both of which increase urinary excretion of this cation by decreasing renal tubular expression of phosphate cotransporter proteins (NPT2A, NPT2C). Synthesis of FGF23, a product of osteoblasts and osteocytes, is depressed by low serum phosphate levels and by phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX), dentin matrix protein 1 (DMP1), and ectonucleotide pyrophosphate -phosphodiesterase 1 (ENPP1) all three of which proteins are also synthesized by bone cells. PTH increases whereas FGF23 depresses synthesis of calcitriol (1,25 dihydroxyvitamin D). (See text for details.) (From Minisola, S., Peacock, M., Fukumoto, S., et al. (2017). Tumour-induced osteomalacia. *Nat Rev*, 3, 17044. With permission)

TABLE 9.1 Human Genes Involved in Mineral Homeostasis and Bone Metabolism

Protein	Gene	Chromosome	OMIM ^a
Adaptor-related protein complex 2	<i>AP2S1</i>	19q13.31	602242
Adenylyl cyclase	<i>ADCY3</i>	2p23.3	600291
Adhesion G-protein-coupled receptor A2	<i>ADGAR2</i>	8p11.23	606823
Adipocyte-, C1q-, and collagen domain-containing (Adiponectin)	<i>ADIPOQ</i>	3q27.3	605441
Aggrecan 1	<i>AGC1</i>	15q26.1	155760
Alkaline phosphatase	<i>ALPL</i>	1p36.2	171760
APC gene	<i>APC</i>	5q22.2	611731
β-arrestin 1	<i>ARRB1</i>	11q13.4	107940
ATPase, Ca(2+)-transporting, plasma membrane, 1	<i>ATP2B1</i>	12q21.33	108731
ATPase, Ca(2+)-transporting, Slow twitch	<i>ATP2A2</i>	12q24.11	108740
Axis inhibitor 1	<i>AXIN1</i>	16p13.3	603816
β1-Catenin	<i>CTNNB1</i>	3p22-p21.3	116806
Bone morphogenetic protein-1	<i>BMP1</i>	8p21.3	112264
Bone morphogenetic protein-2	<i>BMP2</i>	20p12	112261
Bone morphogenetic protein-4	<i>BMP4</i>	14q22.2	112262
Bone morphogenetic protein-7	<i>BMP7</i>	20q13.31	112267
BMP Receptor 1A	<i>BMPR1A</i>	10q22.3	601299
BMP Receptor 2	<i>BMPR2</i>	2q33	600799
Bromodomain adjacent to zinc-finger domain, 1B (WSTF)	<i>BAZ1B</i>	7q11.23	605681
BSND gene (Barttin)	<i>BSND</i>	1p32.3	606412
C-type natriuretic protein	<i>NPPC</i>	2q24-qter	600296
Calbindin (9K)	<i>S100G</i>	Xp22.2	302020
Calcitonin	<i>CALCA</i>	11p15.2	114130
Calcitonin receptor	<i>CALCR</i>	7q21.3	114131
Calcium release-activated calcium modulator 1	<i>CRACM1</i>	12q24	610277
Calcium sensing receptor	<i>CASR</i>	3q13.3-q21.1	601199
Calcium channel, voltage dependent, L-type, alpha 1C subunit	<i>CACNA1C</i>	12p13.33	114205
Calmodulin 1	<i>CALM1</i>	14q32.11	114180
Carbonic anhydrase II	<i>CA2</i>	8q21.2	611492
Cartilage-associated protein	<i>CRTAP</i>	3p22.3	605497
Cartilage oligomeric matrix protein	<i>COMP</i>	19p13.11	600310
β-Catenin	<i>CTNNB1</i>	3p22.1	116806
Cathepsin K	<i>CTSK</i>	1q21.3	603959
Caveolin 1	<i>CAV1</i>	7q31.2	601047
Chloride channel 5	<i>CLCN5</i>	Xp11.22	300008
Chloride channel 7	<i>CLCN7</i>	16p13.3	602727
Chloride channel, kidney, B	<i>CLCNKB</i>	1p36.13	602023
Claudin 2	<i>CLDN2</i>	Xq22.3-23	300520
Claudin 10	<i>CLDN10</i>	13q32.1	617579
Claudin 14	<i>CLDN14</i>	21q22.13	605608
Claudin 16 (Paracellin-1)	<i>CLDN16</i>	3q28	603959
Claudin 19	<i>CLDN19</i>	1p34.2	610036
Cocaine amphetamine regulated transcript	<i>CART</i>	5q13.2	602606
Collagen triple helix repeat-containing protein 1	<i>CTHRC1</i>	8q22.3	6100635
Collagen type I(α1)	<i>COL1A1</i>	17q21.33	120150
Collagen type I(α2)	<i>COL1A2</i>	7q22.1	120160
Collagen type II(α1)	<i>COL2A1</i>	12q13.11	120140
Collagen type III(α1)	<i>COL3A1</i>	2q31	120180
Collagen type IV(α1)	<i>COL4A1</i>	13q34	120130
Collagen type IX(α1)	<i>COL9A1</i>	6q13	120210
Collagen type X(α1)	<i>COL10A1</i>	6q22.1	120110
Collagen type XI(α1)	<i>COL11A1</i>	1p21	120280
Colony stimulating factor—Macrophage	<i>CSF1</i>	1p13.3	120420
CSF receptor—Macrophage (c-Fms)	<i>CSF1R</i>	5q32	164770
Cholesterol 25-hydroxylase	<i>CH25H</i>	10q23.31	604551
Core binding factor, beta subunit	<i>CBFB</i>	16q22.1	121360
Cyclic AMP-responsive binding protein	<i>CREB1</i>	2q33.1	123810
Cyclic AMP response element-binding protein 3-like 1	<i>CREB3L1</i>	11p11.2	616215
Cytochrome P450, III A, 4	<i>CYP3A4</i>	7q22.1	124010
Cytochrome P450, subfamily IIR, polypeptide 1 (25-Hydroxylase)	<i>CYP2R1</i>	11p15.2	608713
Cytochrome P450, subfamily XXVIII, polypeptide 1 (25OHD-1α hydroxylase)	<i>CYP27B1</i>	12q14.1	609506
Cytochrome P450, family 24, subfamily A, polypeptide 1 (25OHD-24 hydroxylase)	<i>CYP24A1</i>	20q13.2	126065
7-Dehydrocholesterol reductase	<i>DHCR7</i>	11q13.4	602858
Dentin matrix acidic phosphoprotein 1	<i>DMP1</i>	4q22.1	600980
Dikkopf, xenopus, homolog of, 1	<i>DKK1</i>	10q21.1	605189
Disheveled 1	<i>DVL1</i>	1p36.33	601365
Distal-less homeobox 5	<i>DLX5</i>	7q21.3	600028
Ectonucleotide pyrophosphatase/phosphodiesterase 1	<i>ENPP1</i>	6p23.2	173335
Elastin	<i>ELN</i>	7q11.23	130160
Endogenous retroviral family W, member 1	<i>ERVW1</i>	7q21.2	604659
Ephrin-B1	<i>EFNB1</i>	Xq13.1	300035

Continued

TABLE 9.1 Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd

Protein	Gene	Chromosome	OMIM
Ephrin-B2	<i>EFNB2</i>	13q33.3	600527
Ephrin receptor EPHA1	<i>EPHA1</i>	7q34-q35	179610
Epidermal growth factor	<i>EGF</i>	4q25	131530
Family with sequence similarity 20, member A	<i>FAM20A</i>	17q24.2	611062
Family with sequence similarity 20, member C	<i>FAM20C</i>	7p22.3	611061
Fibroblast growth factor-1 (acidic)	<i>FGF1</i>	5q31	131220
Fibroblast growth factor-2 (basic)	<i>FGF2</i>	4q28.1	134920
Fibroblast growth factor-5	<i>FGF5</i>	4q21	165190
Fibroblast growth factor-7	<i>FGF7</i>	15q21.2	148180
Fibroblast growth factor-18	<i>FGF18</i>	5q35.1	603726
Fibroblast growth factor-21	<i>FGF21</i>	19q33.33	609436
Fibroblast growth factor-23	<i>FGF23</i>	12p13.32	605380
Fibroblast growth factor receptor-1	<i>FGFR1</i>	8p11.2-p11.1	136350
Fibroblast growth factor receptor-2	<i>FGFR2</i>	10q26.13	176943
Fibroblast growth factor receptor-3	<i>FGFR3</i>	4p16.3	134934
Fibroblast growth factor receptor-4	<i>FGFR4</i>	5q35—qter	134935
FK506-binding protein	<i>FKBP10</i>	17q21.2	607063
Four-and-a-half Lim domains	<i>FHL1</i>	Xq26.3	300163
Frizzled, drosophila, homolog of	<i>FZD1</i>	7q21.13	603408
Frizzled-related protein-4	<i>FRP4</i>	7p14-p13	606570
Gamma-carboxyglutamic acid protein, bone (Osteocalcin)	<i>BGLAP</i>	1q22	112260
GATA-binding protein 3	<i>GATA3</i>	10p14	131320
Glial cells missing, Drosophila, homolog of, 2	<i>GCM2</i>	6p24.2	603716
Glycogen synthase kinase 3-alpha	<i>GSK3A</i>	19q13.2	606784
Group-specific component (DBP)	<i>GC</i>	4q13.3	139200
Growth hormone 1	<i>GH1</i>	17q23.3	139250
Growth hormone receptor	<i>GHR</i>	5p13-p12	600946
Guanine nucleotide binding protein, alpha stimulating	<i>GNAS1</i>	20q13.2	139320
Guanine nucleotide binding protein, alpha 11	<i>GNA11</i>	19p13.3	139313
Hairless	<i>HR</i>	8p21.1	602302
Hematopoietic transcription factor PU.1	<i>SPI1</i>	11p11.2	165170
Heterogeneous nuclear ribonucleoprotein D	<i>HNRPD</i>	4q21.1-q21.2	601324
HNF1 homeobox B	<i>HNF1B</i>	17q12	189907
Indian hedgehog	<i>IHH</i>	2q35	600726
Inhibitor of kappa light polypeptide gene enhancer ... gamma	<i>IKBKG</i>	Xq28	300248
Inositol trisphosphate receptor	<i>ITPR1</i>	3p26.1	147265
Insulin-like growth factor-I	<i>IGF1</i>	12q22-q24.1	147440
IGF-I receptor	<i>IGF1R</i>	15q25-q26	147370
Integrin α v	<i>ITGAV</i>	2q31	193210
Integrin β 3	<i>ITGB3</i>	17q21.32	173470
Integrin-binding sialoprotein	<i>IBSP</i>	4q22.1	147563
Interferon-induced transmembrane protein family 5	<i>IFITM5</i>	11p15.5	614757
Interleukins-1-beta	<i>IL1B</i>	2q14.1	147720
Interleukin-6	<i>IL6</i>	7p15.3	147620
Interleukin 6-dependent DNA binding protein	<i>CEBPB</i>	20q13.13	189965
Klotho	<i>KL</i>	13q13.1	604824
Leptin	<i>LEP</i>	7q31.3	164160
Lipocalin 2	<i>LCN2</i>	9q34.11	600181
Low-density lipoprotein receptor-related protein 2 (Megalyn)	<i>LRP2</i>	2q31.1	600073
Low-density lipoprotein receptor-related protein 5	<i>LRP5</i>	11q13.2	603506
Low-density lipoprotein receptor-related protein 6	<i>LRP6</i>	12p13.2	603507
V-Mak musculoaponeurotic fibrosarcoma oncogene family, protein B	<i>MAFB</i>	20q12	608968
Matrilin 3	<i>MATN3</i>	2p24.1	602109
Matrix extracellular phosphoglycoprotein	<i>MEPE</i>	4q21.1	605912
Matrix gamma carboxyglutamic acid (Gla protein)	<i>MGP</i>	12p12.3	154870
Matrix metalloproteinase 9	<i>MMP9</i>	20q13.12	120361
Mediator complex subunit 4 (vitamin D interacting protein)	<i>MED4</i>	13q14.2	605718
Membrane-bound transcription factor protease site 2	<i>MBTPS2</i>	Xp22.12	300294
Methyl-CpG-binding domain protein 4	<i>MBD4</i>	3q21.3	603574
Mitochondrial RNA-processing endoribonuclease, RNA component of	<i>RMRP</i>	9p13.3	157660
Mothers against decapentaplegic, drosophila, homolog of, 2	<i>SMAD2</i>	18q21.1	601366
Natriuretic peptide precursor C	<i>NPPC</i>	2q37.1	600296
Natriuretic peptide receptor B	<i>NPR2</i>	9p13.3	108961
Natriuretic peptide receptor C	<i>NPR3</i>	5p13.3	108962
Noggin, mouse, homolog of	<i>NOG</i>	17q22	602991
Notch, drosophila, homolog of 1	<i>NOTCH1</i>	9q34.3	190198
Nuclear factor- κ B, Subunit 1	<i>NFKB1</i>	4q24	164011
Nuclear factor- κ B, Inhibitor (I κ B)	<i>NFKB1A</i>	14q13.2	164008
Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1	<i>NFATC1</i>	18q23	600489
ORAI calcium release-activated calcium modulator 1	<i>ORAI1</i>	12q24.31	610277

TABLE 9.1 Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd

Protein	Gene	Chromosome	OMIM
Osteoclast-associated receptor	<i>OSCAR</i>	19q13.42	606862
Osteocrin	<i>OSTN</i>	3q28	610280
Osteopetrosis-associated transmembrane protein 1	<i>OSTM1</i>	6q21	607649
Paired box gene 1	<i>PAX1</i>	20p11.22	167411
Parathyroid hormone	<i>PTH</i>	11p15.2	168450
PTH-related/like protein	<i>PTHLLH</i>	12p11.22	168470
PTH 1 receptor	<i>PTH1R</i>	3p21.31	168468
PTH 2 receptor	<i>PTH2R</i>	2q34	601469
Patched 1	<i>PTCH1</i>	9q22.3	601309
Peptidyl-prolyl isomerase B (CypB)	<i>PPIB</i>	15q22.31	123841
Peroxisome proliferator-activated receptor gamma	<i>PPARG</i>	3p25.2	601487
Phosphate-regulating gene with homologies to endopeptidases on the X chromosome	<i>PHEX</i>	Xp22.1	300550
Phosphodiesterase 3A, cGMP-inhibited	<i>PDE3A</i>	12p12.2	123805
Phosphodiesterase 4D, cAMP-specific	<i>PDE4D</i>	5q11.2-q12.1	600129
Phospholipase C β -1	<i>PLCB1</i>	20p12.3	607120
Plastin 3	<i>PLS3</i>	Xq23	300131
Pleckstrin homology domain containing protein, family M, member 1	<i>PLEKHM1</i>	17q21.31	611466
Potassium channel, inwardly rectifying, subfamily 1, member 1	<i>KCNJ1</i>	11q24.3	600359
Potassium channel, inwardly rectifying, subfamily J, member 10	<i>KCNJ10</i>	1q23.2	602208
Potassium channel, voltage-gated, Shaker-related subfamily, member 1	<i>KCNA1</i>	12p13.32	176260
Pregnane X receptor	<i>NR112</i>	3q13-q21	603065
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	<i>PLOD1</i>	1p36.22	153454
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	<i>PLOD2</i>	3q24	601865
Prolyl 3-hydroxylase 1	<i>P3H1</i>	1p34.2	610339
Protein disulfide isomerase, family A, member 3 (MARRS-BP)	<i>PDI3</i>	15q14.3	602406
Protein kinase A	<i>PRKAR1A</i>	17q24.3	188830
Protein phosphatase 1, regulatory subunit 1B	<i>PPP1R1B</i>	17q12	604399
Protein phosphatase 3, catalytic subunit, alpha isoform (calcineurin A)	<i>PPP3CA</i>	4q24	114105
Receptor activator of NF- κ B (RANK)	<i>TNFRSF11A</i>	18q22.1	603499
RANK-Ligand (RANKL)	<i>TNFSF11</i>	13q14.11	602642
Retinoid X receptor α	<i>RXRα</i>	9q34.3	180245
Reversion-inducing cysteine-rich protein with kazal motifs	<i>RECK</i>	9p13.3	605227
Runt-related transcription factor 2	<i>RUNX2</i>	6p21.1	600211
Sclerostin	<i>SOST</i>	17q21.31	605740
Secreted frizzled-related protein 1	<i>SFRP1</i>	8p11.21	604156
Secreted phosphoprotein 1 (Osteopontin)	<i>SPP1</i>	4q22.1	166490
Secreted protein, acidic, cysteine-rich (Osteonectin)	<i>SPARC</i>	5q33.1	182120
Serpine peptidase inhibitor, clade H, member 1 (Heat shock protein 47)	<i>SERPINH1</i>	11q13.5	600943
Sirtuin 1	<i>SIRT1</i>	10q21.3	604479
Smoothed	<i>SMOH</i>	7q31-q32	601500
Sodium phosphate cotransporter Solute carrier family 34, member 1 (NPT2a/NaPi-IIa)	<i>SLC34A1</i>	5q35.3	182309
Sodium phosphate cotransporter Solute carrier family 34, member 2 (NPT2b/NaPi-IIb)	<i>SLC34A2</i>	4p15.2	604217
Sodium phosphate cotransporter Solute carrier family 34, member 3 (NPT2c/Na-Pi-IIc)	<i>SLC34A3</i>	9q34.3	609826
Solute carrier family 4 (anion exchanger), member 2	<i>SLC4A2</i>	7q36.1	109280
Solute carrier family 8 (sodium-calcium exchanger), member 1 – cardiac	<i>SLC8A1</i>	2p22.1	182305
Solute carrier family 8 (sodium-calcium exchanger), member 2 – skeletal	<i>SLC8A2</i>	19q13.32	601901
Solute carrier family 9, member 3	<i>SLC9A3</i>	5p15.3	182307
Solute carrier family 9, member 3, regulator 1 (NHERF1)	<i>SLC9A3R1</i>	17q25.1	604990
Solute carrier family 12 (sodium/potassium/chloride transporter), member 1	<i>SLC12A1</i>	15q21.1	600839
Solute carrier family 12 (sodium/chloride transporter), member 3	<i>SLC12A3</i>	16q13	600968
Solute carrier family 20 (phosphate transporter), member 2	<i>SLC20A2</i>	8p11.21	158378
Sonic hedgehog	<i>SHH</i>	7q36.3	600725
Sorting nexin 10	<i>SNX10</i>	7p15.2	614780
Sphingosine-1-phosphate	<i>S1PR1</i>	1p21.2	601974
SRY-box 9	<i>SOX9</i>	17q24.3	608160
Stromal interaction molecule 1	<i>STIM1</i>	11p15.4	605921
T-Box 1	<i>TBX1</i>	22q11.2	602054
T-cell factor/lymphoid enhancer binding factor	<i>LEF1</i>	4q25	153245
T-cell immune regulator 1	<i>TClRG1</i>	11q13.2	604592
Tartrate-resistant acid phosphatase	<i>ACP5</i>	19p13.2	171640
Transcription factor 3/VDR interacting repressor	<i>TCF3</i>	19p13.3	147141
Transcription factor Sp7 (Osterix)	<i>SP7</i>	12q13.13	606633
Transforming growth factor, Beta 1	<i>TGFB1</i>	19q13.2	190180
Transient receptor potential cation channel, subfamily M, member 6	<i>TRPM6</i>	9q21.13	607009
Transient receptor potential cation channel, subfamily M, member 7	<i>TRPM7</i>	15q21.2	605692
Transient receptor potential cation channel, subfamily V, member 4	<i>TRPV4</i>	12q24.1	605427

Continued

TABLE 9.1 Human Genes Involved in Mineral Homeostasis and Bone Metabolism—cont'd

Protein	Gene	Chromosome	OMIM
Transient receptor potential cation channel, subfamily V, member 5	<i>TRPV5</i>	7q34	606679
Transient receptor potential cation channel, subfamily V, member 6	<i>TRPV6</i>	7q34	606680
Transmembrane protein 38B	<i>TMEM38B</i>	9q31.2	611236
Triggering receptor expressed on myeloid cells 2	<i>TREM2</i>	6p21.1	605086
Tumor necrosis factor- α	<i>TNF</i>	6p21.33	191160
Tumor necrosis factor ligand superfamily, member 11 (OPGL, RANKL, TRANCE)	<i>TNFSF11</i>	13q14.11	602642
Tumor necrosis factor receptor superfamily, Member 11A	<i>TNFRSF11A</i>	18q21.33	603499
Tumor necrosis factor receptor superfamily, member 11B (osteoprotegerin)	<i>TNFRSF11B</i>	8q24.12	602643
Tumor necrosis factor-associated factor 6	<i>TRAF6</i>	11p12	602355
Tuberoinfundular peptide 39	<i>TIP39</i>	19q13.33	608386
UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyl-transferase 3	<i>GALNT3</i>	2q24.3	601756
V-Fos FBJ murine osteosarcoma	<i>FOS</i>	14q24.3	164810
Vascular endothelial growth factor	<i>VEGF</i>	6p12	192240
Vitamin D binding protein	<i>GC</i>	4q13.3	139200
Vitamin D receptor	<i>VDR</i>	12q12-q14	601769
Voltage-gated calcium channel α_1	<i>CACNA1C</i>	12p13.3	114205
Wingless-type mouse mammary tumor virus (MMTV) integration site, member 1	<i>WNT1</i>	12q13.12	164820
Wingless-type mouse mammary tumor virus (MMTV) integration site, member 9	<i>WNT9A</i>	1q42	602863
WNT inhibitory factor 1	<i>WIF1</i>	12q14.3	605186
Xenotropic and polytropic retrovirus receptor	<i>XPR1</i>	1q25.3	605237

^aOnline Mendelian Inheritance in Man - <https://www.omim.org/>

cytoplasm, leading to further depolarization of the membrane and activation of cell function.⁹ Voltage-gated channels 2+ may be activated by high or low electrical voltage. For example, the skeletal muscle high voltage-activated calcium channel is composed of five subunits: α_1 , α_2 , β , γ , and δ (one voltage-activated α_1 subunit is encoded by *CACNA1C*). The voltage-sensing, pore-forming Ca^{2+} -binding α_1 subunit has four repeated domains, each with six transmembrane spanning regions (or helices) and intracytoplasmic amino and carboxyl terminals; transmembrane helix 4 serves as the voltage sensor; and the α_1 subunit also has a sequence of amino acids between transmembrane helices 5 and 6 that is partially inserted into the membrane to serve as a “selectivity filter.”⁹ The assembly, intracellular movement, interaction with other proteins, activation, and kinetic properties of the α_1 subunit are modified by the extracellular, glycosylated α_2 subunit, the β subunit (a cytoplasmic globular protein), a small membrane spanning δ subunit that is disulfide-linked to form dimeric $\alpha_2\delta$, and a second transmembrane subunit γ . The voltage-gated calcium channels are designated in accord with their cloned specific α_1 subunits and have been termed *Cav 1.1*, *Cav 1.2*, *Cav 1.3*, ...*Cav 3.3*. The high voltage-dependent *Cav1.1* through *Cav 1.4* calcium channels are present in skeletal, cardiac, and vascular smooth muscle cells; endocrine cells, neurons; and fibroblasts. These calcium channels are activated by the guanine triphosphate (GTP)-binding α_q subunit of $G_{\alpha q}$ -proteins through stimulation of phospholipase C (PLC), leading to phosphorylation of the channel protein. The *Cav1.1* through *Cav 1.4* calcium channels modulate the growth and proliferation of fibroblasts and smooth muscle cells, the synthesis of extracellular matrix collagen proteins, and the activation of specific transcription factors.

Ca^{2+} also enters the cytosol through the plasma membrane store-operated Ca^{2+} channel—ORAI1 (encoded by *ORAI1*).^{8,10,11} Store-operated Ca^{2+} channels are active in cells that are electrically nonexcitable, such as lymphocytes and other immune cells.⁵ Both voltage-gated and store-operated Ca^{2+} channels often coexist in the same cell. The type of Ca^{2+} channel used in a specific cell is determined by the expression of *STIM1* (see Table 9.1) encoding stromal

interaction molecule 1—a protein that transverses the membrane of the endoplasmic reticulum. When the Ca^{2+} content (e.g., “store”) of the endoplasmic reticulum is depleted, *STIM1* unfolds and bridges the cytosolic distance between the endoplasmic reticulum and the cell’s plasma membrane where it binds to and opens the ORAI1 channel, thereby permitting Ca^{2+} influx into the cytoplasm and the restoration of Ca^{2+} stores within cytoplasmic organelles.¹⁰ At the same time that it activates the ORAI1 Ca^{2+} channel, *STIM1* inhibits the voltage-gated Ca^{2+} channel.^{5,12,13} Loss-of-function mutations in *ORAI1* have been identified in patients with severe combined immune deficiency and tubular myopathy.^{14,15}

In addition to traversing rapidly across the cell’s plasma membrane through voltage-gated and store-operated channels, Ca^{2+} enters the cytosol, but at a markedly slower rate, through bifunctional membrane-associated IP_3 receptors that also serve as calcium channels.^{8,16} Encoded by *ITPR1*, the tetrameric IP_3 receptor has six transmembrane domains and a pore-forming loop between the fifth and sixth transmembrane segments. Whereas many IP_3 receptor/channels are expressed in the membranes of the endoplasmic reticulum where IP_3 activation leads to depletion of Ca^{2+} stored in the endoplasmic reticulum, thereby triggering store-operated calcium channel activation, only one to two such channels are expressed in the plasma membrane of each cell. Ca^{2+} is translocated not only through specific Ca^{2+} channels but also through paracellular transport channels. Ca^{2+} is extruded from cell cytoplasm by calcium and energy-dependent adenosine triphosphate (ATP)-driven calcium pumps and in exchange for sodium (Na^+) through H^+ /ATPase and Na^+ / Ca^{2+} exchangers.⁴ The Ca^{2+} -ATPases are encoded by four genes: *ATP2B1* encodes a 1220-amino-acid (AA) membrane protein with a cyclic adenosine monophosphate (AMP)-dependent protein kinase domain and a carboxyl domain to which a calbindin (a calcium binding protein) attaches that links hydrolysis of ATP with transport of cations across membranes. The Ca^{2+} -ATPases have the capacity to transport Ca^{2+} against high concentration gradients. The Na^+ / Ca^{2+} exchangers are widely distributed plasma membrane Ca^{2+} export proteins.^{2,4} A homolog of the human Na^+ / Ca^{2+} exchanger is comprised of 10 transmembrane domains

consisting of two sets of five transmembrane helices in inverted reverse order, with the amino and carboxyl termini within the cytoplasm.^{17,18} Transmembrane helices 2, 3, 7, and 8 form an ion-binding pocket with three low-affinity Na^+ and one high-affinity Ca^{2+} binding sites symmetrically arranged in a diamond formation. Through conformational changes of the structure of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, the Na^+ and Ca^{2+} binding sites are alternately exposed to either the intracellular or extracellular surfaces of the cell's plasma membrane. As Na^+ from the extracellular space occupies its three binding sites, the binding affinity for Ca^{2+} decreases, thereby releasing this cation into the cytosol and reversing the orientation of the $\text{Na}^+/\text{Ca}^{2+}$ channel; inasmuch as cytosolic levels of Na^+ are low, these cations are then released into the cytosol, restoring the affinity of the exchanger for Ca^{2+} . The human cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchanger is encoded by *SLC8A1* and the human skeletal muscle $\text{Na}^+/\text{Ca}^{2+}$ exchanger by *SLC8A2*.¹⁷ Within the cell, cytosolic Ca^{2+} is transported across the membrane and into the endoplasmic reticulum of muscle cells through Ca^{2+} -ATPase channels encoded by *ATP2A2*; linkage of small ubiquitin-like modifier type 1 (sumoylation) to this Ca^{2+} -ATPase extends its half-life and increases its intrinsic activity.¹⁹

The circulating concentration of Ca^{2+} is detected by the CaSR, encoded by *CASR*, a G-protein-coupled transmembrane heptahelical receptor (GPCR) expressed on the plasma membrane of the PTH-secreting chief cells of the parathyroid glands, epithelial cells of the renal tubules, osteoblasts, and other tissues. When the ambient Ca^{2+} concentration declines, CaSR signaling in the parathyroid glands through $\text{Gq}_{11\alpha}$ (encoded by *GNA11*) activates the intracellular phosphatidylinositol 4,5 bisphosphate signal transduction pathway, leading to rapid release of Ca^{2+} from intracellular storage sites and subsequent increase in synthesis and secretion of PTH. In turn, PTH (and PTHrP) may act through binding to its stimulatory GPCR (encoded by *PTH1R*) present on the plasma membranes of osteoblasts to enhance expression of *TNFSF11* encoding the receptor activator of nuclear factor kappa-B (NF- κ B) ligand (RANK-ligand) thereby stimulating osteoclastogenesis and increasing the rate of bone resorption, thus releasing calcium and increasing its extracellular concentration. PTH also acts on epithelial cells lining the distal renal tubule to enhance renal reabsorption of filtered Ca^{2+} and synthesis of calcitriol (thereby increasing intestinal absorption of Ca^{2+}) while suppressing proximal renal tubular reabsorption of filtered phosphate directly and indirectly by enhancing osteocytic synthesis of fibroblast growth factor-23 (FGF23). Increased serum concentrations of Ca^{2+} reciprocally suppress CaSR activity and PTH synthesis. Intracellular signaling induced by PTH proceeds primarily through the stimulatory guanosine phosphate-binding protein composed of alpha (G_{src}), beta, and gamma subunits.²⁰ After substitution of GTP for guanosine diphosphate (GDP) on the α subunit, G_{src} dissociates from its linked $\beta\delta$ subunits. G_{src} then stimulates intracellular adenylyl cyclase (encoded by *ADCY3*) that converts ATP to cyclic AMP and releases it from the cytoplasmic surface of the plasma membrane of the PTH-responsive cell. Cyclic AMP in turn binds to the regulatory alpha subunit of protein kinase A (encoded by *PRKARIA*), thereby initiating intracellular signaling.²⁰ The catalytic subunits of protein kinase A phosphorylate a number of intracellular proteins, including the cyclic AMP response binding protein (encoded by *CREB1*) that in turn initiates transcription of cyclic AMP target genes. CREB1 is deactivated by one of several phosphodiesterases, including those encoded by *PDE4D* and *PDE3A*. By increasing activity of 25-hydroxyvitamin D-1 α hydroxylase, PTH stimulates renal synthesis of calcitriol, the active metabolite of vitamin D; calcitriol increases calcium absorption in the proximal small intestinal tract and distal

renal tubule, phosphate absorption in the intestine and kidney, and calcium mobilization from bone stores by enhancing osteoclastogenesis; calcitriol downregulates the synthesis and secretion of PTH (see Fig. 9.1).

In the gastrointestinal tract, calcium is absorbed by both active transcellular and passive paracellular processes.^{21,22} During growth in younger subjects and when calcium intake and consequently intraintestinal calcium concentrations are low, active transcellular absorption of calcium predominates; when calcium intake is adequate or high, passive paracellular absorption of calcium in the proximal intestine predominates.^{1,21,23} Transcellular gastrointestinal absorption of calcium is an active and saturable process that is primarily stimulated by the active metabolite of vitamin D₃ (calcitriol) by regulating the availability of transmembrane calcium "pumps" and channels on the luminal/apical and basolateral surface membranes of duodenal enterocytes.^{2,22} Calcitriol acting through the vitamin D receptor (VDR) increases duodenal expression of calcium transporter protein-1 (encoded by transient receptor potential cation channel 6 [*TRPV6*])—a luminal calcium channel with six transmembrane domains and intracytoplasmic amino and carboxyl terminals and a pore region between the fifth and sixth transmembrane domains that forms a homotetramer or heterotetramer combined with TRPV5; TRPV5 and TRPV6 specifically permit Ca^{2+} permeation at physiological levels of this cation.²³ Calcium is also actively absorbed in the intestinal tract by mechanisms that are independent of the VDR.²⁴ Thus the expression of *TRPV6* is increased by both estrogen and prolactin; prolactin increases transcellular absorption of calcium independently of calcitriol.²⁵ After entering the cytoplasm of the enterocyte, Ca^{2+} travels from its apical to its basolateral membrane either within a lysosomal vesicle bound to vitamin D-dependent calbindin D_{9k} (encoded by *S100G*), a protein with two high-affinity calcium binding sites, or within the cytoplasm linked to calmodulin (encoded by *CALM1*). Calmodulin 1 is a 149 AA protein with an amino-terminal "lobe" linked to a carboxyl-terminal "lobe" that can assume more than 30 three-dimensional conformations.⁹ Each lobe has two globular calcium binding domains consisting of two helix-loop-helix motifs connected by an α -helical linker; calcium binding exposes hydrophobic "pockets" that allow calmodulin to bind to and regulate the activity of target proteins.²⁶ When bound to a target protein, calmodulin can assume an exceedingly large number of conformations. This versatility enables calmodulin to act as a calcium sensor for many different proteins subserving distinct processes within a single cell, including voltage-gated calcium channels and calcium/calmodulin-dependent protein kinases. At the basolateral membrane, Ca^{2+} dissociates from calbindin and calmodulin and is then extruded into the extracellular space across the basolateral membrane through a basolateral Ca^{2+} - Mg^{2+} -dependent ATPase calcium channel (encoded by *ATP2B1*). When calcium intake is adequate or high, the bulk of ingested calcium is passively absorbed through paracellular channels between enterocytes in the jejunum and ileum. Calcitriol regulates synthesis of several tight junction proteins, including claudins-2 and -12, cadherin-17, and aquaporin 8.^{21,22,27}

Intestinal calcium absorption is influenced by vitamin D status, its food source (the bioavailability of calcium in cow milk formulas is 38%, that in human breast milk is 58%); the form of the calcium salt in dietary supplements; and the presence in food of inhibitors of calcium absorption, such as phytates, oxalates, or phosphates (e.g., cola beverages).²⁸ Intestinal calcium absorption is increased during adolescence, pregnancy, and lactation, and depressed in patients with nutritional or functional vitamin D deficiency, chronic renal disease, and hypoparathyroidism. The amount of calcium ingested

influences the net amount of calcium absorbed; the lower the calcium intake, the greater is the efficiency of its absorption. In the adult, when the dietary calcium intake is less than 200 mg/d, fecal calcium excretion exceeds intake; thus active absorption of calcium cannot compensate for very low intake. As dietary calcium increases from 200 to 1000 mg/d, active calcium absorption increases but at a progressively decreasing rate. When dietary calcium exceeds 1000 mg/d, active calcium absorption remains relatively constant at 400 mg/d, but passive absorption of calcium through paracellular channels continues to increase.² Thus hypercalcemia may result from dietary calcium excess as in the milk-alkali syndrome. PTH indirectly increases intestinal calcium absorption by enhancing renal 25-hydroxyvitamin D-1 α hydroxylase activity and hence calcitriol synthesis. Growth hormone (GH) and estrogens also increase intestinal absorption of calcium; glucocorticoids and thyroid hormone inhibit this process. Calcium is excreted into the intestinal tract in the ileum and in pancreatic and biliary secretions.

Low calcium intake is associated with increased fracture rate in children and adolescents, and therefore adequate dietary intake of calcium during infancy, childhood, and adolescence is necessary to attain a peak bone mass that may lessen the risk of fracture and the development of osteoporosis.²⁹ To ensure optimal mineralization of the developing skeleton, age-related dietary intakes of elemental calcium for infants, children, and adolescents have been recommended (Table 9.2).^{30,31} As a nutrient essential for bone mineralization, intake of calcium is optimal when skeletal calcium content is maximal for the age and gender of the subject.³ In normally weighted adolescents, a calcium intake of or above 1600 mg per day results in maximal calcium retention (approximately 35%).³² Calcium retention and bone mass increase as body mass index (BMI) rises when calcium intake is sufficient. The retention of oral calcium is dependent not only on the level of nutrient intake but also upon gender and race: white adolescent males retain more calcium than do white girls (37% vs. 30%); black adolescent girls retain more calcium than do white females (38% vs. 30%). Besides dietary intake of calcium and vitamin D status, the most significant modifiable determinant of bone mineralization is weight-bearing physical activity.³³

Calcium is excreted primarily by the kidney. After the ultrafiltrable portion (ionized, complexed, chelated) of plasma calcium crosses the renal glomerular basement membrane, more than 98% of filtered calcium is reabsorbed—primarily in the proximal convoluted tubule (60%) but also in the thick

ascending limb of the loop of Henle (TALH) (20%), distal convoluted tubule (10%), connecting tubule (3%), and collecting duct (10%).¹ In the proximal convoluted tubule, the bulk of calcium is passively reabsorbed through paracellular channels; a small amount of calcium is reabsorbed actively, a process regulated by both PTH and calcitonin. In the cortical TALH, resorption of filtered calcium is regulated in part through the influence of Na⁺-K⁺-2Cl⁻ ion channels and the CaSR upon the permeability of paracellular channels to calcium. Active transcellular movement of calcium in the TALH is stimulated by PTH and calcitonin.^{1,34} In the distal convoluted renal tubule, active transcellular reabsorption of 5% to 10% of filtered calcium occurs through a calcium channel encoded by *TRPV5* that is expressed on the apical membrane of renal tubular epithelial cells, a process regulated by calcitriol and PTH.²³ In the cytoplasm, calbindin D_{9k} then shuttles Ca²⁺ across the cell for extrusion by ATP2B1 channels and the Na⁺-Ca²⁺ exchanger type 1 (encoded by *SLC8A1*) on the basolateral membrane of the renal tubular cell. Calcitriol, PTH, low dietary calcium, estrogens and androgens, and acid/base balance increase expression of *TRPV5* and, thus renal tubular reabsorption of calcium. PTH also enhances posttranslational phosphorylation of TRPV5 and hence its movement to and insertion into the apical membrane of the renal tubular cell and inhibits its endocytosis, the paired effects thus resulting in increased numbers of TRPV5 channels on the apical surface of the renal tubular cell.²³ FGF23 and α kltho (encoded by *KL*), factors essential for normal phosphate homeostasis, increase the intracellular retention and hence decrease the abundance and hence the activity of the TRPV5 calcium channel on the apical surface of the renal tubular cell.^{22,35,36} Calmodulin, an intracellular Ca²⁺-binding protein that also binds to and inhibits the action of TRPV5, modulates renal tubular reabsorption of Ca²⁺, a process reversed by PTH-mediated phosphorylation of the TRPV5 calbindin-binding site.³⁷

In the TALH, calcium and magnesium are reabsorbed through voltage-driven paracellular channels (in part through paracellin-1, a tight junction protein channel). Paracellular transport of solute involves passive movement of ions along channels in the tight junctions between adjacent cells that are in complete contact with one another. Claudins are proteins present in the plasma membranes of the adjacent cells of the TALH that have formed tight junctions; claudins have four extracellular domains that associate with one another and selectively influence paracellular solute permeability and movement.³⁸ Claudins-14, -16, and -19 (encoded by *CLDN14*, -16, -19, respectively) are expressed in the tight junctions of the cells lining the TALH; the expression of *CLDN14* is partially regulated by the CaSR.^{1,34} Cells of the TALH also express *CASR* on their basolateral membrane; when activated by peritubular Ca²⁺, the CaSR decreases renal tubular reabsorption of calcium through the paracellular channels by inhibiting activity of the Na⁺-K⁺-2Cl⁻ transporter and lowering lumen-positive voltage. Increased glomerular filtration and/or decreased renal tubular reabsorption increase renal excretion of calcium, phosphate, and magnesium. Urinary excretion of calcium is augmented by increased dietary intake, hypercalcemia of diverse pathophysiology (with the exception of that associated with familial hypocalcemic hypercalciuria), expansion of extracellular volume, metabolic acidosis, and loop diuretics (e.g., furosemide).² PTH and PTHrP increase renal tubular reabsorption of Ca²⁺, whereas glucocorticoids, mineralocorticoids, and Ca²⁺ itself suppress Ca²⁺ reabsorption (see Fig. 9.1).

Plasma Ca²⁺ concentrations are monitored by the CaSR, a 1078 AA, seven transmembrane GPCR encoded by *CASR* whose extracellular domain recognizes and binds Ca²⁺,

TABLE 9.2 Recommended Dietary Intakes of Calcium and Vitamin D in Infants, Children, and Adolescents

Age (y)	Calcium (mg/day)	Calcium (Upper Limit)	Vitamin D (IU/day)	Minimum [25(OH)D] (ng/mL)
<0.5	200	1000	400	20
0.5–1	260	1500	400	20
1–3	700	2500	600	20
4–8	1000	2500	600	20
9–13	1300	3000	600	20
14–18	1300	3000	600	20
Pregnant or lactating	1300	3000	600	20

Amounts of calcium and vitamin D are the Recommended Dietary Allowances (RDA) for $\geq 97.5\%$ of population. (Modified from Golden, N.H., Abrams, S.A., and Committee on Nutrition. (2014). *Pediatrics*, 134, e1229–e1243.)

Mg²⁺, gadolinium, aromatic AA, antibiotics, and other compounds.^{39,40} The CaSR is expressed on the cell's plasma membrane as a homodimer linked by disulfide bonds between extracellular domain cysteine residues 129 and 131 of each CaSR; its extracellular domain of 612 AA binds Ca²⁺.³⁴ Homodimerization of the CaSR takes place in the endoplasmic reticulum after core N-linked glycosylation; homodimeric CaSR is then packaged within the Golgi apparatus where it is further glycosylated and transported to the cell membrane.⁴¹ The amino-terminal extracellular domain of the CaSR is composed of 612 AA with five Ca²⁺ binding sites constructed in a "Venus flytrap" configuration; there are 250 AA in the transmembrane domain composed of seven transmembrane helices and three alternating extracellular and intracellular loops, and 216 AA in the carboxyl-terminal intracellular domain.⁴⁰ The second extracellular loop contains sites (Asp758, Glu759, Glu767) that modulate the "sensitivity" of the CaSR to Ca²⁺. Within the intracellular domain of the CaSR are three protein kinase C phosphorylation sites; phosphorylation of Thr888 suppresses Ca²⁺-mediated CaSR intracellular signal transduction.³⁹ Through its binding of Ca²⁺, the CaSR finely regulates the extracellular concentration of this cation by modulating the secretion of PTH and the renal tubular reabsorption of filtered calcium. The CaSR is a member of the C family of GPCRs with extremely long extracellular domains (500–600 AA residues) and homologies with receptors that bind glutamate, γ -amino butyric acid (GABA), and pheromones, as well as with the taste receptors. The very long extracellular domain is extensively glycosylated, a posttranslational modification essential for efficient movement of the receptor to the cell surface. The CaSR is located on the plasma membrane of parathyroid chief cells, at the apical or basolateral membranes of most renal tubular cells—particularly those in the TALH and the collecting ducts, on the cell membranes of the calcitonin-synthesizing parafollicular C cells of the thyroid gland, in cartilage and bone, lungs, adrenal glands, breast, intestines, skin, lens, placental cytotrophoblasts, and nervous tissue.⁴² Expression of CaSR is regulated in part by glial cell missing homolog 2 (*GCM2*), encoding a transcription factor essential for formation of the parathyroid glands, and enhanced by calcitriol, high Ca²⁺ levels, and interleukin-1 β .⁴³ Insertion of the CaSR into the cell's plasma membrane is mediated by an agonist-driven insertional pathway antagonized by the cooperative interaction of β -arrestin 1 (encoded by *ARRB1*) and an adaptor-related protein complex 2 (*AP2S1*), the latter complex facilitating retrograde movement (endocytosis) of the CaSR from the cell surface and its return to the cytoplasm.^{34,44}

The intracellular signal of the CaSR is transduced by the G α_q and G α_{11} (*GNA11*) family of GPCRs that activates PLC β -1 (encoded by *PLCB1*), that then cleaves phosphatidylinositol 4,5-bisphosphate to diacylglycerol (DAG) and IP₃. In the parathyroid gland, IP₃ mobilizes stored intracellular calcium, whereas DAG activates the mitogen-activating protein kinase (MAPK) signal transduction pathway that leads to decrease in PTH secretion. Activation of the CaSR also stimulates activity of phospholipases A₂ and D and PKB (AKT) and PKC but inhibits that of adenylyl cyclase, the latter through stimulation of adenylyl cyclase-inhibitory G α_i activity.⁴⁰ Increases in plasma Ca²⁺ and rising cytosolic levels of Ca²⁺ within the parathyroid chief cells suppress expression of *PTH* and increase degradation of *PTH* messenger ribonucleic acid (mRNA), thereby limiting synthesis and secretion of *PTH*; CaSR signaling also inhibits proliferation of chief cells. Decline in Ca²⁺ leads to decrease in CaSR activity, lower intracellular Ca²⁺ values, and thence to increased *PTH* secretion, thus enabling the CaSR to exercise minute-to-minute control

over the release of *PTH* and hence of the plasma concentration of Ca²⁺.

The serum Ca²⁺ concentration is related to polymorphic variants of the CaSR; 70% of individuals are homozygous for alanine at AA 986 within the intracellular domain, 3% are homozygous for serine, and the remainder are heterozygous for the two amino acids. In heterozygous Arg986Ser and homozygous Ser986 subjects, Ca²⁺ levels are significantly higher than in those who are homozygous for Ala986.⁴⁵ The CaSR also serves as a Mg²⁺ sensor and modulates renal tubular reabsorption of this cation decreasing its reabsorption when Mg²⁺ levels rise. By binding to the CaSR, aromatic L-amino acids appear to "sensitize" the receptor to a given level of Ca²⁺.³⁹

In the kidney, CaSRs are present on the apical (luminal) brush-border membranes of cells in the proximal renal tubule where they are exposed to intratubular Ca²⁺, on basolateral cell membranes in cells in the TALH, and on the apical surface of cells in the distal renal collecting ducts.⁴⁰ Functioning through binding to local CaSRs, increasing peritubular concentrations of Ca²⁺ and Mg²⁺ inhibit renal tubular reabsorption of filtered Ca²⁺ and Mg²⁺, respectively. In the kidney, binding of Ca²⁺ to the CaSR decreases not only transcellular transport of filtered Ca²⁺ but also its paracellular transport in the TALH. Inactivating mutations in *CASR* result in familial hypocalciuric hypercalcemia, whereas gain-of-function mutations in *CASR* are associated with autosomal dominant hypoparathyroidism ("familial hypercalciuric hypocalcemia"). Stimulation of the CaSR suppresses *PTH*-mediated renal tubular Ca²⁺ reabsorption.^{40,46} In addition to effects on renal tubular cation transport, the Ca²⁺/CaSR complex inhibits antidiuretic hormone-induced renal tubular permeability to water by decreasing the number of apical aquaporin-2 water channels in the inner medullary collecting ducts, thus leading to polyuria. Stimulation of the CaSR expressed in cells of the renal distal collecting ducts also increases local H⁺-ATPase activity, resulting in urine acidification.⁴⁶

Acting through the CaSR, increasing serum Ca²⁺ concentrations stimulate release of calcitonin from thyroid C cells. The CaSR is expressed throughout the intestinal tract, where it may modulate the changes in intestinal motility that accompany low (increased) and high (depressed) serum Ca²⁺ values.³⁹ In addition, in gastric cells, stimulation of CaSR activity increases release of gastrin into the circulation and thereby intragastric acidity.⁴⁰ Expression of the CaSR in the brain suggests a mechanism whereby Ca²⁺ may influence neural function locally by modulating neurotransmitter and neuroreceptor function. The CaSR is present on cell membranes of osteoblasts, osteocytes, and osteoclasts, and agonist (Ca²⁺, neomycin, gadolinium) activation of the CaSR stimulates intracellular signal transduction in these cells. Increase in CaSR activity in osteoblasts enhances their proliferation.⁴⁰ The CaSR may mediate recruitment of osteoblast precursor cells to sites of high Ca²⁺ levels, the residue of local osteoclast activity, one of the factors linking the bone remodeling processes of resorption and formation.⁴²

Calcimimetics are agonist drugs that activate the CaSR; calcilytics are antagonists of the CaSR.⁴⁷ The CaSR binds and responds not only to Ca²⁺ but also to Mg²⁺, selected L-amino acids, and some antibiotics; the latter are designated type I calcimimetics. Synthetic compounds (e.g., phenylalkylamines) that bind to the second and third extracellular loops of the transmembrane domain of the CaSR and allosterically modulate (increase or decrease) the sensitivity of the CaSR to ambient Ca²⁺ are designated type II calcimimetic agonists or calcilytic antagonists, respectively.^{39,40} A widely used calcimimetic is cinacalcet

[N-[1-(R)-(-)-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl]-1-aminopropane hydrochloride]; this agent has been effective in decreasing secretion of PTH in patients with either primary or secondary hyperparathyroidism.⁴⁸ Calcilytics decrease the sensitivity of the CaSR to Ca^{2+} and thus increase the secretion of PTH and depress renal tubular reabsorption of Ca^{2+} . Variants of CASR may prevent its normal biosynthesis (class I), disturb movement of the CaSR protein to the plasma membrane (class II), interfere with binding of ligand to the CaSR (class III), impair activation of the CaSR (class IV), or disrupt receptor function by a currently unknown mechanism (class V).

PHOSPHATE

Second to calcium in abundance, phosphorus is present in humans in organic forms associated with fats, sugars, and proteins and in inorganic forms complexed to sodium, calcium, or magnesium.^{49,50} It is an essential component of deoxyribonucleic acid (DNA) and RNA nucleotides, critical for generation, storage, and utilization of energy as ATP, and irreplaceable as a part of cell membranes, signal transduction pathways, and bone mineral. Serum concentrations of phosphate reflect the balance between the amount of this anion ingested into and absorbed by the duodenum and jejunum of the gastrointestinal tract; the quantity filtered through the renal glomerulus and subsequently reabsorbed by the proximal renal tubule or excreted in urine; its movement between intracellular and extracellular spaces; and the intraosseous interactive dynamics of calcium, phosphate, and the formation and resorption of hydroxyapatite.

Some 85% of body phosphate is deposited in bone as hydroxyapatite. Some 14% of phosphate is intracellular (in the cytosol or mitochondria in the form of inorganic phosphate esters or salts, cell and organelle membrane phospholipids, and phosphorylated metabolic intermediate compounds involved in energy metabolism and formation of ATP and in signal transduction and in the cytosol and nucleus as an essential component of RNA and DNA) or in interstitial fluid or serum (approximately 1%). In serum, phosphate circulates as free orthophosphate anions HPO_4^{2-} and H_2PO_4^- (55%), bound to proteins (10%), or complexed to calcium, magnesium, or sodium (35%).^{2,51} At pH 7.4, serum HPO_4^{2-} and H_2PO_4^- are present in a molar ratio of 4:1; in alkalotic states the ratio increases, with acidosis it declines (at pH 7.4, 1 mmol/L of orthophosphate = 1.8 mEq/L = 3.1 mg/dL). The serum concentrations of calcium and phosphate are reciprocally related under normal circumstances, and the calcium \times phosphate product approximates 30. Intracellularly, cytosolic free phosphate concentrations approximate those in serum—3 to 6 mg/dL.

The serum phosphate concentration is determined by oral intake, intestinal absorption and excretion, renal tubular reabsorption and excretion of filtered phosphate, and release from bone by dissolution of hydroxyapatite by PTH and calcitriol, and fluctuates with age, gender, growth rate, diet, and serum calcium levels (see Fig. 9.2).^{1,2,50,52-54} In as much as phosphate is found in all cells and foods, dietary deficiency is unusual. Dietary phosphate is absorbed across the intestinal brush border as HPO_4^{2-} in direct proportion to its intake, principally in the duodenum and jejunum. Phosphate is absorbed through the apical membranes of cells lining the small intestine and proximal renal tubule by both passive and active transport pathways.^{49,50} Paracellular transport is a passive mode of phosphate movement from lumen to the extracellular space through the tight junctions between the sides of the epithelial cells that line the small intestine and proximal renal tubule. Phosphate is actively transported across the apical membranes of epithelial

cells lining the small intestine through the sodium/phosphate channel designated NPT2b encoded by *SLC34A2* (one of three type II sodium/phosphate cotransporters), the sodium-hydrogen exchanger 3 (NHE3 encoded by *SLC9A3*), and the sodium-dependent phosphate transporter encoded by *SLC20A2*—members of the type III sodium/phosphate cotransporter family. Expression of *SLC34A2* is increased by low dietary phosphate intake and calcitriol and suppressed by PTH. However, PTH also increases intestinal absorption of ingested phosphate (and calcium) by increasing expression of *CYP27B1* encoding renal 25-hydroxyvitamin D-1 α -hydroxylase, thereby enhancing synthesis of calcitriol, whereas *FGF23* has the opposite effect. When dietary phosphate intake falls below 310 mg/d in the adult, net phosphate absorption is negative. At low phosphate intakes, absorption is active in the duodenum, jejunum, and distal ileum, whereas at high intakes, 60% to 80% of ingested phosphate is absorbed primarily by diffusion. Phosphate absorption can be impaired by its intraluminal precipitation as an aluminum or calcium salt and by intestinal malabsorption disorders.

The kidney regulates moment-to-moment phosphate homeostasis. After passage through the renal glomerular membrane, 85% of filtered phosphate is reabsorbed by active transport against an electrochemical gradient across the apical membrane of the cells lining the proximal renal tubule through the type II sodium/phosphate cotransporters encoded by *SLC34A1* (NPT2a) and *SLC34A3* (NPT2c) with the aid of a Na^+ , K^+ -ATPase pump.^{2,51,55} Phosphate is also reabsorbed to a limited extent in the loop of Henle (10%), distal convoluted tubule (3%), and connecting tubule (2%). Expression of *SLC34A1* is increased by hypophosphatemia and suppressed by hyperphosphatemia. PTH, PTHrP, FGF23, and glucocorticoids decrease proximal renal tubular reabsorption of filtered phosphate by lowering expression of *SLC34A1* (NPT2a) and *SLC34A3* (NPT2c).^{49,56} In the mouse, NPT2a actively transports approximately 70% and NPT2c 30% of proximal renal tubular reabsorbed phosphate; in humans, however, NPT2c may be the major renal tubular transporter of phosphate. The Na^+ - H^+ exchanger regulatory factor 1 (NHERF1—encoded by *SLC9A3R1*) is a scaffolding protein that is essential for trafficking of NPT2a and NPT2c to the luminal membrane of cells in the proximal renal tubule; NHERF1 also interacts with PTH1R.⁵⁵ The family of phosphate transporters encoded by *SLC20A2* (designated PiT2) has also been identified in the renal proximal tubule. Phosphate exits the proximal renal tubular cell with sodium by cation exchange for potassium through channels (encoded by *XPR1*) located on the cell's basolateral membrane. The maximal tubular reabsorption of phosphate approximates the filtered load. Tubular phosphate reabsorption is increased by low phosphate intake and hypophosphatemia (because of decrease in filtered load), hypercalcemia (by decrease in the glomerular filtration rate), depletion of ECF volume, and metabolic alkalosis. Renal tubular reabsorption of phosphate is depressed by high phosphate intake, PTH (and PTHrP), and the phosphatonin FGF23—and is caused by rapid internalization and subsequent destruction of the NPT2a and NPT2c phosphate cotransporter proteins.⁵¹ The PTH-dependent degradative processes are mediated through the type 1 PTH/PTHrP receptor and transduced by intracellular signaling systems utilizing protein kinase A, protein kinase C, and MAPK. Phosphate is deposited in bone as hydroxyapatite dependent on local levels of calcium, phosphate and alkaline phosphatase activity and reabsorbed by osteoclasts whose activity is stimulated by PTH, calcitriol, and other osteoclast activating factors. There are age-related variations in serum concentrations (mg/dL) of phosphate: less than 5 days, 4.8–8.2;

1–3 years, 3.8–6.5; 4–11 years, 3.7–5.6; 12–15 years, 2.9–5.4; 16–19 years, 2.7–4.7; adult, 2.5–4.5.

Phosphatonins

The phosphatonins are a group of proteins that inhibit renal tubular reabsorption of phosphate. Increasing serum concentrations of phosphate and calcitriol stimulate osteocyte/osteoblast production of the predominant phosphatonin—FGF23—that then depresses synthesis of the phosphate cotransporters NPT2a and NPT2c and decreases renal expression of *CYP27B1* (encoding 25-hydroxyvitamin D-1 α -hydroxylase) and thus formation of calcitriol and consequently, intestinal absorption of calcium and phosphate. Simultaneously, FGF23 enhances expression of renal *CYP24A1* (encoding 25-hydroxyvitamin D 24-hydroxylase) and hence the synthesis and urinary excretion of water-soluble 24,25-dihydroxyvitamin D and 1,24,25-trihydroxyvitamin D.⁴⁹ FGF23 is generated as a 251 AA protein with a 24 AA signal sequence; for optimal biological function FGF23 must be posttranslationally glycosylated by UDP-N-acetyl-alpha-D-galactosamine:Polypeptide N-acetylgalactosyltransferase 3 (encoded by *GALNT3*). *GALNT3* is an enzyme that O-glycosylates the threonine-178 and serine-180 residues of FGF23; failure to glycosylate the threonine-178 site leads to accelerated degradation (and hence inactivation) of FGF23. FGF23 is proteolytically degraded by intracellular subtilisin-like proprotein convertase acting between AA 179 and 180 (... Arg₁₇₆-His₁₇₇-Thr₁₇₈-Arg₁₇₉-Ser₁₈₀ ...) that separates the amino-terminal FGF-like domain from the carboxyl-terminal tail. Spontaneously occurring gain-of-function mutations within this sequence of *FGF23* slow the inactivation of FGF23, thus prolonging its biological life, resulting in autosomal dominant hypophosphatemic rickets (ADHR). *FGF23* is expressed primarily by osteoprogenitor cells, osteoblasts, and osteocytes and to a lesser extent by the thymus, brain, thyroid, parathyroid glands, cardiac muscle, liver, and small intestines. In osteoblasts and osteocytes, expression of *FGF23* is stimulated by high serum levels or dietary intake of phosphate, PTH, and calcitriol acting through VDR.^{51,57,58} Inasmuch as calcitriol promotes synthesis of FGF23 whereas FGF23 inhibits production of calcitriol, there is a reciprocal feedback relationship between calcitriol and FGF23.

FGF23 links to the “c” isoform of tyrosine kinase FGF receptors (FGFR) 1, 2, and 3. The genes encoding the FGFRs consist of 19 exons that may be alternatively spliced to include or to exclude either exon 8 or 9 (encoding the third extracellular immunoglobulin-like domain of the FGFR which partially specifies the ligand bound by the receptor); when exon 8 is included in the mRNA transcript the “b” isoform of the FGFR is formed; when exon 9 is included in the transcript, the “c” isoform is produced. FGF23 acts through the dimeric coreceptor quaternary complex—FGF23/ α Klotho/FGFR1 (IIIc)/heparan sulfate—expressed on the plasma membrane of the target cell to depress renal tubular reabsorption of phosphate, intestinal absorption of phosphate, synthesis of calcitriol, and secretion of PTH.^{49,59,60} Although it is likely that FGF23 binds to multiple FGFR “c” isoforms, it is the multifunctional transmembrane protein α Klotho (encoded by *KL*) that serves as a coreceptor, thereby converting FGFR into specific FGF23 receptors in the parathyroid glands and kidneys.^{54,55,61}

α Klotho is a 1012 AA protein (encoded by *KL* whose expression is increased by calcitriol) with extracellular, transmembrane, and intracellular domains synthesized by renal tubular cells that serves as a coreceptor with FGFR1(cIII) for FGF23.⁶² Klotho was so named because its loss in mice is

associated with a phenotype of premature aging. There are membrane bound and soluble secreted forms of α Klotho; soluble α Klotho is a 549 AA protein composed of the ectodomain of the intact protein and is present in the circulation and excreted by the kidney.⁵⁹ The transmembrane form of α Klotho binds FGF23, FGFR1(IIIc), and heparan sulfate to form a quaternary complex that transmits the FGF23 signals that decrease renal tubular cell membrane expression of the genes encoding the phosphate transporters NPT2a (*SLC34A1*) and NPT2c (*SLC34A3*), thereby limiting renal tubular reabsorption of filtered phosphate and increasing its urinary excretion. The complex of FGF23/FGFR3,4/ α Klotho/heparan sulfate depresses transcription of renal *CYP27B1* encoding 25-hydroxyvitamin D-1 α hydroxylase necessary for synthesis of calcitriol while enhancing expression of *CYP24A1* encoding vitamin D-24 hydroxylase, thereby increasing the solubility and renal excretion of calcitriol and lowering its serum levels.^{59,60,62,63}

KL is also expressed in the parathyroid gland; there, FGF23 interacting with α Klotho and FGFR3,4 decreases transcription of *PTH* and thereby reduces PTH synthesis and secretion.⁶² Reciprocally, both calcitriol and PTH increase synthesis of FGF23 by bone cells, establishing a feedback loop regulating FGF23 synthesis and secretion.⁶⁴ After binding of the FGF ligand to the FGFR, the receptors dimerize, autophosphorylate, and then phosphorylate intracellular proteins involved in signal transduction, including PLC γ , growth factor receptor bound (GRB)-14, son of sevenless, FGF receptor substrate (FRS)-2—a docking protein that links the activated FGFR to the phosphoinositide-3-kinase (PI3K), and MAPK signaling pathways.⁶⁵ *FGF23* expression is increased by iron deficiency by interrupting the negative feedback effect of hypophosphatemia upon FGF23 synthesis.⁵⁶ The truncated, alternatively spliced form of α Klotho with 549 AA is secreted into the circulation, cerebrospinal fluid, and urine.⁶³ Soluble α Klotho regulates signal transduction by growth factors (insulin, insulin-like growth factor-1) independently of FGF23 through binding to sialogangliosides—components of intramembraneous lipid rafts. Soluble α Klotho enhances renal calcium absorption by cells lining the renal distal convoluted and connecting tubules by increasing the number of calcium transport channels encoded by *TRPV5* expressed on the apical membrane of these cells.⁶³

Serum values of FGF23 are increased in patients with X-linked hypophosphatemic rickets, autosomal recessive and dominant hypophosphatemic rickets, tumor-induced osteomalacia, and fibrous dysplasia associated with hypophosphatemia.⁶⁶ In patients with autosomal dominant hypophosphatemic rickets, gain-of-function mutations (e.g., Arg179Trp) in *FGF23* result in resistance to degradation of FGF23, a protein that is normally cleaved between Arg179 and Ser180, whereas in subjects with tumor induced osteomalacia, the rate of production of FGF23 is greatly increased. In familial tumoral calcinosis (OMIM 211900), loss-of-function mutations in *FGF23* lead to accelerated intracellular degradation of FGF23 that prevents secretion of intact protein resulting in decreased renal excretion of phosphate, hyperphosphatemia, relatively increased calcitriol levels, and diffuse ectopic calcification.^{54,67}

Phosphatonins were first identified by analysis of tumors associated with hypophosphatemic osteomalacia.^{54,68} Among other phosphatonins are matrix extracellular phosphoglycoprotein (encoded by *MEPE*) and secreted frizzled-related protein 4 (encoded by *SFRP4*).⁶⁹ *MEPE* is expressed by osteoblasts, osteocytes, and odontoblasts. Expression of *MEPE* is inhibited by calcitriol. *MEPE* is a 525 AA, 58-kDa protein that is a member of the short-integrin-binding, ligand-interacting

glycoprotein family whose genes are clustered on chromosome 4q22.1. MEPE modulates osteoblast and osteoclast function and may both inhibit and support bone mineralization.⁵⁴ MEPE enhances phosphaturia by increasing expression of *FGF23*.⁶⁴ MEPE (and other phosphatonins) contains within its structure an acidic serine aspartate-rich MEPE-associated (ASARM) peptide that may be released from its parent structure by cathepsins B and K in phosphorylated or nonphosphorylated forms. Phosphorylated ASARM and MEPE bind avidly to hydroxyapatite and prevent further deposition of calcium and phosphate; ASARM also inhibits phosphate uptake by the intestinal tract and the renal tubule (MEPE also is involved in the osteocytic response to a mechanical load). MEPE and ASARM are substrates for the X-linked phosphate-regulating endopeptidase (encoded by *PHEX*); inactivating variants of *PHEX* result in X-linked hypophosphatemic rickets.^{64,70}

Other SIBLING noncollagenous matrix proteins include dentin-matrix acidic phosphoprotein 1 (encoded by *DMP1*), dentin sialo-phosphoprotein (*DSPP*), osteopontin (also termed *secreted phosphoprotein 1* [SPP1]), and integrin-binding sialoprotein (*IBSP*).⁶⁴ In addition to sharing the ASARM sequence near their carboxyl terminals, these proteins have in common an Arg-Gly-Asp (RGD) tripeptide motif that interacts with and binds to integrins on the surface of interacting cells. The basic ASARM motif consists of 23 AA; several of its serine residues are specifically phosphorylated by casein serine kinase. Phosphorylated and nonphosphorylated ASARMS released by *DMP1*, *DSPP*, and *SPP1* are also resistant to proteolysis, except by the zinc metalloendopeptidase *PHEX*. Inactivating mutations of *PHEX* results in X-linked hypophosphatemic rickets, in part, by inability to degrade the ASARM peptide coating of hydroxyapatite.⁶⁴ *PHEX* may also be involved in the expression of *FGF23* and the stability of *FGF23*. Carboxyl to the ASARM motif of *DMP1* is an additional sequence of 35 AA that is termed the *minfostin* motif; a mutation in this region of *DMP1* results in one form of autosomal recessive hypophosphatemic rickets. The ASARM region of *DMP1* binds to *PHEX* sited on the plasma membrane of the normal osteocyte suppressing expression of *PHEX*; displacement of binding by excessive free ASARM peptides (as in patients with hypophosphatemic rickets) further increases *FGF23* expression.⁶⁴ In addition to patients with tumor-induced osteomalacia and X-linked hypophosphatemic rickets, increased serum concentrations of *FGF23* are found in subjects with autosomal dominant hypophosphatemic rickets because of activating mutations in *FGF23* and autosomal recessive hypophosphatemic rickets types 1 and 2 because of loss-of-function mutations in *DMP1* or *ENPP1*, respectively.^{52,54}

Secreted Frizzled-related protein 4 (*FRP4*) is a 346 AA glycosylated protein that shares the structure of the extracellular domain of the transmembrane Frizzled GPCR. The natural ligands of Frizzled receptors are wingless (WNT) proteins, and their coreceptors are cell-surface low-density lipoprotein receptor-related proteins; these heterotrimeric complexes stabilize intracellular β -catenin and the attendant signal transduction systems that are essential for many transformative processes including cartilage and bone formation. Secreted *FRP4* serves as a “decoy” receptor for WNT proteins and thereby competes with the Frizzled receptor for WNT binding and by so doing inhibits the function of the Frizzled receptor. *FRP4* is expressed in bone cells and in large amounts by tumors with associated osteomalacia. *FRP4* inhibits sodium-dependent renal tubular phosphate reabsorption by inhibition of WNT signaling leading to hypophosphatemia; it also reduces expression of *CYP27B1*, the gene encoding 25-hydroxyvitamin D₃-1 α -hydroxylase.⁷¹

Immunoreactive species of *FGF23* are measurable in normal adult sera but values vary depending on the epitopes recognized by the antibodies used, that is, whether they are specific for the full-length *FGF23* protein or only for its carboxyl-terminal fragment, and on the assay methodology (e.g., enzyme-linked immunosorbent assay or chemiluminescent immunoassay).⁷²⁻⁷⁴ In assays that recognize full-length *FGF23* and carboxyl-terminal fragments of *FGF23*, intact *FGF23* values vary diurnally and there is marked interindividual variability in *FGF23* levels.⁷³ The serum *FGF23* concentration is inversely related to that of phosphate, and values rise when dietary phosphate increases (or during infusion of phosphate) and decline with phosphate restriction.⁵⁴

MAGNESIUM

Magnesium (Mg^{2+}) is the fourth most abundant of the body cations. It is an intracellular and extracellular divalent cation that is critical for DNA and protein synthesis, neuroexcitability, intracellular signaling, oxidative phosphorylation, and bone hydroxyapatite development.¹ In serum, magnesium is present in the bound and free states. Approximately 1% of body magnesium is present in the ECF compartment, 14% in muscle and soft tissue, and 85% in bone, where it is found on the surface of the hydroxyapatite crystal and of which 50% is freely exchangeable.^{2,4,75} In blood, magnesium (1.7–2.4 mg/dL = 0.7–1.0 mmol/L) is partially bound to proteins (approximately 30%), complexed to phosphate and other anions (15%), and free Mg^{2+} (55%). As with calcium, magnesium levels rise as pH declines (with increased acidity). Intracellularly, magnesium (0.5 mmol/L) is bound to ATP and other molecules; 10% is in the ionic form and 60% is within mitochondria. Mg^{2+} is a cofactor in many enzymatic reactions, including those that consume or produce ATP. Magnesium alters free radicals and influences nitric oxide synthase, cyclic guanosine monophosphate generation, endothelin production, and immune function. Magnesium decreases membrane excitability in nerve and muscle cells and blocks the excitatory N-methyl D-aspartate receptor. It is a necessary cofactor for the regulation of neuromuscular excitability, nerve conduction, oxidative metabolism by mitochondria, glycolysis, phosphorylation, and transcription and translation of DNA. It is essential for the secretion (but not the synthesis) of PTH by the parathyroid chief cell.

Net intestinal magnesium absorption is directly related to dietary intake and independent of calcitriol.² The bulk of ingested magnesium is absorbed in the small intestine (and colon) in proportion to the intestinal luminal concentration of this cation through voltage-dependent paracellular channels within tight intercellular junctions, a process assisted by claudins. There is a small component of active, transcellular intestinal magnesium absorption through ion channels encoded by transient receptor potential cation channel, subfamily M, member 6 (*TRPM6*) linked to the transcript of *TRPM7*, a magnesium-permeable cation channel. The *TRPM6/TRPM7* heterooligomer is expressed on the surface of cells lining the intestinal tract.^{23,76} Loss-of-function mutations in *TRPM6* result in autosomal recessive hypomagnesemia with secondary hypocalcemia because of impairment of intestinal (and renal tubular) absorption of this cation. The intestinal absorption of magnesium may be impaired by high phosphate intake, intestinal dysfunction, or chronic laxative abuse. Magnesium is also excreted into the intestinal tract.

Some 70% of serum magnesium is ultrafiltrable and passes through the renal glomerular membrane; approximately 95% of filtered magnesium is reabsorbed—15% in the proximal

renal tubule, 70% in the cortical TALH, and 10% in the distal convoluted tubule.^{2,73} Thus 3% to 5% of filtered magnesium is excreted in the urine. In the proximal renal tubule and TALH, magnesium reabsorption is primarily passive through paracellular tight junction channels using claudins 10, 16, and 19. In the proximal renal tubule, reabsorption of water increases intratubular magnesium concentration, thereby facilitating its paracellular reabsorption. Tight junction claudin proteins bridge intercellular gaps and regulate the movement of ions across an epithelial sheet. Claudin 10 (encoded by *CLDN10*) is a 226/228 AA protein. Biallelic inactivating mutations in *CLDN10* result in the HELIX syndrome (OMIM 617671) of anhidrosis, alacrima, xerostomia, malformed teeth, hypermagnesemia, renal wasting of sodium and chloride, and secondary hyperparathyroidism. Paracellin-1 (encoded by *CLDN16*) is a 305 AA protein expressed in the renal cortical TALH and distal convoluted tubule; paracellin-1 has four transmembrane domains with intracellular carboxyl and amino terminals. Paracellin-1 is also employed for paracellular reabsorption of calcium in the TALH. Homozygous or compound heterozygous loss-of-function mutations of *CLDN16* lead to familial, autosomal recessive renal hypomagnesemia (type 3) characterized by renal wastage of magnesium in association with hypercalciuria and nephrocalcinosis (OMIM 248250).⁷⁷ Claudin 19 is a 224 AA tight junction protein. Biallelic inactivating mutations in *CLDN19* result in renal wasting of magnesium, hypomagnesemia, hypercalciuria, and nephrocalcinosis, leading to renal failure (hypomagnesemia type 5—OMIM 248190); in addition, because *CLDN19* is expressed in the eye, affected subjects have decreased vision because of retinal malformation.

In the TALH, magnesium reabsorption occurs through a paracellular pathway that is impermeable to water and is propelled by a transepithelial voltage gradient that is lumen-positive and generated by a $\text{Na}^+\text{-K}^+\text{-ATPase}$.⁶⁷ The voltage gradient requires the integrated action of a $\text{Na}^+\text{-K}^+\text{-Cl}^-$ -coupled cotransporter (NKCC2 encoded by *SLC12A1*), a Cl^- channel (CLC-Kb encoded by *CLCNKB*), and a K^+ channel (ROMK encoded by *KCNJ1*). Barttin, encoded by *BSND*, is an essential beta subunit of the CLCNKB Cl^- channel. Active transcellular movement of magnesium through the TALH is accomplished utilizing the NKCC2 cotransporter, a pathway inhibited by activation of the CaSR.¹ In the renal distal convoluted tubule, magnesium is actively reabsorbed through a channel composed of a heterooligomer of TRPM6 linked to TRPM7 expressed on the surface of cells lining this renal segment.^{23,75,78} It is primarily in the distal convoluted tubule that magnesium is actively transported through apical (luminal) membrane TRPM6 channels. The renal tubular expression of *TRPM6* and intracellular movement of TRPM6 is regulated by dietary magnesium, estrogens, acid-base balance, and proepidermal growth factor (pro-EGF). *TRPM7* is also expressed on the luminal surface of renal cells in the distal convoluted tubule where it too facilitates reabsorption of magnesium.⁷⁸ Other magnesiotropic proteins expressed in the distal convoluted tubule are the thiazide sensitive $\text{Na}^+\text{:Cl}^-$ cotransporter, potassium channels Kv1.1 and Kir4.1, and hepatocyte nuclear factor 1B (HNF1B). Biallelic inactivating mutations in *TRPM6* result in hypomagnesemia type 1 with secondary hypocalcemia (OMIM 602014); this disorder presents in early infancy with tetany and seizures and is caused by both impaired absorption of intestinal magnesium and reabsorption of renal tubular magnesium. Pro-EGF regulates the availability and activity of TRPM6 by enabling its movement from intracellular sites of storage to the luminal plasma membrane of renal distal convoluted tubular cells and colonic epithelial cells; biallelic loss of *EGF* leads to hypomagnesemia type 4 (OMIM 611718). Loss of *SLC12A3* and its encoded product—the thiazide sensitive $\text{Na}^+\text{:Cl}^-$

cotransporter—leads to reduced TRPM6 activity or abundance and the Gitelman syndrome (OMIM 263800) of hypomagnesemia, hypocalciuria, and hypokalemic metabolic alkalosis. Loss of the voltage-gated K^+ channel Kv1.1 (encoded by *KCNA1*) that colocalizes with TRPM6 alters the polarization of the renal epithelial cell of the distal convoluted tubule, a functional abnormality that impairs activity of TRPM6 and hence of magnesium transport resulting in autosomal dominant hypomagnesemia with episodic ataxia, tetany, muscular cramping, and myokymia (involuntary, localized quivering of a few muscle fibers within a muscle) (OMIM 160120). The pathogenesis of hypomagnesemia (OMIM 612780) that results from the loss-of-function mutations in *KCNJ10* encoding the inwardly rectifying K^+ channel—Kir4.1—is similar to that described for loss of *KCNA1*. Inactivating mutations of *KCNJ10* lead not only to hypomagnesemia but are also associated with the SESAME syndrome (seizures, sensory neural deafness, ataxia, mental retardation, electrolyte imbalance; OMIM 612780).

PTH increases magnesium reabsorption in the renal TALH and distal convoluted tubule; hypermagnesemia and hypercalcemia (acting through the CaSR) decrease renal tubular magnesium reabsorption as do expansion of ECF volume, metabolic alkalosis, phosphate depletion, loop diuretics, aminoglycoside antibiotics, and impaired renal function.²

ALKALINE PHOSPHATASE

The alkaline phosphatases are a group of transmembrane glycoproteins tethered to cell plasma membranes that remove phosphate groups from organic substrates; the extracellular (ecto) domain may be released from the cell membrane into the circulation by a phospholipase. There are three tissue-specific alkaline phosphatases (placental, germ cell, intestinal) and a tissue nonspecific alkaline phosphatase (TNSALP) (encoded by *ALPL*), the latter composed of several isoforms with similar protein composition of 507 to 524 AA but differing carbohydrate configurations that are present in the skeleton, teeth, liver, and kidney.⁷⁹ Bone TNSALP is generated by chondroblasts and osteoblasts by alternative processing of *ALPL* utilizing one of two leading exons during transcription and translation, permitting the osteoblast to synthesize and release a specific bone isoform. Functional as a homodimer, TNSALP removes phosphate residues (organically bound to a serine residue) from many phosphoprotein substrates including inorganic pyrophosphate, a substance that inhibits mineralization of bone organic matrix, phosphoethanolamine, and pyridoxyl 5'-phosphate, the precursor of pyridoxine in the absence of which seizures recalcitrant to treatment may develop.

Although TNSALP circulates as a homodimer, in tissue, it is a homotetrameric ectoenzyme (ectophosphatase) located on the cell surface of the osteoblast anchored through its carboxyl terminal to cell membrane phosphatidylinositol-glycan. In bone, alkaline phosphatase: (1) binds to collagen type I and prepares skeletal matrix for mineralization; (2) transports inorganic phosphate and free calcium into the cell; and (3) hydrolyzes organic phosphates, principally pyrophosphate and phosphoethanolamine, thereby increasing the local concentration of phosphate to values that exceed the calcium \times phosphate product encouraging deposition of calcium phosphate as hydroxyapatite on collagen type I fibers. Inactivation of pyrophosphate and other inhibitors of mineralization (e.g., phosphorylated ASARM derived from SIBLING proteins by removing their phosphate moieties by PHEX) permits formation of hydroxyapatite. The hepatic form of TNSALP is formed by alternative splicing of dual exons 1 of *ALPL*. Hepatic alkaline

phosphatase converts pyridoxyl-5'-phosphate (the major circulating form of vitamin B₆) to pyridoxal, a compound essential for normal synthesis of neural GABA, an inhibitory neurotransmitter; without pyridoxal-5'-phosphate central nervous system levels of GABA are low and seizures may occur. Loss-of-function mutations in *ALPL* lead to infantile, childhood, and adult forms of hypophosphatasia. The three genes encoding tissue-specific intestinal, placental, and germ cell alkaline phosphatase isoenzymes are clustered on chromosome 2q34-q37.

PARATHYROID HORMONE; PARATHYROID HORMONE-RELATED PROTEIN; PTH/PTHrP RECEPTORS

Parathyroid Hormone

PTH increases concentrations of plasma Ca²⁺, lowers serum phosphate values, stimulates synthesis of calcitriol, and exerts both anabolic and catabolic effects on bone. The chief cells of four paired parathyroid glands are derived from the endoderm of the dorsal segments of the third (paired inferior glands) and fourth (paired superior glands) pharyngeal pouches; occasionally, there may be a fifth parathyroid gland embedded within the substance of the thyroid gland or in the mediastinum.⁸⁰ The thymus is formed by the endoderm of the third pharyngeal pouch and calcitonin-synthesizing parafollicular C cells of the thyroid by endoderm of the fourth pharyngeal pouch. Transcription factors required for the development of the pharyngeal pouches and differentiation and organization of the parathyroid glands include T-box 1 (encoded by *TBX1*), GATA-binding factor/protein 3 (encoded by *GATA3*), glial cell missing, drosophila, homolog 2 (encoded by *GCM2*), and V-MAF musculoaponeurotic fibrosarcoma oncogene family, protein B (encoded by *MAFB*), a factor that aids in the movement of the parathyroid glands to sites on the periphery of the thyroid gland⁶ (Fig. 9.3). *GATA3*, *GCM2*, and *MAFB* are essential for the expression of *PTH* and synthesis of PTH postnatally.^{6,81} Variants of *GATA3* are associated with the syndrome of hypoparathyroidism, sensorineural deafness, and renal disease (OMIM 146255), whereas mutations in *GCM2* result in either familial hypo- or hyperparathyroidism (OMIM 146200/617413, respectively). In addition, four-and-a-half Lim domains (encoded by *FHL1*) is a gene that is also required for parathyroid gland formation as an inactivating variant of this gene is associated with aplasia of these structures.⁸² *PTH* is composed of three exons; exon 1 is transcribed but not translated; the second and third exons of *PTH* encode the prepro-PTH sequence of 115 AA that is processed to intact 84 AA PTH that is released from

the parathyroid glands in response to declining serum concentrations of Ca²⁺, detected by the CaSR expressed on the plasma membrane of the parathyroid gland chief cell.

PTH is recognized by the heptahelical PTH1R GPCR expressed on the plasma membranes of target tissue cells, including those that line the intestinal tract and renal tubules. In the distal renal tubule and collecting ducts, PTH increases reabsorption of filtered calcium. PTH inhibits reabsorption of filtered phosphate in the proximal renal tubule. PTH also stimulates expression of *CYP27B1* encoding renal 25-hydroxyvitamin D-1-alpha hydroxylase and consequently renal synthesis of calcitriol. In the intestinal tract, calcitriol increases absorption of calcium. PTH stimulates osteoblastogenesis and indirectly osteoclastogenesis, the latter by enhancing osteoblast expression of *TNFSF11* encoding the receptor activator of NF-κB-ligand (RANK-ligand) and suppressing that of *TNFRSF11B* encoding osteoprotegerin, a decoy receptor for RANK-ligand.⁸³ The intracellular message of PTH is transmitted by activation of signal transduction systems, including the cyclic AMP-protein kinase A, calcium-related protein kinase C, and MAPK pathways and those utilizing phospholipases A and C.

The synthesis and secretion of PTH by parathyroid gland chief cells is regulated by Ca²⁺ concentrations acting through the CaSR that either enhance (when Ca²⁺ concentrations are low) or repress (when they are high) transcription of *PTH* and secretion of PTH; Ca²⁺ values also modulate the rate of chief cell proliferation, a response also mediated by the CaSR. Calcimimetic drugs have inhibitory effects on PTH secretion, a property that has made these agents useful in the management of patients with excess secretion of PTH, such as mild hyperparathyroidism and autosomal dominant familial hypocalciuric hypercalcemia. Low serum concentrations of phosphate inhibit transcription of *PTH*, whereas rising serum phosphate values enhance production of PTH not only indirectly by lowering of Ca²⁺ values but also by stabilizing PTH mRNA, permitting greater translation of PTH and by stimulation of chief cell replication.⁸⁰ Calcitriol inhibits transcription of *PTH*, secretion of PTH, and proliferation of chief cells; FGF23 also decreases PTH secretion. In response to acute hypocalcemia PTH stored in secretory vesicles is rapidly released. When hypocalcemia is prolonged, the secretion of PTH¹⁻⁸⁴ is augmented by decrease in its rate of intracellular degradation and increase in the rate of transcription of *PTH*; when hypocalcemia is extended PTH synthesis and secretion are amplified by increase in chief cell number. When Ca²⁺ values rise, the secretion of PTH is decreased acutely by degradation of stored PTH¹⁻⁸⁴ by calcium-responsive proteases within the parathyroid glands; if prolonged, hypercalcemia leads to decrease in expression of *PTH* and to decline in the number of chief cells.⁸⁰

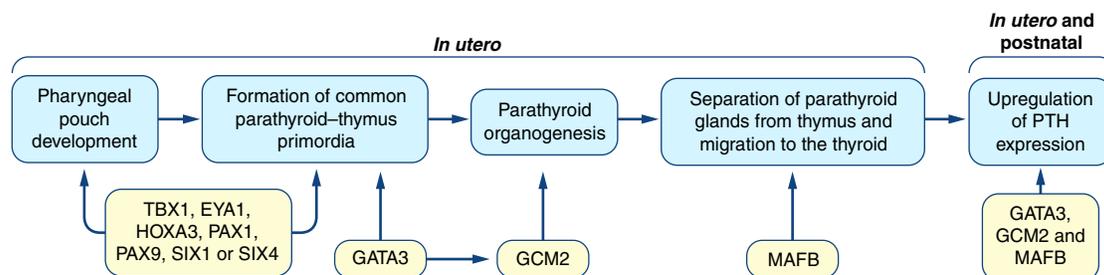


Fig. 9.3 Genetic regulation of the development and function of the parathyroid glands in the mouse. The parathyroid glands develop together with the thymus from the third pharyngeal pouch in mice (whereas in man, the parathyroid glands develop from the third and fourth pharyngeal pouches) under the sequential guidance of multiple transcription factors. *GCM2* is essential for initial differentiation of recognizable parathyroid tissue. GATA-binding protein 3 (*GATA3*), glial cell missing homolog 2 (*GCM2*), and V-Mak musculoaponeurotic fibrosarcoma oncogene family, protein B (*MAFB*) synergistically induce expression of *PTH*. (From Mannstadt, M., Bilezikian, J.P., Thakker, R.V., et al. (2017). Hypoparathyroidism. *Nat Rev Dis Primers*, 3, 17055. With permission).

The amino-terminal 25 AA signal sequence (encoded by exon 2) of 115 AA prepro-PTH is removed by furin, a prohormone convertase, as the polypeptide leaves the endoplasmic reticulum forming the 90 AA pro-PTH (encoded by exon 3) that is then further processed in the Golgi apparatus by furin to mature human 84 AA PTH¹⁻⁸⁴ that is stored in secretory vesicles and granules and from which it is either secreted by an exocytic mechanism or degraded by calcium-sensitive proteases (cathepsins B and H colocalized within the vesicles) to smaller amino and carboxyl-terminal fragments of PTH. PTH degradation represents an important mechanism that regulates the release of intact bioactive PTH¹⁻⁸⁴ and is accelerated when the plasma Ca²⁺ concentration is elevated. The full biological activity of PTH¹⁻⁸⁴ is found in its first 34 AA sequence and all of its skeletal and renal effects (vide infra) are localized within the segment PTH¹⁻³¹. Amino acids numbers 1 and 2 (ser-val) comprise an activation sequence that is essential for bioactivity of the amino-terminal portion of PTH¹⁻⁸⁴. Amino acids 20 to 26 constitute a core sequence necessary for binding to the amino-terminal ectodomain of PTHR1; within this seven AA sequence, Arg²⁰ and Trp²³ are critically important for binding of the PTH ligand to its receptor.⁸⁴ In addition to intact PTH¹⁻⁸⁴, the parathyroid glands secrete a phosphorylated form of PTH¹⁻⁸⁴ and carboxyl-terminal PTH fragments of varying length, but they do not secrete amino-terminal fragments of PTH¹⁻⁸⁴.^{85,86} The half-life of circulating PTH¹⁻⁸⁴ is approximately 2 minutes; it is rapidly metabolized by the liver and excreted by the kidney. In the hepatic Kupffer cells, PTH¹⁻⁸⁴ is cleaved usually either between AA 33 and 34 or between AA 36 and 37 to carboxyl-terminal PTH peptides with half-lives of approximately 15 to 20 minutes. In the kidney, intact and carboxyl-terminal fragments of PTH are filtered by the glomerulus, reabsorbed across the apical membrane of proximal renal tubular cells, and then degraded to smaller fragments. Megalin, a multifunctional low-density lipoprotein-related receptor (encoded by *LRP2*) expressed in coated pits on the luminal/apical surfaces (as well as endocytic vacuoles and lysosomes) of proximal renal tubular cells, specifically recognizes intact PTH¹⁻⁸⁴ and fragments of PTH and mediates the renal tubular endocytosis of PTH¹⁻⁸⁴ that has been filtered through the glomerulus.

The classical functions of PTH¹⁻⁸⁴, as well as its shorter amino peptide derivatives PTH¹⁻³⁴ and PTH¹⁻³¹ upon regulation of calcium and phosphate homeostasis are carried out through the seven transmembrane, G-protein-coupled type 1 PTH/PTHrP receptor sited on cell membranes in the renal tubule and osteoblast. Thus with equal potency PTH¹⁻⁸⁴, PTH¹⁻³⁴, and PTH¹⁻³¹: (1) inhibit renal tubular reabsorption of phosphate, thereby increasing its urinary excretion; (2) increase renal tubular reabsorption of Ca²⁺; (3) augment renal synthesis of calcitriol by enhancing expression of *CYP27B1* (encoding 25-hydroxyvitamin D₃-1 α hydroxylase), thereby amplifying intestinal absorption of calcium; (4) stimulate osteoblast production of RANK-ligand, thus enhancing osteoclastogenesis and osteoclastic reabsorption of calcium; and (5) mediate the anabolic effects of PTH on the volume and microarchitecture of bone.⁸⁷ PTH¹⁻⁸⁴, PTH¹⁻³⁴, and PTH¹⁻³¹ stimulate intracellular signal transduction through several G-protein-coupled pathways, including the G_{s α} -adenylyl cyclase-cyclic AMP-PKA and the G_{q/11}-PLC- β -inositol triphosphate (IP₃)/DAG-Ca²⁺-protein kinase C (PKC)-MAPK signal transduction pathways.⁸⁸ PTH¹⁻⁸⁴ and PTH¹⁻³⁴ also induce cell function through β -arrestin that does not involve a G-protein that in turn activates intracellular signaling pathways mediated by MAPK, protein kinase B (PKB), and PI3K.^{86,89}

Multiple carboxyl-terminal peptides derived from PTH¹⁻⁸⁴ are either secreted directly by the parathyroid chief cells

or return to the circulation after metabolism of intact PTH¹⁻⁸⁴ by hepatic Kupffer cells.⁸⁶ Indeed, carboxyl-terminal fragments of PTH¹⁻⁸⁴ are secreted in greater abundance from the parathyroid glands than is intact PTH¹⁻⁸⁴, and the proportion of carboxyl-terminal fragments secreted increases as the ambient Ca²⁺ concentration rises. Amino-terminal fragments generated by hepatic catabolism of PTH¹⁻⁸⁴ are degraded further within the liver and do not recirculate. PTH¹⁻⁸⁴ and the carboxyl-terminal fragments of PTH are filtered by the kidneys; the carboxyl-terminal fragments are reabsorbed by the renal tubules and further degraded intracellularly. Among the circulating carboxyl-terminal fragments of PTH are PTH⁷⁻⁸⁴, 24-84, 34-84, 37-84, 41-84, 43-84

In addition to their classical effects on mineral homeostasis, several nonclassical actions of PTH¹⁻⁸⁴ have been identified including: (1) rapid and direct stimulation of intestinal calcium absorption independent of its effects on vitamin D metabolism, (2) stimulation of hepatic gluconeogenesis, (3) acute natriuresis and calciuresis, and (4) enhancement of neutrophil movement in vitro.⁸⁶ Inasmuch as many of these nonclassical biological effects of intact PTH are not replicated by the amino-terminal fragment PTH¹⁻³⁴, it has been suggested that they may be related to the carboxyl-terminal portion of the protein. Indeed, the carboxyl-terminal fragments of PTH may have biological functions that oppose those of the amino-terminal portion of PTH, namely, hypocalcemic properties that are perhaps mediated by proapoptotic effects on both osteoclasts and osteocytes.⁴⁵

Because there are multiple circulating forms of PTH, its immunological measurement is dependent on the specificity of the antibody(ies) used in the assay. When a "first-generation" polyclonal PTH radioimmunoassay is used, both intact and carboxyl-terminal fragments of PTH are usually measured. Use of "second-generation" immunoradiometric and immunochemoluminometric assays using dual monoclonal antibodies, the first directed toward an epitope within the amino terminal of PTH (AA 20–25) and the second specific for an epitope in the carboxyl terminal of PTH (e.g., AA 59–78), improves the specificity of immunological assays for intact PTH¹⁻⁸⁴. Nevertheless, most of these assays detect both intact and selected carboxyl-terminal fragments of PTH and consequently measured PTH values may be inappropriately high, particularly in patients with chronic renal insufficiency in whom circulating levels of carboxyl-terminal fragments of PTH are high.⁹⁰ Furthermore, the comparability of PTH assays from different commercial sources is often limited.⁹¹ Using a two-site immunochemiluminescent assay, serum PTH concentrations average approximately 11 to 13 pg/mL and range between 2.3 and 24.5 pg/mL in children and adolescents 2 to 16 years of age; values do not vary with age but are a bit higher in girls than boys.⁹² Development of "third-generation" PTH assays that use antibodies that are directed to an epitope of the first four amino acids of PTH has improved the specificity and stability of assays for PTH¹⁻⁸⁴.^{93,94} A "fourth generation" of PTH assays uses immunoaffinity, in situ digestion, liquid chromatography, and tandem mass spectrometry to separate PTH¹⁻⁸⁴ from smaller PTH fragments.⁹⁰

The synthesis and secretion of PTH¹⁻⁸⁴ and its various fragments are modulated for the most part by the serum Ca²⁺ concentration acting through the CaSR expressed on the plasma membrane of the parathyroid chief cell. Rapidly declining, as well as steady-state low serum concentrations of Ca²⁺, increase PTH secretion by accelerating its release from storage sites in secretory vesicles; hypocalcemia also raises intracellular levels of PTH mRNA by increasing the transcription rate of *PTH* and enhancing the stability of PTH mRNA by its posttranscriptional binding to cytosolic proteins.^{4,80} Hypercalcemia decreases *PTH* transcription and destabilizes and thence lowers cellular levels of PTH mRNA. The serum Ca²⁺ concentration

also determines the form of PTH released by the parathyroid gland; during hypocalcemia PTH¹⁻⁸⁴ is the predominant form secreted; in hypercalcemic states, carboxyl-terminal fragments of PTH are released in abundance.

Low serum phosphate concentrations exert an independent and direct inhibitory effect on the transcription of *PTH*, post-transcriptional *PTH* mRNA stability, *PTH* secretion, and proliferation of parathyroid chief cells; hyperphosphatemia increases *PTH* secretion by lowering plasma concentrations of Ca^{2+} and by enhancing the stability of *PTH* mRNA.⁸⁰ Prolonged hyperphosphatemia contributes to hyperplasia of the parathyroid glands frequently encountered in patients with chronic renal disease. Calcitriol directly inhibits *PTH* transcription acting through the nuclear VDR. Calcitriol also controls expression of *CASR* and of *VDR* and decreases proliferation of parathyroid cells. However, chronic hypocalcemia overcomes the suppressive effects of calcitriol on *PTH* transcription by decreasing VDR number in the parathyroid chief cell. FGF23 released by osteocytes and osteoblasts directly impedes synthesis and secretion of *PTH*. Both hypomagnesemia and hypermagnesemia inhibit release but not synthesis of *PTH*. Other agents that increase *PTH* release include: β -adrenergic agonists, dopamine, prostaglandin E, potassium (by decreasing cytosolic Ca^{2+} levels within the parathyroid chief cell), prolactin, lithium (by “resetting” the set-point for *PTH* release), glucocorticoids, estrogens, and progestins. Prostaglandin F_{2 α} α -adrenergic agonists, and fluoride suppress *PTH* release by increasing cytosolic Ca^{2+} values.

PTH regulates the serum concentration of calcium by stimulating its reabsorption in the distal renal tubule and from the skeleton and indirectly by augmenting the intestinal absorption of calcium by increasing the synthesis of calcitriol. In bone, *PTH* enhances osteoclast differentiation and maturation indirectly by acting on and through stromal cells and osteoblasts to increase synthesis of RANK-ligand, an activator of osteoclastogenesis, and to suppress that of osteoprotegerin, a decoy receptor for RANK-ligand. When administered intermittently, however, PTH¹⁻⁸⁴ and PTH¹⁻³⁴ exert anabolic effects upon skeletal mass by increasing osteoblast number by accelerating their differentiation from progenitor cells and from inactive bone-lining cells. They do so in part by decreasing the stimulatory effects of the peroxisome proliferator activator receptor gamma (PPAR γ) upon mesenchymal stem cell differentiation into adipocytes permitting their differentiation into osteoblasts, by augmenting the rate of osteoblast proliferation, and by reducing the rate of apoptosis of osteoblasts.⁸⁷ The anabolic effects of *PTH* on bone formation are further mediated by enhancing synthesis and release of matrix-embedded growth factors, such as locally generated insulin-like growth factor-I (IGF-I), which has positive effects on differentiation and survival of osteoblasts, by stimulating the WNT-Frizzled- β -Catenin pathway of osteoblastogenesis, and by inhibiting antagonists to bone formation, such as sclerostin. In addition, through promotion of β -arrestin-mediated intracellular signal transduction, *PTH* increases trabecular number and volume.^{87,89} Thus *PTH* stimulates anabolism of bone mineralization whereas bisphosphonates exert an antiresorptive effect.⁹⁵ *PTH* stimulates calcitriol synthesis by increasing renal tubular expression of *CYP27B1*, the gene encoding 25-hydroxyvitamin D₃-1 α -hydroxylase, the enzyme that catalyzes the synthesis of calcitriol from calcidiol. *PTH* depresses proximal and distal renal tubular reabsorption of phosphate by decreasing expression of *SLC34A1*, encoding a Na⁺-HPO₄ cotransporter, thus increasing the urinary excretion of this anion. In addition, *PTH* directly stimulates secretion of bone-derived FGF23, thereby further increasing urinary phosphate excretion; *PTH* also indirectly stimulates FGF23 release by lowering serum phosphate concentrations and increasing those of calcitriol.⁹⁶

Parathyroid Hormone-Related Protein

PTHrP also termed *PTH-like protein (PTHLP)* (encoded by *PTHLP*) was initially identified as a major cause of humoral hypercalcemia of malignancy. *PTH* and PTHrP have evolved from a common ancestral gene through gene duplication, and they are structurally and functionally related.⁹⁷ Thus the sequence of AA in the preproportion of the translated products and the first eight of 13 AA at the beginning of their first coding exons are identical; although their AA sequences diverge thereafter, the predicted three-dimensional structures of the next 21 AA of both peptides are similar, as is the PTH1R to which the two proteins bind and through which they transmit their intracellular signals. However, *PTH* binds more avidly to PTH1R and elicits a greater hypercalcemic response than does PTHrP.⁹⁷ Following transcription, *PTHLP* is translated into isoforms composed of the first 139, 141, and 178 AA. PTHrP transmits its message both distally as an endocrine hormone and locally as both a paracrine and intracrine messenger. Variable parts of PTHrP are also secreted by keratinocytes, epithelial cells of the breast, and other tissues in sequences that include: AA 1 to 36 (which binds to and activates PTH1R), AA 38 to 94, and AA 107 to 138. Acting through the vitamin D nuclear receptor, calcitriol inhibits transcription of *PTHLP* and decreases the stability of PTHrP mRNA, thereby increasing the rate of intracellular degradation of the PTHrP protein.

Because transcription of *PTHLP* may begin in an exon downstream of the exon that encodes its signal peptide, after synthesis PTHrP can remain in the cytoplasm and be directly imported into the nucleus.⁹⁷ A nuclear localization sequence is present between AA 84 and 93 in the PTHrP molecule. After synthesis, PTHrP retained within the cytoplasm of the synthesizing cell is bound to importin β 1 and shuttled between cytosol and nucleus where it links to and regulates movement of targeted (m)RNA into the ribosome and translation of the selected gene(s), thus serving as an intracrine signaling transducer. PTHrP also transmits its message in a paracrine manner between cells within tissues. PTHrP is synthesized by many fetal and adult tissues (cartilage, bone, smooth, cardiac and skeletal muscle, skin, breast, intestines, parathyroid glands, pancreatic islets, pituitary, placenta, and central nervous system) and plays a crucial role in chondrocyte differentiation and maturation, formation of the mammary gland and eruption of teeth, and epidermal and hair follicle growth. In the fetus, periarticular chondrocytes located at the ends of long bones synthesize PTHrP in response to Indian hedgehog (encoded by *IHH*), a protein secreted by late proliferative-early prehypertrophic chondrocytes that functions through its receptor—Patched 1 (encoded by *PTCH1*).⁹⁸ PTHrP enters into and diffuses through the growth plate and signals prehypertrophic chondrocytes through PTH1R to slow their rate of differentiation to hypertrophic chondrocytes—thus prolonging the stage of chondrocyte proliferation and delaying ossification, thereby increasing the length of the cartilaginous growth plate and the long bone.⁹⁷ In addition to stimulating the secretion of PTHrP, the rate of growth plate chondrocyte proliferation is increased by *IHH*, which also directs differentiation of perichondrial cells to osteoblasts. PTHrP additionally influences osteoblast-induced bone formation and tooth eruption. PTHrP secreted by the fetal parathyroid gland and the PTHrP³⁷⁻⁹⁴ midregion fragment synthesized by the placenta increase placental calcium transport^{97,99} (Table 9.3). Both *PTH* and PTHrP are required for normal mineral homeostasis in the fetus.

Normal nipple and mammary gland formation is dependent upon local synthesis of PTHrP. In utero, this protein promotes differentiation of mesenchymal tissue surrounding the budding epithelial mammary ducts; PTHrP is also important for terminal differentiation of the mammary system during

TABLE 9.3 Parathyroid Hormone-Related Protein: Sites of Expression and Action

Site	Action
Mesenchyma Subarticular cells/ prechondrocytes	PTHrP depresses the rate of differentiation of late proliferating chondrocytes to hypertrophic chondrocytes and delays ossification, thus permitting increased longitudinal growth of the cartilaginous growth plate
Bone	Enhances or depresses bone resorption
Smooth muscle	Relaxation
Vascular system Myometrium Urinary bladder Cardiac muscle Skeletal muscle	Positive chronotropic and inotropic effects
Epithelia Skin Breast	Regulates proliferation of keratinocytes Induces ductal branching and formation of breast epithelium, secreted into milk, drives mobilization of calcium from maternal bone for transfer to nursing infant
Teeth	Stimulates resorption of overlying bone enabling eruption
Endocrine system Parathyroid glands Pancreatic islets Placenta	Stimulates transport of calcium Stored and cosecreted with insulin Enhances calcium transport from mother to fetus
Central nervous system	Neuroprotective by antagonizing excessive calcium

(Modified from Wysolmerski, J.J. (2008). Parathyroid hormone-related protein. In: Rosen, C.J. (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 7th ed, The American Society of Bone and Mineral Metabolism, Washington DC, p 127–133; Wysolmerski, J.J. (2012). Parathyroid hormone-related protein: an update. *J Clin Endocrinol Metab*, 97, 2947–2956.)

puberty.⁹⁷ During lactation, mammary expression of *PTHLH* and secretion of PTHrP increases while production of estrogens declines permitting unopposed PTHrP-induced mobilization of maternal skeletal calcium necessary to meet the calcium requirement of the breastfed infant but substantially decreasing maternal bone mineral content, a process that is reversed when lactation ceases.¹⁰⁰

Biallelic loss of PTHrP in “knock-out” mice is lethal because of bony malformations. In *Pthlh*^{-/-} mice, there is decrease in the number of resting and proliferating chondrocytes; the columnar organization of the growth plate is disrupted, there is premature acceleration of chondrocyte maturation and apoptosis, and inappropriate ossification resulting in a dwarfing phenotype (a domed and foreshortened cranium, short limbs, small thorax) that is similar to that of Blomstrand chondrodysplasia (OMIM 215045), a disorder associated with loss-of-function mutations of *PTH1R*. Mice in which expression of *Pthlh* has been maintained only in chondrocytes survive but are short with cranial chondrodystrophy and failure of tooth eruption. In the heterozygous state (*Pthlh*+/-), mouse fetal development is normal but by 3 months of age, the trabeculae of the long bones are osteopenic; a similar bone phenotype is noted when loss of *Pthlh* expression is confined to osteoblasts.⁹⁹ For comparison, in mice in which *Pth* has been “knocked out,” there is decreased mineralization of cartilage matrix, expression of vascular endothelial growth factor (VEGF)

and neovascularization, osteoblast number, and trabecular bone volume.¹⁰¹ Thus both PTH and PTHrP are necessary for normal fetal endochondral bone development. Cortical thickness of long bones is increased in both *Pth*- and *Pthlh*-null mouse models, indicating that the regulation of endochondral and periosteal osteoblast function differs.

Serum concentrations of PTHrP are low except when it is secreted by tumors, leading to humoral hypercalcemia of cancer. There are high levels of PTHrP in breast milk and low concentrations of PTHrP may be measurable in the serum of lactating women.

Parathyroid Hormone/Parathyroid Hormone-Related Protein Receptors

PTH and PTH-rP bind to the PTH receptor, PTH1R.⁸⁸ PTH1R is a 585 AA protein that is a member of the Class B family of GPCRs whose ligands include calcitonin, GH-releasing hormone, secretin, glucagon, glucagon-like peptide 1, gastric inhibiting polypeptide, vasoactive intestinal polypeptide, corticotropin-releasing hormone, and pituitary adenylate cyclase activating protein that are characterized by long extracellular amino-terminal domains (~160 AA residues) with multiple cysteine residues forming disulfide bridges. PTH1R has an extracellular domain, seven transmembrane spanning segments connected by three extracellular and three intracellular loops, and an intracellular domain with which the signaling G-protein interacts. *PTH1R* is composed of 15 exons; there are three promoters that regulate expression of *PTH1R*. As a result of alternative splicing of its mRNA, there are several isoforms of the PTH/PTHrP receptor.

The amino terminals of both PTH¹⁻³⁴ and PTHrP¹⁻³⁶ bind to the transmembrane and the extracellular loop sequences of the transmembrane segments of PTH1R, whereas their carboxyl terminals bind to the amino-terminal extracellular portion of PTH1R.^{4,86,88,102} Ligand-bound PTH1R transmits its message through several intracellular signal transduction pathways, including the G_{sα}-adenylyl cyclase-cyclic AMP-PKA system, the G_{q/11}-PLCβ-inositol trisphosphate-cytoplasmic Ca²⁺-PKC path, the G_{12/13}-phospholipase D-transforming protein RhoA system, and the β-arrestin-MAPK pathway.⁸⁹ Interaction of the specific PTH1R ligand and PTH1R determines the signaling pathway and duration of response of these two components; thus PTH has a greater affinity for PTH1R than does PTHrP and coupling of PTH with PTH1R leads to a more stable, prolonged, and intense signaling response than does interaction of PTHrP and PTH1R. Indeed, even after internalization, PTH-PTH1R signaling continues.⁸⁸

After inducing intracellular signaling, PTH1R is phosphorylated by a GPCR kinase (GRK) and bound to β-arrestin-1 (encoded by *ARBB21*), leading to its internalization, and subsequent destruction or reutilization.⁸⁹ Once internalized, PTH1R may be degraded, recycled to the cell membrane, or directed to the nucleus by importins-α₁ and -β where it is present in the nucleoplasm.¹⁰³ The physiological functions of nuclear PTH1R and its ligands PTH and PTHrP are as yet unknown.

In the kidney, *PTH1R* is expressed on the apical and basolateral surfaces of cells in the proximal renal tubule; binding of PTH to apical PTH1R preferentially signals through cyclic AMP-PKA, whereas interaction of PTH with basolateral PTH1R activates the PLC-PKC signal transduction pathway. Through both PKA and PLC, PTH/PTH1R activates the MAPK signal transduction system of extracellular signal-regulated kinases (ERK)-1,2, allowing regulation of cell differentiation, division, growth, and apoptosis. In addition to participating in the internalization of the PTH-PTH1R complex, β-arrestin enables it to stimulate intracellular signaling in a manner that is

independent of G-proteins; the GRK-mediated patterns of proline phosphorylation of PTH1R and β -arrestin likely determine their conformations and functional activities.¹⁰⁴ Thus interaction of PTH-PTH1R with β -arrestin may also activate the MAPK signaling system and facilitate PI3K and PKB (AKT) intracellular signal transduction pathways.⁸⁹ PTH-PTH1R activation of the MAPK pathway through its GPCR is rapid but relatively brief lasting only a few minutes, whereas activation through the β -arrestin pathway is slow and sustained over hours. Another mechanism through which PTH/PTH1R interaction affects cellular function is by the binding of PTH1R to the Na^+ - H^+ exchanger regulatory factor 2 (NHERF2—encoded by *SLC9A3R2*) with consequent stimulation of PLC activity and inhibition of adenylyl cyclase activity.¹⁰⁵ In the kidney, PTH1R binds to NHERF1 (encoded by *SLC9A3R1*), an essential factor for trafficking of the phosphate channel NPT2a to the luminal membrane of cells in the proximal renal tubule.⁵⁴ In the proximal renal tubule, linking of PTH to PTH1R increases cyclic AMP production, leading to decrease in the internalization and expression of the sodium-phosphate cotransporters and increase in urinary phosphate excretion; association of PTH1R with NHERF1 blunts the phosphaturic effect of PTH. Therefore disruption of PTH1R-NHERF1 interaction leads to renal wasting of phosphate. Agonists of PTH have been synthesized that can “bias” the selection of the signal transduction pathway used; a “biased” agonist might stabilize a receptor conformation that stimulates a specific signal transduction sequence or activate one pathway while simultaneously inhibiting another.^{88,89,104}

PTH1R is expressed in renal tubular cells and osteoblasts, skin, breast, heart, and pancreas among other tissues—the latter sites reflecting the paracrine targets of PTHrP. PTH, PTHrP, and calcitriol decrease expression of *PTH1R*. Targeted loss of *PTH1R* is accompanied by impaired proliferation of chondrocytes and acceleration of chondrocyte maturation and calcification, an outcome mimicked by targeted loss of $\text{G}_{\alpha s}$ in chondrocytes.^{86,99} Clinically, inactivating mutations in *PTH1R* result in hypocalcemia and Blomstrand chondrodysplasia (OMIM 215045), whereas constitutively activating mutations of *PTH1R* lead to hypercalcemia and Jansen metaphyseal chondrodysplasia (OMIM 156400).

A second PTH receptor (PTH2R) is selectively activated by PTH but does not recognize PTHrP; PTH specificity is determined by Ile⁵ and Trp²³ in native PTH, sites that affect activation and binding, respectively.⁸⁶ *PTH2R* encodes a 539 AA GPCR with 50% homology to PTH1R that activates adenylyl cyclase; it is expressed predominantly in brain, testis, placenta, and pancreas, but not in bone or kidney; its physiological role is uncertain. In response to PTH¹⁻⁸⁴, PTH2R enhances both cyclic AMP generation and Ca^{2+} mobilization. However, the naturally occurring endogenous ligand for PTH2R is not PTH but the 39 AA PTH-related hypothalamic tuberoinfundibular peptide (TIP39); this protein is also expressed in the testis and various central nervous system regions.⁹⁷

Although as yet not molecularly characterized, receptors for carboxyl-terminal fragments of PTH¹⁻⁸⁴ have been functionally identified as renal and bone cell binding sites for PTH¹⁻⁸⁴ from which intact hormone can be only partially displaced by PTH¹⁻³⁴ but can be further displaced by PTH⁵³⁻⁸⁴ and PTH⁶⁹⁻⁸⁴.⁸⁶ In addition, in osteocytes, osteoblasts, and chondrocytes from which the PTH/PTHrP receptor has been “knocked out” and to which intact PTH¹⁻⁸⁴ but not PTH¹⁻³⁴ binds, labeled PTH¹⁻⁸⁴ can be displaced by carboxyl-terminal PTH¹⁹⁻⁸⁴, ²⁸⁻⁸⁴, ³⁹⁻⁸⁴ fragments. Important determinants for binding of PTH to the carboxyl-terminal selective receptor(s) appear to be AA 24 to 27 (Leu-Arg-Lys-Lys) and 53 to 54 (Lys-Lys). Furthermore, carboxyl-terminal fragments of PTH exert biological effects in intact and *PTH1R*-null cells, such as regulation of alkaline phosphatase

activity in osteosarcoma cells and osteoblasts (but not generation of collagen type I), stimulation of Ca^{2+} uptake by osteosarcoma cells and chondrocytes, and cell survival in vitro. Thus the physiological actions of PTH likely reflect the integrated sum of the individual functions of the intact hormone and its carboxyl-terminal fragments. Carboxyl-terminal fragments of PTHrP are not recognized by the sites that bind carboxyl-terminal fragments of PTH.

CALCITONIN

Calcitonin, a 32 AA peptide encoded by *CALCA*, is synthesized and released by the neural crest-derived parafollicular C cells of the thyroid gland in response to rising concentrations of Ca^{2+} (a reaction mediated by activation of the CaSR present on the plasma membrane of the C cell) and in response to several gastrointestinal hormones, including gastrin.^{4,106} *CALCA* is a six exon gene that by alternative transcription and translation forms a 141 AA protein from which calcitonin (exon 4) and katalcacin, a 21 AA hypocalcemic peptide adjacent to the carboxyl terminus of calcitonin, are derived, and a 128 AA protein from which is gleaned the 37 AA calcitonin gene-related-peptide (CGRP)- α (exon 5), a vasodilator and neurotransmitter that also interacts with the calcitonin receptor. Calcitonin lowers serum concentrations of calcium by inhibiting osteoclast-mediated bone resorption and by increasing renal calcium excretion. After binding of calcitonin to its GPCR expressed on the osteoclast cell membrane, the morphology of the ruffled membrane of the functioning osteoclast flattens causing it to withdraw from the bone reabsorption site, thereby decreasing this process and consequently lowering peripheral calcium concentrations.¹⁰⁷ Calcitonin also decreases renal tubular reabsorption of filtered calcium. However, calcitonin enhances proximal renal tubular synthesis of calcitriol and thereby increases intestinal absorption of calcium. In turn, calcitriol suppresses synthesis and secretion of calcitonin; thus calcitriol has the same reciprocal relationship with calcitonin as it does with PTH and FGF23.

Calcitonin is produced in excess by medullary carcinoma of the thyroid and, at times, by other neuroendocrine tumors. The calcitonins of multiple species share similar structures including: five of the first seven amino-terminal AA, a disulfide bridge between AA 1 and 7, glycine at AA residue 28, and a proline amide residue at carboxyl-terminal AA 32. In the interior of the peptide, species other than human have several basic amino AA that make them more stable and easily recognized by the human calcitonin receptor and thus more biologically potent, for example, therapeutically useful salmon calcitonin.

Calcitonin secretion is stimulated primarily by increasing concentrations of Ca^{2+} transduced by the CaSR expressed on the plasma membrane of the C cell.⁴ Members of the gastrin-cholecystokinin intestinal peptide hormone family (gastrin, glucagon, pancreaticozym, glucocorticoids, and β adrenergic agonists) are also potent calcitonin secretagogues.^{4,107} Calcium, pentagastrin, and glucagon are effective stimuli used to assess calcitonin secretion clinically. Somatostatin, calcitriol, and chromogranin A¹⁻⁴⁰ inhibit and chromogranin A⁴⁰³⁻⁴²⁸ stimulates calcitonin secretion. Calcitonin secretion declines as the Ca^{2+} concentration declines. The half-life of calcitonin is brief; it is metabolized primarily by the kidney and also by liver, bone, and thyroid gland. Serum levels of calcitonin are high in the fetus (when serum total calcium concentrations are normally 12–13 mg/dL) and newborn, fall rapidly after birth as Ca^{2+} values decline and then more slowly until 3 years of age remaining relatively constant thereafter (<12 pg/mL). After 10 years of age, serum concentrations of calcitonin are higher in males than in females. The physiological role of calcitonin is unclear as serum calcium concentrations are normal in patients with both decreased (primary congenital or acquired

hypothyroidism) and increased (medullary carcinoma of the thyroid) secretion of this peptide. However, disposal of a calcium load is slower in the calcitonin-deficient subject. Calcitonin values are slightly higher in males than in females. Immunoassayable concentrations of calcitonin are increased in patients with medullary carcinoma of the thyroid, chronic renal insufficiency, and pycnodysostosis.¹⁰⁷

The biological effects of calcitonin are mediated through its 490 AA class B GPCR encoded by *CALCR*. Intracellular signaling of the *CALCR* is transduced through the $G_{s\alpha}$ adenylyl cyclase-cyclic AMP-PKA and $G_{\alpha q}$ -PLC-IP₃ signal transduction pathways. Alternative splicing of *CALCR* results in two isoforms of the calcitonin receptor, one of which has an additional 16 AA inserted into its first intracellular loop between transdomains I and II. The calcitonin receptor is expressed in osteoclasts; when exposed to calcitonin, the osteoclast shrinks and bone resorbing activity declines quickly. Thus calcitonin lowers serum calcium and phosphate levels particularly in patients with hypercalcemia. In lactating women, serum levels of calcitonin rise and calcitonin is excreted in breast milk. Women who are breastfeeding their infants lose 5% to 10% of their trabecular bone mineral during 6 months of lactation that is recouped rapidly when lactation ceases, much more quickly than when

bone mass is lost because of other problems (e.g., glucocorticoid excess, bed rest). Inasmuch as calcitonin values increase during pregnancy and lactation, calcitonin may play a role in the remineralization of bone in lactating women after completion of breastfeeding.

VITAMIN D

Synthesis and Biological Activity of Vitamin D

Cholecalciferol (vitamin D₃) may either be generated endogenously by exposure of the skin to sunlight or consumed (in a diet consisting of oily fish, such as wild salmon, tuna, herring, cod, and mackerel; fungi, such as shiitake mushrooms; and egg yolks or as a dietary supplement). (Ergocalciferol [vitamin D₂] is a plant and yeast sterol that may either be consumed or ingested as a dietary supplement.) In the cutaneous stratae basale and spinosum, vitamin D₃ is synthesized from cholesterol: 7-dehydrocholesterol is photoisomerized to previtamin D₃ by ultraviolet B radiation at 290 to 315 nm and heat (37° C) from sunlight and then spontaneously isomerizes to cholecalciferol (vitamin D₃) (Fig. 9.4).^{23,108,109} Previtamin D₃ is also metabolized into products, such as lumisterol₃

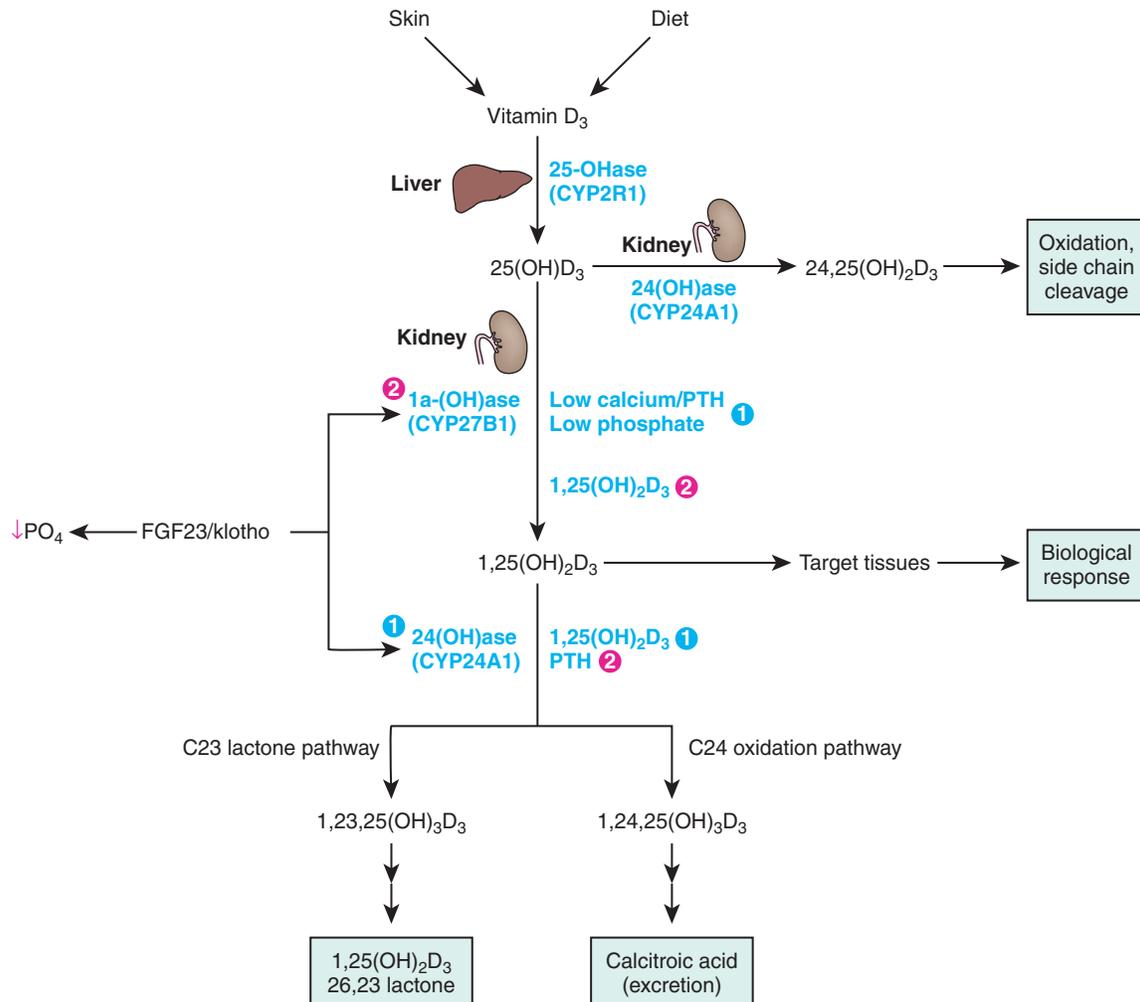


Fig. 9.4 Synthesis and metabolism of vitamin D. In skin, 7-dehydrocholesterol is metabolized to cholecalciferol (vitamin D₃) that is then hydroxylated at carbon 25 in the liver to form calcidiol (25OHD₃) and at carbon 1 in the kidney to form calcitriol [1,25(OH)₂D₃]. Calcitriol is inactivated by hydroxylation at carbon 24 or carbon 23. Parathyroid hormone (PTH) enhances synthesis of calcitriol and impedes its catabolism. Fibroblast growth factor-23 (FGF23) antagonizes synthesis of calcitriol and hastens its degradation. (From Christakos, S., Dhawan, P., Verstuyf, A., et al. (2016). Vitamin D: metabolism, molecular mechanisms of action, and pleiotropic effects. *Physiol Rev*, 96, 365–408. With permission).

and tachysterol₃ and to inactive photoproducts when exposure to sunlight is excessive, thereby preventing vitamin D intoxication by exposure to sunlight alone. Cutaneously synthesized vitamin D₃ enters the circulation directly. Ergocalciferol (vitamin D₂) derived from ergosterol differs structurally from vitamin D₃ by the presence of a double bond between carbons 22 and 23 and a methyl group on carbon 24. Vitamins D₃ and D₂ exert reasonably similar biological effects when ingested in equivalent physiological doses; however, the biological potency of vitamin D₂ is less than that of vitamin D₃ because it is rapidly cleared from the circulation, restricting its further processing into a biologically active form.^{108,110,111} The latitude, season of the year, and time of day influence the rate of cutaneous synthesis of vitamin D₃ in response to exposure to sunlight; in higher latitudes, the path through which ultraviolet B radiation from the sun travels is longer and more of the ultraviolet radiation is absorbed by the ozone layer; thus less reaches the surface of the earth. Exposure of the back of a white adult to intense summer sun (mid-July) for 10 to 12 minutes in the northeastern United States generates around 10,000 to 20,000 IU of vitamin D₃ over the next 24-hour interval.¹¹² (For persons of color, 30–120 minutes of exposure to sunlight may be required for comparable effects.) Sun-blocking topical creams and aging also decrease the cutaneous formation of cholecalciferol in response to sunlight. Both forms of vitamin D undergo similar chemical modifications to become bioactive metabolites. Orally ingested vitamin D is packaged into chylomicrons and absorbed into the intestinal lymphatic system and then enters the circulation. Vitamins D₃ and D₂ and their metabolites are transported in serum linked to vitamin D-binding protein (DBP), a polymorphic variant of the serum α 2-globulin synthesized by the liver and encoded by *GC*. DBP binds vitamin D and its hydroxylated metabolites with high affinity and capacity.¹¹³

Sequentially, vitamin D is twice hydroxylated, first in the liver at carbon 25 primarily by the vitamin D 25-hydroxylase encoded by microsomal *CYP2R1* to form 25-hydroxyvitamin D₃ (25OHD₃ = calcidiol), and secondly in the mitochondria of the proximal renal tubule at carbon 1 to form 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃ = calcitriol].^{22,110} From serum, the 25-hydroxyvitamin D/DBP complex is transported into the cytoplasm of cells in the proximal renal tubule bound to a transmembrane protein termed *megalyn* (encoded by *LRP2*) and acted upon by mitochondrial 25-hydroxyvitamin D-1 α -hydroxylase (encoded by *CYP27B1*). The 1 α -hydroxyl group of calcitriol is essential for its binding to the VDR.¹¹⁴ The nine exons of *CYP27B1* encode a 507 AA protein with a mitochondrial signal sequence at its amino terminal and ferredoxin and heme-binding sites within its structure. As a class I mitochondrial cytochrome P450 enzyme, 25OHD-1 α -hydroxylase requires for its catalytic activity electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH) that are ferried to the enzyme protein by the electron transport proteins—NADPH-ferredoxin and ferredoxin reductase.^{110,115} (*CYP27B1* is also expressed by the placental deciduous and trophoblastic cells, epithelial cells of the skin, parathyroid glands, pancreatic islet cells, thyroid, chondrocytes, and osteoblasts and by macrophages and T/B cells of the immune system.¹¹⁰)

Renal tubular expression of *CYP27B1* is stimulated by hypocalcemia, hypophosphatemia, PTH, calcitonin, GH, IGF-I, and prolactin and suppressed by calcitriol and FGF23/Klotho. In monocytes and macrophages, expression of *CYP27B1* may be induced by inflammatory cytokines, such as interferon- γ but is not regulated by concentrations of Ca²⁺, phosphate, PTH, or calcitriol.^{109,110} Reciprocally, hypercalcemia and hyperphosphatemia directly suppress expression of *CYP27B1*. Calcitriol exerts a direct autoinhibitory effect upon renal *CYP27B1* expression acting through the VDR and thus upon its own

synthesis; it also indirectly inhibits *CYP27B1* expression by impairing PTH synthesis in the parathyroid gland.¹⁰⁸ FGF23 depresses 25OHD-1 α -hydroxylase activity, whereas calcitriol increases *FGF23* expression effectively establishing a reciprocal control system for these compounds.^{57,64,116} Calcitriol acts in the parathyroid gland to increase expression of *CASR* thereby suppressing expression of *PTH* and indirectly that of *CYP27B1*. Both calcidiol and calcitriol are inactivated by hydroxylation at carbon 24 by renal tubular cells expressing *CYP24A1* (encoding vitamin D 24-hydroxylase) whose expression is stimulated by calcitriol and FGF23/Klotho and inhibited by PTH; calcitriol may also be inactivated by hydroxylation at carbon 23 and conversion to a lactone (see Fig. 9.4).²²

Inasmuch as calcidiol exerts only a minimal inhibitory effect on its production, serum concentrations of calcidiol reflect available body stores of vitamin D. However, genetic variants of *DHCR7* encoding 7-dehydrocholesterol reductase, *CYP2R1*, *GC*, and *VDR* encoding the VDR may affect the serum concentration of calcidiol.¹¹⁷ Although lacking consensus because of variability in calcidiol assay methodology, the current recommendation for interpretation of the 25OHD concentration is: less than 12 ng/mL deficiency of vitamin D; 12 to 20 ng/mL insufficiency; more than 20 to 50 ng/mL sufficiency; and toxicity over 100 ng/mL if associated with hypercalcemia, hypercalciuria, and suppressed serum PTH concentration.^{118,119} It is currently recommended that all infants (whether breast- or formula-fed) receive a vitamin D supplement of 400 IU/day and that children between 1 and 18 years of age receive 600 to 1000 IU of vitamin D daily, depending upon skin color and latitude of residence.^{118,120} It has been recommended that preterm infants ingest 200 to 800 IU of vitamin D daily, that the serum 25OHD concentrations of these subjects be measured serially, and that the dose of vitamin D readjusted as indicated. The recommended upper limit of vitamin D intake is 4000 IU/day for all age groups above 8 years (see Table 9.2).³⁰ In vitamin D-deficient pregnant women, vitamin D supplementation decreased the rates of premature delivery, maternal preeclampsia, and gestational diabetes mellitus.¹²¹ In middle-aged and older adults, intake of 400 to 800 IU per day of cholecalciferol can slightly reduce all-cause mortality.¹²² However, supplementation has had no effect upon the risk of cardiovascular disorders, neoplasia, altered glucose metabolism and adiposity, disorders of mood, and a number of other infectious and noninfectious diseases. Although subnormal serum concentrations of 25OHD may be recorded in many patients with a variety of illnesses, it is likely that hypovitaminosis D in these circumstances is the consequence of the disease rather than its cause.

Calcitriol circulates bound to DBP and enters the target cell (intestinal tract, kidney, parathyroid gland, bone, and other sites) where it binds to the cytosolic VDR, translocates to the nucleus, heterodimerizes with the retinoid X receptor (RXR), binds to the VDR response element (VDRE) of the selected gene, and stimulates or represses gene expression by recruiting site-specific transcriptional regulators. The VDREs have diverse nucleotide compositions and, while often present in the 5' sequence upstream of the target gene, may also be located far distant from the transcription start site.²²

The major functions of calcitriol are to increase intestinal and renal tubular absorption of calcium and phosphate, thereby increasing their serum concentrations and enhancing bone mineralization and to increase bone resorption by stimulating osteoblast production of RANK-ligand, thereby activating osteoclastogenesis.²² During periods of negative calcium balance, calcitriol impedes bone mineralization by increasing production of inhibitors of this process, such as pyrophosphate and osteopontin. Among the more than 1000 gene targets of calcitriol/VDR are: *TNFSF11* (RANK-Ligand—activator of

osteoclastogenesis), *LRP5* (lipoprotein receptor cofactor 5 for WNT/Frizzled receptor interaction), *TRPV6* (intestinal calcium channel), *FGF23*, *SLC34A3* (renal phosphate channel—NPT2c), *PTH*, *PTH1LH*, *KL* (Klotho), *CYP24A1* (25-Hydroxyvitamin D-24 hydroxylase), and *CA2* (Carbonic anhydrase 2).⁶²

Calcitriol is inactivated in bone, intestine, liver, and kidney by glucuronidation, sulfation, multisite (carbons-23, -24, -26) hydroxylation, and lactone formation to water-soluble compounds (such as calcitroic acid) that are excreted in urine and bile.^{108,109,110} In the kidney, 25OHD (calcidiol) and 1,25(OH)₂D (calcitriol) are converted to 24R,25(OH)₂D and 1,24R,25(OH)₃D, respectively, by mitochondrial 25OHD-24 hydroxylase encoded by *CYP24A1*, the first in a series of hydroxylations that increase the water solubility of the products, enabling their biliary and renal excretion (see Fig. 9.3). The expression of *CYP24A1* is increased by hypercalcemia, hyperphosphatemia, FGF23, and calcitriol, as well as retinoic and lithocholic acids, rifampicin, carbamazepine, and phenobarbital and suppressed by hypocalcemia and PTH.¹¹⁵ *CYP24A1* is also expressed by fibroblasts, lung, intestine, oocytes, brain, thyroid, and other tissues.¹⁰⁹ Cleavage of the side chain between carbons 23 and 24 that have been hydroxylated by hepatic cytochrome P450-3A (encoded by *CYP3A4*) dependent enzymes generates water-soluble calcitroic acid that is excreted in bile.^{108,109} Phenobarbital, phenytoin, carbamazepine, and rifampicin impair bone mineralization by inactivating calcitriol and do so by increasing its state of hydroxylation by binding to and activating the nuclear pregnane X receptor (*NR112*) that in turn increases expression of *CYP3A4*.¹²³ Calcitriol may also induce expression of hydroxylases that use cytochrome P450-3A, thereby enhancing its own hydroxylation and degradation. Inactivating mutations of *CYP24A1* lead to (idiopathic) hypercalcemia of infancy by reducing the rate of degradation and thereby prolonging the biological life of calcitriol.¹²⁴

Although emphasis has been placed on the vitamin D endocrine system in the regulation of mineral homeostasis and bone health, its role as a paracrine factor may also be considered. Adipocytes not only store vitamin D, these cells also express vitamin D-25 hydroxylase and 25-hydroxyvitamin D-1 α hydroxylase activities suggesting that fat may be a site of calcidiol and calcitriol synthesis.¹⁰⁹ The bulk of circulating calcitriol is bound to DBP, but it is its free fraction that is biologically active. Indeed, the serum level of DBP does not affect the calcitriol pool that enters the cell and regulates gene transcription.¹¹⁴ Approximately 0.04% of calcidiol and 0.4% of calcitriol are present in free form in serum. “Normal” ranges of calcitriol concentrations are: neonates 8 to 72 pg/mL, infants and children 15 to 90 pg/mL, and adults 21 to 65 pg/mL.

Calcitriol exerts its biological effects by binding to its cytosolic VDR, heterodimerizing with RXR α , entering the nucleus, and binding to the specific VDRE, thereby regulating the expression of more than 1000 target genes involved in mineral and bone metabolism and other processes that influence cell growth, musculoskeletal, cardiovascular, immune, skin, and pancreatic islet cell functions and energy metabolism.^{108,114,125,126} The nuclear effects of calcitriol take place over hours to days as the processes of transcription, translation, posttranslational modifications to the encoded protein(s), storage, and secretion occur in multiple cytosolic compartments. The VDR also associates with caveolae of the cell plasma membrane—flask-shaped invaginations of the membrane composed of sphingolipids and cholesterol where, after binding to ligand, the VDR is able to interact with intracellular signal transduction systems to exert rapid functional cell responses.^{127,128} The rapid cell responses to calcitriol generated by association with the caveolar VDR are evident within seconds to minutes after contact and include: immediate intestinal

absorption of calcium (transcaltachia), opening of voltage-gated calcium and chloride channels in osteoblasts, endothelial cell migration, and pancreatic β cell secretion of insulin. It is the flexible three-dimensional configuration of calcitriol that enables this ligand to exert both genomic and nongenomic (rapid response) actions.^{114,127} The protean three-dimensional structure of calcitriol is the result of: (1) rotation of side chain carbon pairs 17-20, 20-22, 22-23, 23-24, and 24-25; (2) rotation of the carbon 6-7 bond around the B ring; and (3) chair-chair interconversion with formation of either an α - or β - configuration of the cyclohexane-like A ring. Rotation about the carbon 6-7 bond of the B ring allows calcitriol to assume either an extended 6-*s-trans* configuration or a 6-*s-cis* conformation. It is the *trans* form of calcitriol with the β -chair configuration of the A ring that is recognized and bound by the hydrophobic pocket of the nuclear VDR. Ligand-bound VDR interacts with the RXR forming a heterodimer that is recognized by specific hexameric sequences of DNA bases (the VDRE) in the regulatory region of target genes.¹⁰⁹ The nucleotide sequences of the numerous VDREs are highly variable but in general consist of two halves of similar hexanucleotides connected by three (variable) nucleotides.¹¹⁰ The calcitriol/VDR-RXR-VDRE complex then recruits transcription-activating or transcription-repressing coregulatory proteins enabling its genomic transcriptional effects. The *cis* form of calcitriol permits its rapid cell membrane-related actions.

Calcitriol primarily controls intestinal, skeletal, and renal function by regulating expression of genes encoding calcium transporters (calbindin-D_{28K}, calbindin-D_{9K}), calcium channels (TRPV5—expressed primarily in the kidney; TRPV6—expressed predominantly in the intestinal tract), bone matrix proteins (osteocalcin, osteopontin, type I collagen, alkaline phosphatase), and osteoclast activators (RANK-ligand). Calcitriol promotes endochondral bone formation; it increases length of the long bones by amplifying volume, proliferation, and differentiation of epiphyseal chondrocytes and promotes mineralization of cartilage matrix.¹²⁹ Calcitriol increases trabecular and cortical bone formation by augmenting osteoblast number and function; it increases alkaline phosphatase activity, osteocalcin synthesis, and type I collagen formation and represses bone resorption by osteoclasts. Calcitriol directly suppresses transcription of *PTH* in the parathyroid gland acting through the VDR. Vitamin D is also important for normal muscle development and strength.

Mediated by the nuclear VDR, calcitriol stimulates the absorption or reabsorption of calcium in the intestines, bone, and kidney. In the duodenum and proximal small intestine, calcitriol increases the efficiency of calcium uptake from the intestinal lumen by increasing the number of epithelial calcium transport channels (TRPV6) in enterocytes, its movement through the cytoplasm, and its transfer across the basal lateral membrane into the circulation, in part by the induction of calbindin_{9k}, a calcium binding protein, alkaline phosphatase, Ca-ATPase, calmodulin, and other proteins.¹³⁰ Calcitriol also increases jejunal and ileal absorption of phosphate through a transcellular mechanism utilizing the type II Na⁺-HPO₄²⁻ cotransporter NPT2a (encoded by *SLC34A1*) expressed on the luminal surface of the enterocyte. When vitamin D stores are replete, 40% of dietary calcium and 80% of dietary phosphate may be absorbed. Even greater efficiency of mineral absorption is realized during periods of rapid growth, pregnancy, and lactation. A major task of calcitriol is to maintain calcium and phosphate concentrations in blood at levels sufficient to sustain mineralization of osteoid—collagen-containing bone matrix synthesized by osteoblasts. Paradoxically, in states of calcium deficiency, calcitriol acts indirectly within bone to induce monocytic stem cell differentiation into osteoclasts by stimulating osteoblast/stromal cell synthesis of

osteoclast activating factors, such as RANK-ligand. During periods of calcium deficiency, calcitriol is able to promote bone resorption through increased osteoblast production of osteopontin, a bone matrix noncollagenous protein to which osteoclast cell-surface integrin receptors bind enabling formation of the subosteoclastic lacuna essential for bone resorption. Calcitriol also stimulates osteoblasts to produce osteocalcin, bone-specific alkaline phosphatase, osteoprotegerin, and various cytokines.

A number of synthetic vitamin D₃ analogues have been prepared, including alfacalcidol [1α -(OH)D₃], paricalcitol [19-nor-1,25(OH)₂D₂] calcipotriol [1α ,25-(OH)₂-24-cyclopropyl-D₃], maxacalcitrol [1α ,25-(OH)₂-22-oxa-D₃], and tacalcitol [1α ,24R-(OH)₂D₃] and have been used in the treatment of patients with secondary hyperparathyroidism, osteoporosis, and psoriasis. These vitamin D analogues have been engineered to retain the noncalcemic actions of the parent compound while reducing their calcemic properties. Calcitriol and vitamin D₃ analogues exert many immunomodulatory effects.¹³¹ In animal models of autoimmune diseases, such as experimental autoimmune encephalitis, systemic lupus erythematosus, autoimmune thyroiditis, autoimmune diabetes mellitus, and inflammatory bowel disease, calcitriol exerts a protective effect. Calcitriol and its analogues inhibit T lymphocyte differentiation into Th1 cells that secrete interleukin-2, tumor necrosis factor (TNF)- α , and interferon- γ , thereby experimentally modifying disease induction, course, and severity. Calcitriol and analogues also exert differentiating and antiproliferative effects upon a variety of cells; thus calcitriol induces differentiation of promyelocytes into monocytes and macrophages. These agents enhance differentiation of keratinocytes and when administered orally or topically to patients with psoriasis vulgaris ameliorate the disease; they are particularly effective when coadministered with a topical glucocorticoid. In vitro, calcitriol and its analogues inhibit growth of prostate, breast, and colon cancer cell lines; in vivo, experimentally, calcitriol and its analogues prevent or reduce mammary tumor formation whereas in the absence of the VDR mammary tumor growth is enhanced.¹³¹ Calcitriol prevents hypertension and cardiac hypertrophy in mice in whom VDR or CYP27B1 has been “knocked out.” In humans, inferential data suggest that vitamin D plays a role in the pathophysiology of several nonskeletal disorders, including hypertension and cardiovascular disease, autoimmune disorders, multiple sclerosis, and infectious illnesses.^{62,125,126}

Vitamin D Receptor

The VDR is a 427 AA nuclear transcription factor that is encoded by the 11 exon gene—VDR that is expressed in the intestinal tract, kidney, parathyroid gland, osteoblast, skin fibroblasts and keratinocytes, cells within the brain and cardiovascular system, multiple cells of the immune system, including macrophages, T and B lymphocytes and T-regulatory cells, as well as liver and muscle cells.¹³² Regulation of VDR expression uses promoters that are both proximal to but may also be quite distant from the chromosomal site (chr. 12q12–q14) of VDR; some promoter sites are even located within its introns. Because in exon 2 there are two potential start site codons for transcription of VDR, there is a second isoform of VDR with 423 AA. At its 5′ terminus, VDR has three noncoding exons (1A, 1B, 1C) followed by exons 2 through 9 that encode the active protein enabling transcription of three unique mRNA isoforms, depending on the splicing pattern of exons 1B and 1C. As a member of the steroid-thyroid-VDR gene superfamily of nuclear transcription activating factors, the VDR has multiple domains: a short amino-terminal segment of 24 AA (domains A and B) that houses a ligand-independent

transcription activating function termed activation function-1 (AF-1) that interacts with the general transcription factor IIB, a DNA binding domain (C) with two zinc fingers (exons 2, 3), a “hinge” region (D), and a long carboxyl-terminal domain (E) with ligand and RXR α binding sites and a second transcription activating sequence (AF-2) (exons 7, 8, 9). Two nuclear localization signals are found within and immediately distal to the zinc-finger DNA binding domain, the first of which specifically recognizes the vitamin D response element, whereas the second zinc finger permits heterodimerization with RXR α . Structurally, the carboxyl-terminal E–ligand binding domain is composed of 12 α -helices (H1–H12) and has two ligand-dependent transactivating regions—E1, between AA 232 and 272 and AF2 between AA 416 and 424—that recruit transcriptional coactivating factors (the region is termed a *cistrome*) when the VDR is activated by binding to calcitriol; it also contains sequences that enable heterodimerization with RXR α .¹²⁶

Among the factors that enhance transcription of VDR are calcitonin, retinoic acid, estrogen, and transcription factor SP1. In part, estrogens increase expression of VDR through binding to estrogen receptors present in the caveolae of the cell membrane that then activate the MAPK signal transduction pathway.¹³³ PTH down regulates expression of VDR. The VDR is widely expressed in the intestinal tract, distal renal tubule, osteoblast, keratinocyte, hair follicle, fibroblast, smooth and cardiac muscle, lung, bladder, thyroid, parathyroid, pancreas, adrenal cortex and medulla, pituitary, placenta, uterus, ovary, testis, prostate, activated T and B lymphocytes, macrophages, monocytes, spleen, thymus and tonsil, brain, spinal cord, and sensory ganglia. Inactivating mutations of VDR result in vitamin D-resistant rickets (vitamin D-dependent rickets, type 2A—OMIM 277440).

Whereas most members of the superfamily of nuclear receptor transactivating factors pair as homodimers to bind to their specific hormone response elements of the target gene, the calcitriol-VDR complex teams through its E (ligand binding) domain with its obligate partner—unliganded RXR α (encoded by RXRA) to form a heterodimer that then binds to a VDRE. The endogenous ligand for RXR α is 9-cis-retinoic acid. When unliganded, the bulk of the VDR is cytoplasmic; binding of calcitriol to the VDR leads to heterodimerization with RXR α and translocation of the tripartite complex to the nucleus. Unliganded VDR can also be guided to the VDRE of its target gene by interaction with the Williams syndrome transcription factor (WSTF, encoded by BAZ1B), a component of a multiprotein, chromatin-remodeling complex termed WINAC (WSTF including nucleosome assembly complex); there the VDR remains inactive until bound to calcitriol.¹³⁴ There are several classes of VDREs located in the promoter region of target genes: generic positive, conventional negative, and E-box-like negative.¹³⁵ The generic positive or transcription activating VDRE is composed of two repeated hexanucleotide sequences linked by three nonspecific nucleotide bases—5′...(A/G)GGTCA-nnn-(A/G)TTCA ...3′ (113, 135). RXR α binds to the 5′ half of the VDRE and VDR to its 3′ segment. The conventional negative or transcription repressing VDRE is composed of a single copy of the generic positive VDRE with the nucleotide sequence—5′...(A/G)G(G/T)TCA...3′; it is through the conventional negative VDRE that calcitriol inhibits expression of PTH and PTHLH. The nucleotide sequence of the E-box-like negative VDRE is 5′...CANNTG...3′. After binding to the designated VDRE, the calcitriol-VDR-RXR α -WINAC complex recruits coactivating or corepressing modulating proteins (e.g., runt-related transcription factor 2 [encoded by RUNX2], steroid receptor coactivators [SRC] -1, -2, -3 /p160 family with histone acetyl transferase activity, the chromatin remodeling complex, SWI/SNF, interleukin 6-dependent DNA-binding protein

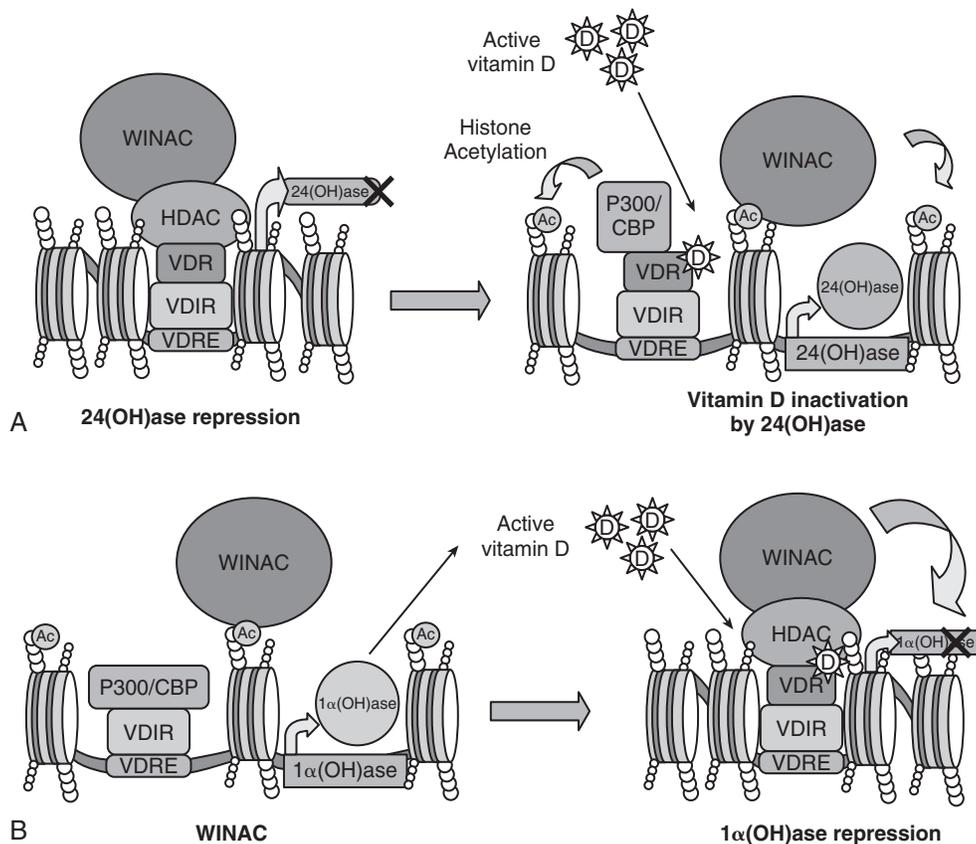


Fig. 9.5 Regulation of vitamin D receptor (VDR) action by interaction with WSTF including nucleosome assembly complex (WINAC) and VDR-interacting repressor (VDIR). A, Under basal conditions, histone deacetylase (HDAC), unliganded VDR-retinoid x receptor (RXR), and VDIR bind to the vitamin D response element (VDRE) of *CYP24A1* encoding 25-hydroxyvitamin D-24 hydroxylase and suppress gene expression; after calcitriol binds to VDR-RXR, HDAC is replaced by a histone acetyltransferase activation complex (p300/CBP), leading to active transcription of *CYP24A1*. B, The autoregulatory effect of calcitriol upon its own synthesis is mediated by its interaction with the VDR-RXR-VDIR-WINAC complex and replacement of histone acetyltransferase by histone deacetylase. (From Barnett, C., Krebs, J.E. (2011). WSTF does it all: a multifunctional protein in transcription, repair and replication. *Biochem Cell Biol*, 89, 12–23. With permission.)

[also designated C/EBP β - encoded by *CEBPB*], TRIP/SUG1, CPB/p300, TIF1) into the promoter site, as well as the general transcription **activating** apparatus of the target gene (TF-IIA, -B, TAF family).^{113,135,136}

Histones are lysine-containing protein components of chromatin, a complex of protein and DNA of which a chromosome is composed that may undergo epigenetic modification; 147 base pairs of DNA wrap around a histone octamer comprised of two copies each of histones H2A, H2B, H3, and H4 forming a nucleosome, a structure that regulates replication, repair, and expression of genes.¹³⁴ Epigenetic regulation of gene expression is achieved, in part, by histone modifying enzyme complexes that remodel chromatin by altering the state of histone methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and proline isomerization.¹³⁷ WSTF is a component of three ATP-dependent chromatin remodeling complexes that alter nucleosome position and thus affect gene exposure to the primary transcription apparatus and accordingly gene expression or silencing; they are directed to sites of action by specific histone modifications.¹³⁴ Within the WSTF protein are a chromatin binding domain (PHD), a tyrosine kinase domain that enables it to phosphorylate histone tyrosine residues, and a binding site for the VDR. In addition to WSTF, the WINAC ATP-dependent chromatin remodeling complex is composed of 12 other subunits; it plays a role in chromatin assembly and cell cycle progression and facilitates activation of *CYP24A1* encoding 25-hydroxyvitamin D-24-hydroxylase

while repressing *CYP27B1* encoding 25-hydroxyvitamin D-1 α -hydroxylase (Fig. 9.5).

Gene activation by the calcitriol-VDR-RXR-WINAC complex is initiated by recruitment of a histone acetyl transferase-helicase complex (e.g., SRC/SWI/SNF) to the promoter segment of the target gene; acetylation of a lysine residue within a histone protein destabilizes the region enabling DNA to unwind, thereby granting basal transcription factors and RNA polymerase II access to the transcription start site thus bringing about RNA modeling of the target gene. Another VDR coactivator is the vitamin D interacting protein (DRIP—encoded by *MED4*) that links the VDRE and the initiation start site of the target gene to RNA polymerase II and its cofactors.¹²⁶ Corepressors cause chromatin to compact by removal of acetyl or addition of methyl groups to lysine residues within a histone protein, thereby silencing gene expression. Inasmuch as the E-box negative VDRE lacks the common hexameric VDRE, this VDRE binds to and gene transcription is inhibited (e.g., *CYP24A1* encoding vitamin D - 24-hydroxylase) by the interaction of unliganded VDR with the VDR-interacting repressor (VDIR encoded by *TCF3*), a process that involves recruitment of a histone deacetylase. Calcitriol inhibits activation of *CYP27B1* encoding 25-hydroxyvitamin D-1 α -hydroxylase by binding to VDR that links to the VDIR-WINAC complex, thereby replacing histone acetyltransferase with a histone deacetylase, thus halting transcription activation (see Fig. 9.5).^{134,135} In addition to regulatory effects upon gene

expression by alterations of histone lysine residues, calcitriol-VDR-RXR-induced methylation of DNA cytosine residues at CpG sites in the promoter region 5' to *CYP27B1* (another epigenetic modification) also represses expression of this gene. Positive and negative regulation of target gene expression by the VDR may also be achieved by interaction of the VDR complex with other transcription factors.¹³⁶ As illustrated in Fig. 9.5, WINAC is involved in both calcitriol-VDR-VDIR-mediated gene transcription and repression.¹³⁵ Deletion or inactivating mutations of *BAXBI* (encoding WSTF) impair the linkage between calcitriol, VDR, and WINAC and thus impede the inhibitory effects of calcitriol upon transcription of *CYP27B1*, resulting in increased synthesis of 25-hydroxyvitamin D-1 α hydroxylase and excessive synthesis of calcitriol, perhaps the pathophysiological process that leads to hypercalcemia in patients with Williams syndrome.

Some of the myriad of genes whose expression is regulated by the VDR are listed in Fig. 9.6.^{109,136} That there may be more than 2000 VDREs within the human genome indicates the potentially widespread effects exerted by the vitamin D system.¹³⁸ Calcitriol acting through the VDR stimulates transcription of genes encoding calcium transport proteins (TRPV5/6), bone matrix proteins (osteopontin, osteocalcin), bone resorption factors (RANK-ligand), and 25OHD-24-hydroxylase and represses those that encode PTH, PTHrP, and 25-hydroxyvitamin D-1 α hydroxylase. The calcitriol-VDR complex also suppresses expression of multiple cytokines (interleukin-2, interferon- γ , granulocyte macrophage-colony stimulating factor) by negatively interacting with transcription factors that enhance their transcription.

Although the VDR is necessary for the actions of calcitriol in the intestines, kidney, bone, skin, and elsewhere, its loss does not interfere with embryogenesis and fetal development in humans with loss-of-function mutations in *VDR* and vitamin D resistance or in mice in which *Vdr* has been "knocked out." In newborn mice, homozygous for targeted deletions that result in truncation of the VDR, osteoid surface is increased, bone mineralization decreased, and epiphyseal plate cartilage formation disorganized with irregular columns of

chondrocytes, increased matrix and excessive vascularity relative to wild-type or heterozygous (*Vdr*^{+/-}) mice.¹³⁹ In addition, hair loss begins at 4 weeks of age and is complete by 4 months of age in homozygous *Vdr*^{-/-} animals and is caused by a defect in the growth cycle of hair follicles, a phenocopy of the generalized alopecia associated with loss of hairless (*HR*), a transcriptional corepressor that interacts with the VDR.¹³⁶ (The hair follicle itself, although dependent on the VDR for physiological cycling, does not require calcitriol; thus maintenance of the normal hair follicle is a ligand-independent function of the VDR.^{62,140}) Maintenance of normal serum concentrations of calcium and phosphate by the feeding of high calcium-phosphate diets to *Vdr*^{-/-} pups beginning at 16 days of age prevents the development of all of the skeletal abnormalities, indicating that the main physiological effects of calcitriol and the VDR are upon the intestinal absorption of calcium and phosphate and the maintenance of normal serum concentrations of these ions.¹³⁹

Calcitriol also has rapid, nongenomic effects mediated through both a cell membrane-associated receptor and the designated nuclear VDR^{128,141} (Fig. 9.7). The cell membrane protein localized to the caveolae (invaginations) that binds 1,25-dihydroxyvitamin D has been designated "membrane-associated rapid response steroid-binding protein" (MARRS-BP encoded by *PDIA3*).^{126,141} After binding of calcitriol to MARRS-BP, its signal is transmitted by several classical intracellular signal transduction systems including: (1) adenylyl cyclase induction of cyclic AMP and PKA; (2) PLC and D-mediated increase in phosphoinositide turnover, resulting in generation of DAG and IP₃ that increase the permeability of Ca²⁺ channels and release Ca²⁺ from storage sites in the endoplasmic reticulum; (3) Gq-protein through PL- β 1 activation and intracellular redistribution of PKC isoforms (α , β , δ); and (4) Jun-activated kinase and the MAPK pathway.¹⁴¹ Within minutes after exposure of a vitamin D responsive tissue (e.g., intestine, chondrocyte, osteoblast) to calcitriol, there is increase in the intracellular concentration of Ca²⁺ (transcalcia) and activation of PLC, PKC, and MAPK.¹²⁷ The complex of calcitriol with its nuclear VDR also has rapid effects mediated

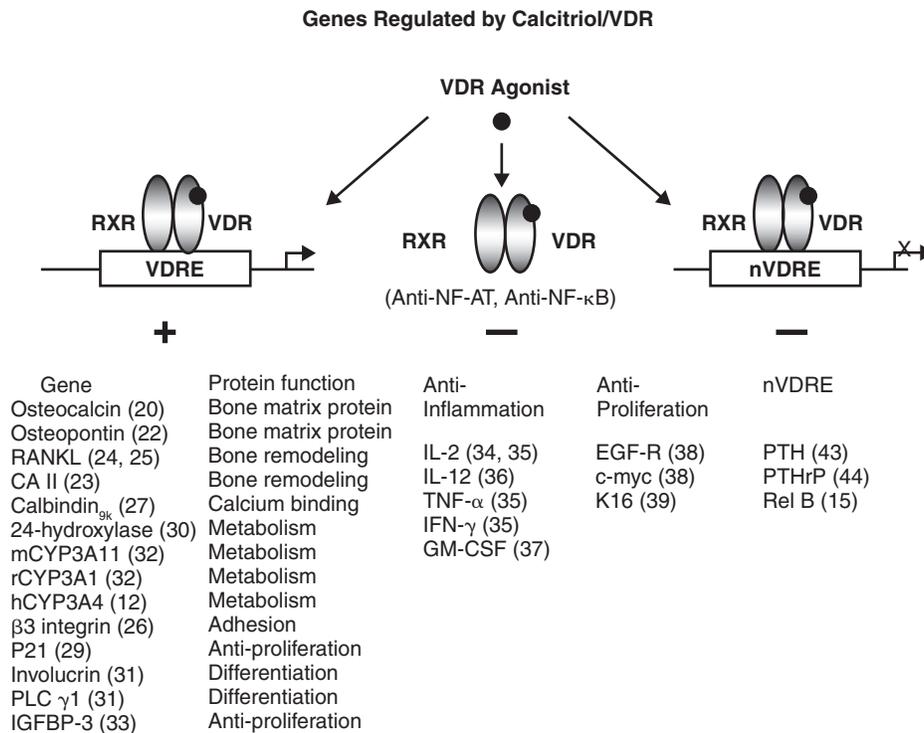


Fig. 9.6 Some of the more than 1000 genes regulated by calcitriol and the vitamin D receptor. (From Nagpal, S., Na, S., Rathnachalam, R. (2005). Noncalcemic actions of vitamin D receptor ligands. *Endocr Re*, 26, 662–687. With permission.)

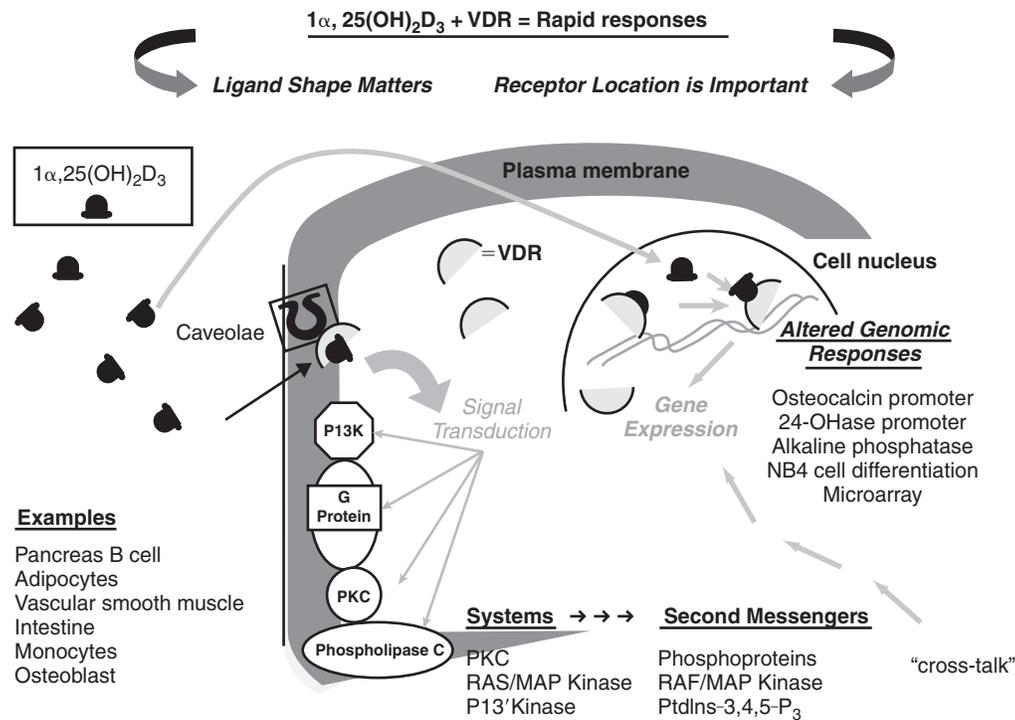


Fig. 9.7 Genomic and nongenomic (rapid) responses to calcitriol. (From Norman, A.W., Bouillon, R. (2010). Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med*, 235, 1034–1045. With permission.)

by the colocalization of nuclear VDR to plasma membrane caveolae and the interaction of both ligand bound nuclear and membrane associated VDRs through linkage to caveolin-1 (encoded by *CAV1*).¹⁴¹ In osteoblasts from nuclear VDR^{-/-} mice and in fibroblasts from patients with inactivating mutations of nuclear VDR, the rapid actions of calcitriol are lost as is the association of the nuclear VDR with caveolae—evidence of the importance of the nuclear VDR for membrane-initiated responses to calcitriol. In chondrocytes, there is MARRS-BP-dependent enhancement of calcium flux, PKC activity, and matrix vesicle mineralization.¹⁴² The plasma membrane caveolae-associated VDR may link to G_{sα} and thence to a calcium channel or adenylyl cyclase or PLC or with caveolin, a protein that interacts with the nonreceptor tyrosine kinase—Src—and in turn with PLC or the kinase h-Ras.^{143,144} The rapid effects of vitamin D may optimize its genomic effects by phosphorylation of proteins required by the VDR transcriptional complex.

SKELETON: CARTILAGE AND BONE

Chondrogenesis and Skeletal Formation

The skeleton is the framework of the body. It is composed of cartilage and bone, specialized cells and forms of connective tissue that provide: (1) mechanical support for muscle/tendon insertion that enables movement; (2) protective shield for soft tissue organs; (3) repository for bone marrow; (4) reserve source of calcium, phosphate, magnesium, and other metabolically important ions; and serves as (5) an endocrine organ as the secreted products of bone cells regulate the function of distant organs—for example, the osteocyte secretes sclerostin (encoded by *SOST*), an inhibitor of bone formation, and FGF23, a factor that depresses renal tubular reabsorption of phosphate and the synthesis of calcitriol.^{116,145} Chondroblasts, osteoblasts, adipocytes, myoblasts, and fibroblasts are derived from a common mesenchymal cell.¹⁴⁶ Skeletal formation begins in utero with transformation of mesenchymal cells

from the lateral plate mesoderm into chondroblasts and chondrocytes that form the long bones of the skeleton (Fig. 9.8).^{146,147} Modeling of bone takes place during intrauterine and postnatal growth when the shape and size of a bone are determined. Mesenchymal stem cells become perichondrial cells, chondroblasts, and chondrocytes under the direction of fibroblast growth factor 2 (*FGF2*), bone morphogenetic protein 4 (*BMP4*), and SRY-Box 5, 6, 9 (*SOX5, 6, 9*). The expression of *SOX9* is stimulated by FGF/FGFR signaling through the MAPK pathway. *SOX9* is a 509 AA protein with an SRY homology domain that is also expressed in the testis where it is responsible for differentiation of Sertoli cells. Target genes of *SOX9* include those that encode collagen type II(α1) and aggrecan (*AGC1*), a chondroitin sulfate proteoglycan core protein that affects the biomechanical strength of cartilage.¹⁴⁸ *SOX9* is expressed not only in chondrocytes in the resting phase but also those in the proliferative phase but not in chondrocytes in the hypertrophic phase of maturation. Chondroblasts are primarily localized to the surrounding perichondrium where they secrete a matrix composed of type II collagen (*COL2A1*), elastin (encoded by *ELN*), and aggrecan. When embedded within cartilage matrix, these cells are termed *chondrocytes*. Chondrocytes pass through sequential developmental stages: (1) resting or basilar chondrocytes are adjacent to and below the secondary ossification center at the end of a long bone; (2) slowly and rapidly proliferating columnar chondrocytes that synthesize collagen type II composed of three cross-linked chains of collagen type II, alpha-1 (*COL2A1*) thus forming collagen type II; (3) prehypertrophic maturing chondrocytes; (4) hypertrophic chondrocytes that synthesize collagen type X consisting of three chains of collagen type X, alpha-1 (*COL10A1*); and (5) terminal chondrocytes that undergo apoptosis (Fig. 9.9). Chondrocyte differentiation, proliferation, and hypertrophy are enhanced by *IHH*, a 336 AA product of prehypertrophic and early hypertrophic chondrocytes that acts through its cell membrane receptor Patched 1 (encoded by *PTCH1*) and coreceptor Smoothed (*SMO*); *IHH* also stimulates the synthesis of

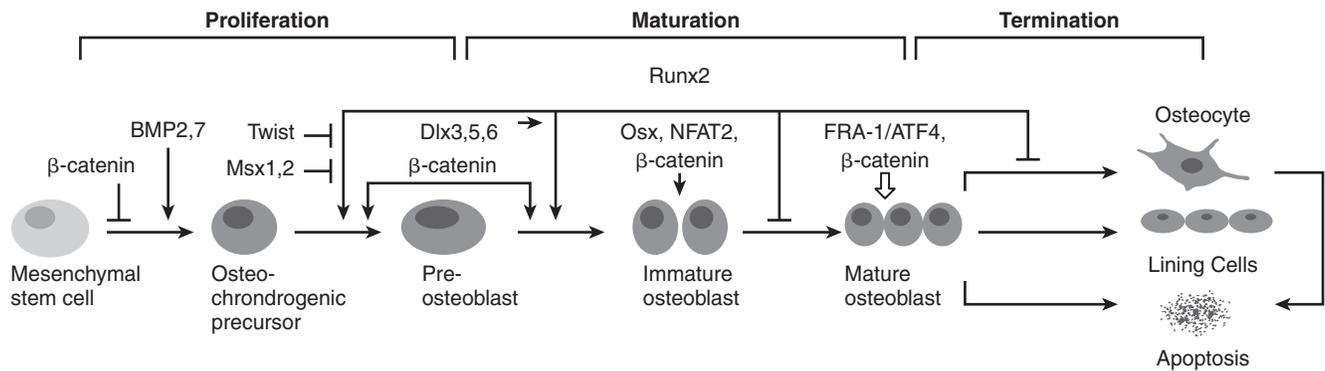
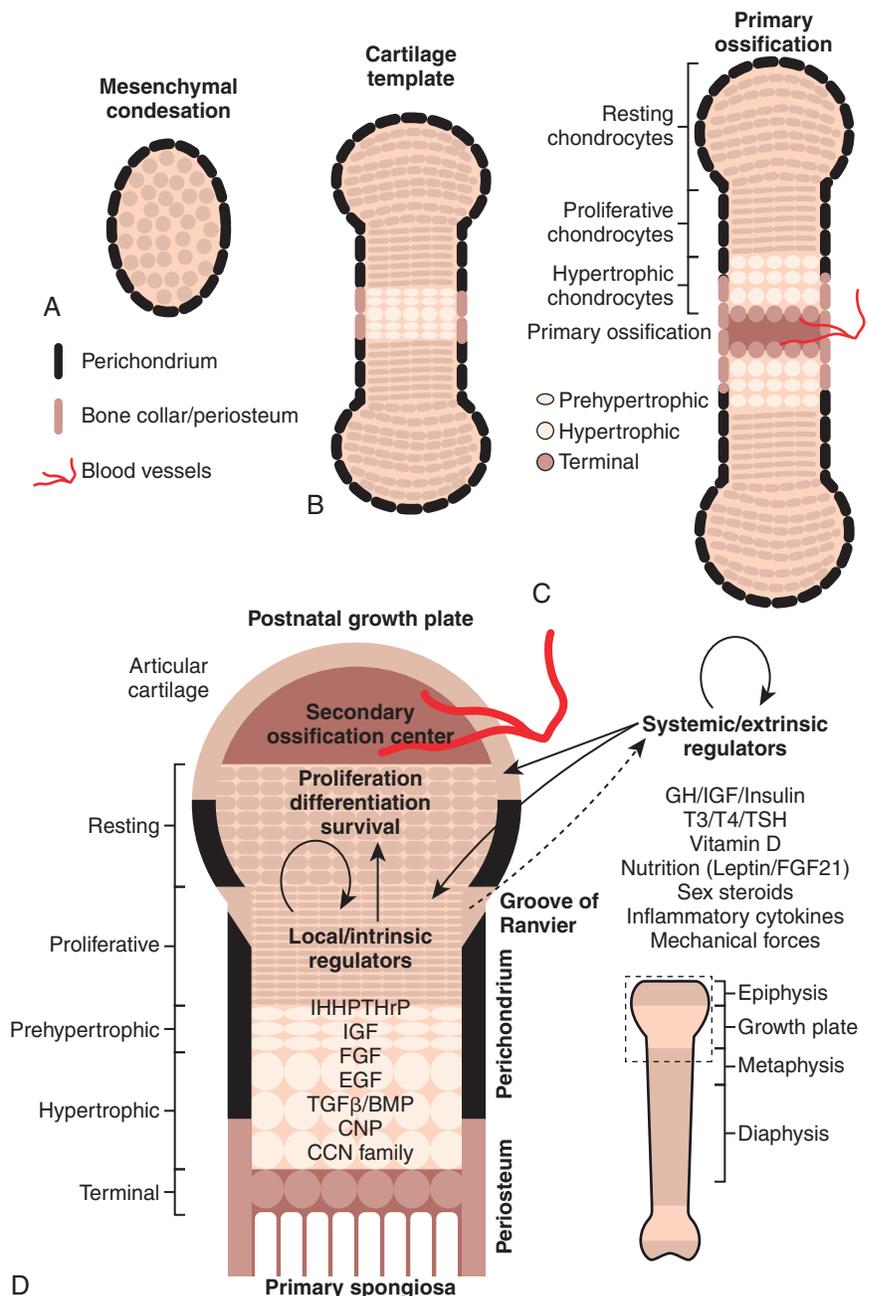


Fig. 9.8 Chondrogenesis/osteoblastogenesis. A common mesenchymal stem cell gives rise to chondroblasts, osteoblasts, myoblasts, fibroblasts, and adipocytes in response to specific differentiating factors. Bone morphogenetic proteins (BMP) are involved in the earliest steps leading to the differentiation of the common mesenchymal precursor cell into chondrocytes and osteoblasts. SRY-box 9 (SOX9) is critically important for differentiation and function of the common progenitor cell of chondrocytes and osteoblasts and for further maturation of osteoblasts. (Peroxisome proliferator-activated receptor γ 2 [PPAR γ 2] stimulates the differentiation of adipocytes. Osteoblasts and adipocytes may be interconverted depending on whether RUNX2 or PPAR γ 2 is the activated transcription factor. MyoD is a muscle-specific transcription factor necessary for development of myoblasts.) (From Krause, C., de Gorter, D.J.J., Karperien, M., ten Dijke, P. (2008). Signal transduction cascades controlling osteoblast differentiation. In: Rosen, C.J. (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 7th ed. American Society for Bone and Mineral Research, Washington, DC, p. 10–16. With permission.)

Fig. 9.9 Differentiation and development of the long bones. The epiphyseal cartilage growth plate is composed of zones of resting, proliferating, prehypertrophic, hypertrophic, and terminal chondrocytes, the latter a transitional zone in which apoptotic hypertrophic chondrocytes and surrounding matrix are replaced by bone, a process mediated by both intrinsic and extrinsic regulatory factors. Indian hedgehog (IHH) is synthesized by prehypertrophic chondrocytes. Receptors for parathyroid hormone-related protein (PTHrP) are expressed by proliferating and transitional chondrocytes. IHH stimulates secretion of PTHrP by periarticular cells and this in turn blocks further differentiation and maturation of late proliferating chondrocytes to hypertrophic chondrocytes, thus prolonging the period of cartilage growth. IHH also acts through bone morphogenetic proteins (BMP) and the Wingless-type mouse mammary tumor virus (MMTV) integration site (WNT)- β -catenin pathway to enhance chondrocyte hypertrophy. Fibroblast growth factors (FGF) also influence chondrocyte proliferation and maturation. (See text for details.) (From Rosello-Diez, A., Joyner, A.L. (2015). Regulation of long bone growth in vertebrates; it is time to catch up. *Endocr Rev*, 36, 646–680. With permission.)



PTHrP by basilar chondrocytes. Differentiation of proliferating chondrocytes into prehypertrophic chondrocytes and of the latter into hypertrophic chondrocytes is inhibited in paracrine fashion by PTHrP functioning through PTH1R; PTHrP is secreted in utero by cells in the proximal periarticular perichondrium and postnatally by chondrocytes in the resting zone of the cartilaginous growth plate and diffuses into the zone of proliferating chondrocytes where it delays further differentiation, thus maintaining their replicative capacity.^{98,149,150} During the process of chondrocyte hypertrophy, individual cell length increases six- to tenfold; as the chondrocyte dies, collagenous fibers are enzymatically digested, the extracellular matrix calcifies, and VEGF is produced.¹⁵¹ Surrounding the cartilage anlagen is a periosteal collar of bone deposited by recently differentiated osteoblasts; in response to VEGF blood vessels, chondroclasts, osteoclasts, osteoblasts, stromal, marrow, and other cells invade the underlying cartilage and establish a primary center of ossification. (IHH is also able to stimulate the conversion of perichondrial cells into osteoblasts.¹⁵⁰) A secondary ossification center forms at the distal ends of long bones (defining the epiphyses) with intervening areas of cartilaginous growth plates that permit bone lengthening by continued proliferation and hypertrophy of chondrocytes and formation of extracellular matrix. Ultimately, the more distal late hypertrophic chondrocytes escape from the inhibitory effects of PTHrP, die, disintegrate, and discharge their contents attracting perichondrial blood vessels with their cellular cargo that invade the extracellular matrix. Chondroclasts and osteoclasts digest the extracellular matrix that has been degraded by proteolysis by matrix metalloproteinases, and crystals of calcium phosphate are deposited in the debris. These are later reabsorbed by osteoclasts after which osteoblasts secrete a matrix of collagen type I into which hydroxyapatite crystals are deposited to form bone. Ultimately, growth plate cartilage disappears as primary and secondary centers of ossification merge, a process regulated by estrogen which appears to accelerate the senescent process in late hypertrophic chondrocytes.¹⁵² Hypertrophic chondrocytes also mediate calcification of cartilage matrix, both of the distal secondary ossification centers at each end of the long bones adjacent to the growth plates and the central primary ossification center. Some hypertrophic chondrocytes develop into trabecular bone osteocytes. FGF18 (*FGF18*) stimulates chondrocyte proliferation, antagonizes chondrocyte differentiation, and induces chondrocyte autophagy.¹⁵³

Endochondral bone formation is the process by which cartilaginous bone is replaced by mineralized bone.¹⁵² The short cuboidal bones of the wrists and ankles, cranial base (ethmoid, sphenoid, occipital, frontal, temporal), and long bones of the limbs (humerus, radius, ulna, metacarpal, femur, tibia, fibula, metatarsal) develop from cartilage templates that are invaded by blood vessels accompanied by chondroclasts, osteoblasts that evolve from perichondrial cells situated in long bones adjacent to the zone of hypertrophic chondrocytes, and osteoclasts.¹⁴⁷ (The flat membranous bones of the skull, scapula, sternum, ribs, and ileum develop directly from osteoblast-mediated trabecular bone formation sandwiched between two layers of compact bone.) The shape of the skeleton is complete by the ninth week of gestation following which, there are many fold increases in skeletal dimensions, volume, and mass.^{152,154–156}

The pattern of endochondral bone development is directed by factors that are independent of bone formation; for example, in transgenic mice in which *Runx2* is inactivated, the cartilage “skeleton” forms normally but is not ossified.¹⁵⁷ Vertebrae evolve from the condensation and segmentation of paraxial mesoderm into somites under the direction and control of *NOTCH1*, *SHH*, and *PAX1* and Notch ligands encoded by *DLL3* and *JAG1*. The appearance of limb buds, proliferating

mesenchymal cells that grow out from the lateral body wall and are capped by an apical ectodermal ridge, heralds development of the cartilage anlagen of the long bones, a process of segmentation directed experimentally by multiple genes (*HOXA1*, *SHH*, *WNT7A*, *FGF4*, *FGFR1*, *BMP4*) among other signaling, receptor, and transcription regulating factors involved in differentiation, paracellular communication, and cell-to-cell interaction.¹⁵⁷ Mitochondrial RNA-processing endoribonuclease encoded by *RMRP* is a ribonucleoprotein that is essential for assembly of ribosomes and cyclin-dependent cell cycle activity, as well as chondrocyte proliferation and differentiation.

Chondrocyte differentiation within the resting zone of the cartilaginous growth plate is stimulated by anterior pituitary GH acting through the cytokine growth hormone receptor (GHR) and the JAK2/STAT5b signal transduction pathway to stimulate hepatic and local synthesis of IGF-I that in turn enhances chondrocyte proliferation and hypertrophy acting through the IGF type 1 receptor (*IGF1R*).¹⁴⁷ The insulin receptor is present in chondrocytes and also enhances their proliferation. Triiodothyronine stimulates chondrocyte maturation and fusion of the growth plate. Androgens act directly in the growth plate to stimulate proliferation of chondrocytes and by conversion to estrogens to accelerate chondrocyte division, their rate of maturation, and epiphyseal fusion. Glucocorticoids inhibit proliferation of chondrocytes and stimulate their apoptosis. In man, inactivating mutations of *PTH1R* result in rapid chondrocyte maturation and Blomstrand chondrodystrophy (OMIM 215045), whereas activating mutations in *PTH1R* lead to impaired chondrocyte maturation and Jansen metaphyseal chondrodysplasia (OMIM 156400). The rate of prehypertrophic and terminal hypertrophic differentiation of chondrocytes is regulated locally by both PTHrP and IHH, the latter by encouraging chondrocyte hypertrophy.¹⁴⁹ Nutritional effects on the growth plate are mediated, in part, by the adipocyte product leptin (*LEP*); obesity accelerates the rate of epiphyseal maturation whereas *FGF21*, whose synthesis is stimulated by suboptimal nutrition, impairs chondrocyte proliferation. Mechanical forces also increase the proliferation of growth plate chondrocytes because immobilization impedes limb growth acting through the MAPK/ERK signal transduction pathway. In addition, signaling through JAK2/signal transducer and activator of transcription (STAT) 5b, mammalian target of rapamycin (mTOR), and HIPPO pathways play important integrative roles in chondrocyte proliferation, hypertrophy, maturation, senescence, and apoptosis.¹⁴⁷

Although *RUNX2* inhibits the initial differentiation of the multipotential mesenchymal stem cell into the chondrocyte pathway, it is essential for progression of the chondrocyte through its later stages of differentiation, as well as for the early stages of osteoblastogenesis.¹⁵⁸ *RUNX2* is the alpha subunit of a transcription factor complex that binds to the promoter region of target genes such as *COLA1* and *MMP13*. As the chondrocyte progresses through its maturational stages of differentiation, proliferation, hypertrophy, and death, the patterns of expression of myriad genes change as do the components of secreted extracellular matrix.^{152,159} For example, experimentally the mesenchymal stem cell expresses among many other genes—*SOX9*, *COL2A1*; the resting chondrocyte—*SRFP5*, *SOX9*, *COL2A1*, *AGC1*, *PTH1R*; the proliferating chondrocyte—*SOX9*, *COL2A1*, *AGC1*, *FGFR3*, *RUNX2*; the prehypertrophic chondrocyte—*BMP*, *COL2A1*, *COL10A1*, *AGC1*, *PTH1R*, *RUNX2*, *VEGF*; the hypertrophic chondrocyte—*COL10A1*, *ALPL*, *IHH*, *RUNX2*, *VEGF*; and the terminal hypertrophic chondrocyte—*COL10A1*, *VEGF*, *MMP13*. During the transition from the resting to the proliferating chondrocyte, functional gene pathways that are involved include the VDR/RXR and BMP signaling systems: in the transition from the

proliferative to the hypertrophic chondrocyte prominent functional gene systems include BMP signaling and components of cellular growth and the cell cycle, such as p53; during the senescent phase of chondrocyte evolution the most prominently expressed signaling pathways are those involving VDR/RXR, MAPK, and WNT/ β -catenin. When the postproliferative chondrocyte begins to hypertrophy, the production of collagen type II declines and that of collagen type IX increases, whereas late hypertrophic and dying chondrocytes synthesize the collagenase—matrix metalloproteinase 13. In addition to IHH, transition from a proliferative to a hypertrophic chondrocyte is stimulated by triiodothyronine working in concert with IGF-I and FGFR3. Triiodothyronine acts through nuclear thyroid hormone receptor α to increase intracellular signaling through the WNT4/ β -catenin pathway. Triiodothyronine also inhibits expression of *PTHLLH*, thus shortening the proliferative phase of chondrogenesis. Increase in activity of FGFR3 accelerates the hypertrophic process. As discussed, IHH inhibits chondrocyte hypertrophy acting through PTHrP that in turn downregulates expression of *RUNX2* and impedes its regulation of hypertrophy associated genes.¹⁵²

FGFs-1, -2, -6, -7, -9, and -18 acting through one of four FGF receptors are also important for normal chondrocyte differentiation and development.¹⁵¹ Normal chondrogenesis is dependent on the balance between the positive regulation of chondrogenesis exerted through FGFR2 and FGFR4 and the negative regulation transmitted through FGFR1 and FGFR3. Mutations in *FGFR1* and *FGFR2* have been associated with syndromes of premature craniosynostosis (Jackson-Weiss, Pfeiffer, Crouzon, and Apert syndromes), whereas variations in *FGFR1* have also been identified in patients with hypogonadotropic hypogonadism. Gain-of-function mutations in *FGFR3*, expressed in chondrocytes in utero and postnatally, are associated with achondroplasia, hypochondroplasia, and related chondrodystrophies. Interestingly, congenital variants of *FGFR4* have not been identified to date. BMPs affect chondrogenesis by increasing production of IHH thus increasing chondrocyte proliferation, but also advancing chondrocyte maturation.¹⁵⁰

Osteoblastogenesis

Osteoprogenitor pluripotent stromal mesenchymal stem cells provide a continuous supply of bone-forming osteoblasts, the network of osteocytes embedded throughout bone that monitors bone integrity and strength, and bone surface lining cells. Osteoblasts are derived from mesenchymal stem cells whose differentiation is under the control of BMP and the WNT1- β -catenin signal transduction pathway.¹⁶⁰ (WNT was derived by combining the names of the *Drosophila* gene *Wingless* with the corresponding mouse gene *Int.*) Bone morphogenetic factors are members of the transforming growth factor (TGF)- β family that bind to TGF β /BMP transmembrane receptors and transmit messages through the MAPK and small mothers against decapentaplegic (SMAD) (*SMAD2*) intracellular signal transduction pathways. SMADs link to and then are released from the TGF β /BMP receptor(s); several may aggregate to form a transcription factor(s) for nuclear target genes that regulate cellular differentiation and growth.¹⁶¹ *WNT1* encodes a secreted glycoprotein that links to a Frizzled receptor (*FZD1*) with seven transmembrane domains on the surface of a mesenchymal multipotential stem cell and directs its differentiation into the osteoblast cell line. *NOTCH1* encodes a cotranscription regulating factor that inhibits initial commitment of the osteoblast precursor cell to further differentiation, but paradoxically enhances proliferation of already committed preosteoblasts, only to block their later progression to mature osteoblasts.¹⁶²

WNT1 also links to low-density lipoprotein receptor-related proteins 5 and 6 (*LRP5/6*), coreceptors that are long chain proteins with a single transmembrane domain expressed on the plasma membrane of the mesenchymal stem cell. LRP5 primarily acts locally to regulate differentiation of osteoblasts and mineralization of bone matrix by enabling the cytoplasmic protein β -catenin (*CTNNB1*) to enter the nucleus of the preosteoblast and further its maturation.¹⁶³ Experimentally, inactivation of both *LRP5* and *LRP6* results in low bone mass, but *LRP6* does so by increasing osteoclastogenesis rather than by decreasing osteoblastogenesis. LRP4, a third member of the LRP family, impairs skeletal mineralization by enabling the inhibitory proteins Dickkopf (encoded by *DKK1*) and sclerostin (encoded by *SOST*) to interact with LRP 5/6 and thereby block the association of WNT1 and LRP5/6 coreceptors. Activating mutations of *LRP5* are associated with an autosomal dominant form of relatively benign high bone mass (OMIM 601884) or in some patients autosomal dominant osteopetrosis type 1 (OMIM 607634), whereas inactivating mutations of *LRP5* result in the autosomal recessive osteoporosis-pseudoglioma syndrome (OMIM 259770) in which, in addition to decreased bone formation because of subnormal rate of osteoblast proliferation, the hyaloid vessels of the embryonic eye fail to regress.¹⁶⁴

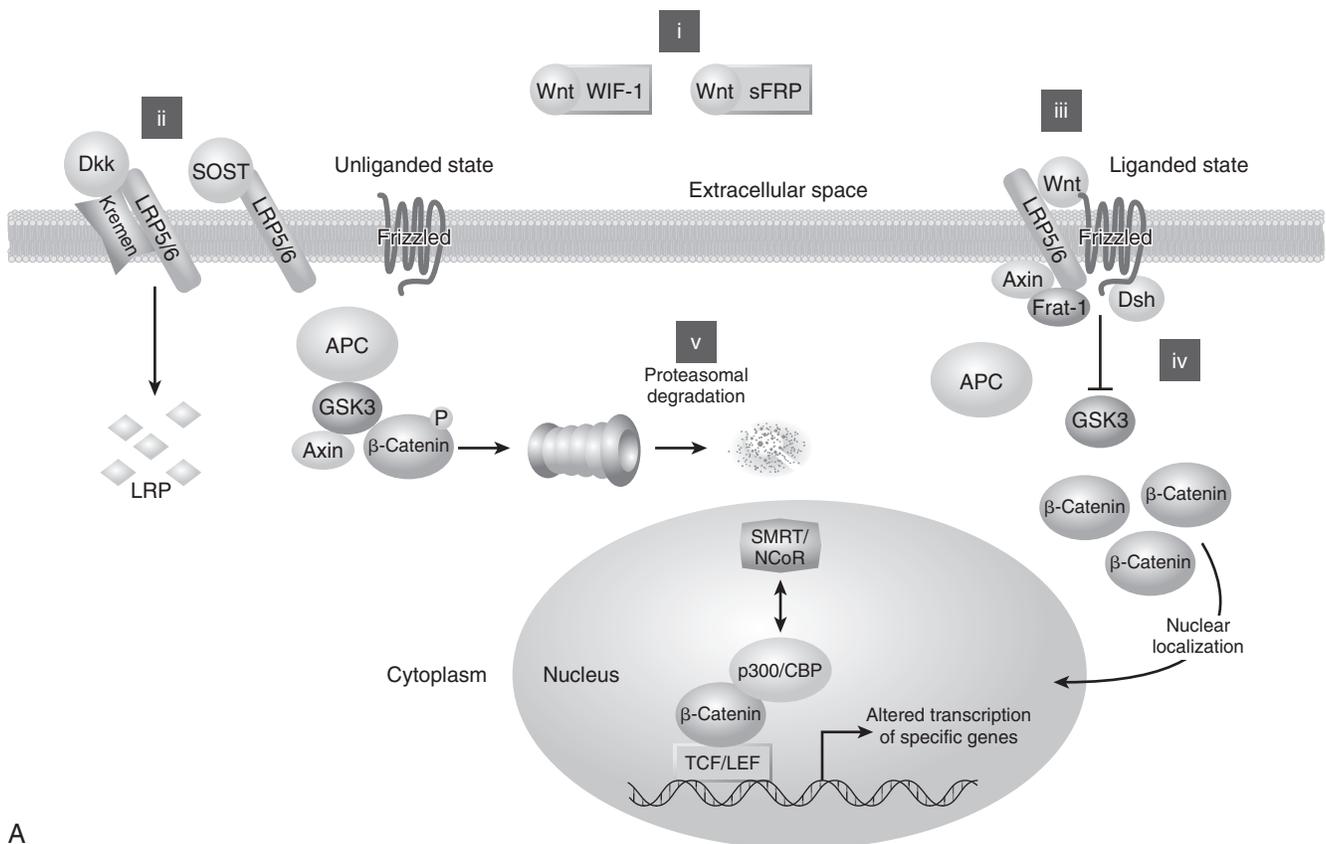
In the resting state of the mesenchymal stem cell, β -catenin is a cytoplasmic protein that is degraded by a “destruction complex” composed of axin (*AXIN1*), the product of *APC*, and glycogen synthase kinase-3 α (*GSK3A*); the latter phosphorylates β -catenin, thereby targeting it for destruction by the ubiquitin-proteasome pathway.^{165,166} When the WNT1 ligand links to the Frizzled membrane receptor and LRP5/6 coreceptors expressed on the plasma membrane of the preosteoblast, the β -catenin “destruction complex” is inhibited, freeing cytoplasmic β -catenin to enter the nucleus and bind to T-cell factor/lymphoid enhancer-binding factor 1 (*LEF1*), a nuclear transcription cofactor that activates WNT1-signaling target genes that result in osteoblast differentiation and function, initially *RUNX2* and its target genes, such as *SP7* (encoding osterix, a 431 AA zinc-finger nuclear transcription factor) (see Fig. 9.8). (Prostaglandin E₂ [PGE₂] is also able to activate *RUNX2* transcription,¹⁶⁷ whereas melatonin too increases differentiation of mesenchymal stem cells into osteoblasts and their synthesis of osterix.¹⁶⁸) Osterix binds to the 5′ promoter of *COL1A1* and is critical for its transcription and hence synthesis of collagen type I(α 1) as well as osteocalcin (*BGLAP*).^{157,158} Osteocalcin plays an important role in normal mineralization of bone matrix. Additional gene targets of *RUNX2* include those encoding *BMP4*, *FGFR1*, *Dickkopf*, *WNT10a*, *WNT10b*, *TGF β 1*, and *FGF18*.¹⁶⁹ *FGF18* is also essential for osteoblast differentiation and proliferation and normal bone development. Expression of *FGF18* is directly stimulated by the WNT-Frizzled-LRP 5/6- β -catenin-*RUNX2* pathway through association with TCF/LEF.¹⁷⁰ *RUNX2* increases expression of the gene encoding TGF β type 1 receptor through which TGF β enhances the transcriptional activity of TCF/LEF.¹⁶⁷ Also involved in the complex process of WNT1 selectivity for the signal transduction pathway(s), leading to osteoblastogenesis are extracellular, transmembrane, and intracellular cofactors, such as *RECK* (reversion-inducing cysteine-rich protein with kazal motifs), *ADGAR2* (adhesion G-protein-coupled receptor A2), and *DVL1* (Disheveled 1).¹⁷¹ WNT signaling also increases production of osteoprotegerin (OPG encoded by *TNFRSF11B*) by the stromal cell/osteoblast, a protein that inhibits osteoclastogenesis, thereby further increasing bone mass. *RUNX2* also mediates the effect of mechanical loading upon osteoblast differentiation and collagen synthesis. After initial osteoblast differentiation, the expression of *RUNX2* declines as the mature osteoblast evolves.

Stromal mesenchymal stem cells are able to differentiate not only into chondroblasts (under the direction of SOX9) and osteoblasts (through WNT-induced signaling of *RUNX2*, *DLX5* [distal-less homeobox 5], and *SP7* [Osterix]), but also into adipocytes (through *PPARG*), myoblasts, and fibroblasts. Committed osteoblasts and adipocytes are capable of redifferentiating into the other cell type depending on whether the expression of *RUNX2* or *PPARG* predominates; this process is directed by WNT10b, which enhances expression of *RUNX2*, *DLX5*, and *SP7* while suppressing that of *PPARG*.^{146,172} The nuclear nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase encoded by *SIRT1* also inhibits the adipocyte differentiating effects of *PPARG* by docking corepressors to this transcription factor, thereby diverting mesenchymal stem cells into the osteoblastogenic pathway.¹⁷³ BMP-2, -4, and -7 induce osteoblastogenesis acting through their heterodimeric cell surface receptors and transduce their intracellular signals through receptor-regulated SMADS 1, 5, and 8 that heterodimerize with DNA-binding SMAD 4 to induce expression of *RUNX2*, *DLX5*, and *SP7*; TGF β signals through SMADs 2 and 3 to promote *RUNX2* expression; platelet-derived growth factor, FGFs, and IGF-I also enhance the proliferation and further differentiation of committed osteoblast precursors.¹⁴⁶ WNT binding to Frizzled-LRP5/6 leads to intracellular signaling not only through the canonical (β -catenin) route but also

through a noncanonical (G α_q -protein and PLC) signal transduction pathway (Fig. 9.10).^{152,174,175}

There are additional factors of importance for bone differentiation and function. Serpin peptidase inhibitor, clade F, member 1 (encoded by *SERPINF1*) is expressed by osteoblasts and is also secreted by liver and bone; it is involved in the processes of endochondral ossification, osteoblast differentiation and maturation, and synthesis of the two subunits of collagen type 1—COL1 α 1 and COL1 α 2.¹⁷⁶ *IFITM5* encodes a protein (interferon-induced transmembrane protein family 5) confined to bone and expressed by osteoblasts whose primary function is uncertain, but may affect stability of *SERPINF1*.¹⁷⁷ Plastin 3 (*PLS3*) is an actin and calcium binding protein critical for the formation of the actin filaments that form the cytoskeleton of the osteocyte, the mature mechanosensory bone cell into which the osteoblast is transformed after being embedded within deeply deposited bone. Dentin matrix acidic phosphoprotein 1 (*DMP1*) is a member of the SIBLING family of secreted phosphoproteins that regulates expression of genes essential for osteoblast development, as well as mineralization of type I collagen.

Several endogenous factors antagonize BMP/WNT1-mediated osteoblastogenesis.¹⁶⁰ Sclerostin (encoded by *SOST*) sequesters and thus antagonizes the effects of BMPs; in the resting state sclerostin binds to the extracellular domains of



A

Fig. 9.10 Influence of Wingless-type mouse mammary tumor virus (MMTV) integration site, member 1 (WNT)- β -catenin signal transduction pathway on osteoblastogenesis. When a WNT ligand binds to its Frizzled receptor and its coreceptor lipoprotein receptor-related proteins (LRP) 5/6, phosphorylation and degradation of β -catenin ceases, enabling its transport into the nucleus where it serves as a cofactor for the T-cell factor/Lymphoid enhancer binding factor (TCF/LEF) transcription factor complex and enhances osteoblast differentiation. The WNT signaling system can be inhibited by binding of sclerostin (SOST) and Dkkopf (DKK) to the coreceptors and of WNT inhibitory factor (WIF1) and secreted Frizzled-related protein (sFRP) to the WNT ligand. (See text for details.) (From Krishnan, V., Bryant, H.U., MacDougald, O.A. (2006). Regulation of bone mass by Wnt signaling. *J Clin Invest*, 116, 1202–1209. With permission.)

Continued

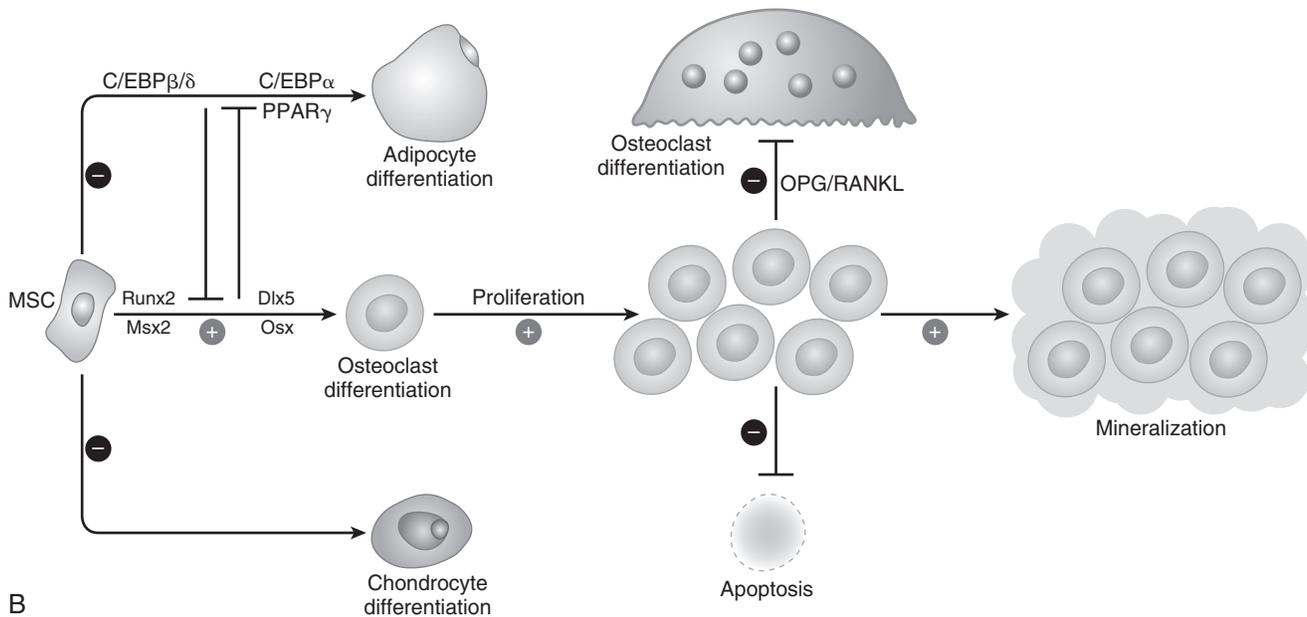


Figure 9.10, cont'd

coreceptors LRP5/6, thereby interfering with their binding to WNT and thus interrupting the WNT-Frizzled GPCR- β -catenin signal transduction pathway, leading to osteoblast differentiation and suppressing bone formation (see Fig. 9.10); sclerostin also binds to LRP4, a linkage that enhances the osteoblast antagonist effects of this 213 AA protein. Sclerostin also increases expression of *TNFSF11* (encoding RANKL), thereby promoting osteoclastogenesis.¹⁷⁸ Sclerostin is primarily expressed and secreted by cortical and trabecular osteocytes in response to decrease in mechanical forces ("unloading") exerted upon the skeleton; as a consequence, the rate of bone formation decreases and that of bone resorption increases.¹⁷⁹ The 232 AA protein noggin (*NOG*) binds to sclerostin, thereby neutralizing its effects on osteoblastogenesis; noggin also antagonizes activity of BMPs for which the inhibitory effect is itself mitigated by binding of sclerostin to noggin. In response to increased mechanical loading, osteocyte expression of *SOST* decreases and the rate of bone formation increases. The inhibitory effect of mechanical loading on osteocyte production of sclerostin may be mediated by paracrine factors, such as prostaglandins, nitric oxide, or oncostatin M. In addition to mechanical forces, the expression of *SOST* and/or synthesis of sclerostin is suppressed by PTH functioning through PTH1R. (There is a high density of binding sites for carboxyl fragments of PTH on osteocyte membranes, suggesting that these sequences may play a role in the mechanosensory activity of the osteocyte network.⁸⁶) Estrogens, cytokines produced by osteoblasts and osteoclasts (oncostatin M, leukemia inhibitory factor, cardiotrophin-1, interleukin-33), prostaglandin E₂, and hypoxia also suppress expression of *SOST*. Calcitonin, osterix, and TNF- α exert a direct stimulatory effect upon osteocyte expression of *SOST* and synthesis of sclerostin. Inactivating mutations in *SOST* result in autosomal recessive sclerosteosis 1 (OMIM 269500) characterized by skeletal overgrowth. Dickkopf-1 (*DKK1*) is a 266 AA protein that binds to the transmembrane LRP5/6 coreceptors for WNT and impedes WNT stimulation of the Frizzled GPCR- β -catenin-mediated signal transduction pathway, leading to osteoblast differentiation from mesenchymal stem cells and hence osteoblast function. Dickkopf-1 is also a ligand for NOTCH1, a single pass

transmembrane receptor that inhibits WNT signaling and RUNX2 activation preventing osteoblast maturation. The structure of the secreted frizzle-related proteins (*SFRP1*) is partially similar to that of the ligand binding domain of the Frizzled heptahelical transmembrane receptors, thereby enabling *SFRP1* to bind to WNT ligand, thereby inhibiting intracellular signaling of the WNT-Frizzled GPCR-LRP5/6- β -catenin complex and therefore osteoblast differentiation. WNT inhibitory factor 1 (encoded by *WIF1*) binds to WNT and also interferes with WNT binding to the Frizzled GPCR and to LRP5/6.

Osteoblasts have a life span of 3 months. They are heterogeneous and express diverse genes that may be dependent or independent of the stage of the cell cycle and extent of differentiation.¹⁷² The heterogeneity of osteoblasts may relate to the many different bone architectures and microenvironments in which they reside and must function. Actively bone-forming osteoblasts have a large nucleus, plentiful Golgi apparatus, and abundant endoplasmic reticulum. When the rate of bone formation is low, osteoblasts are small and quiescent and incorporated into the endosteum separating bone mineral from marrow or into the undersurface of the exterior enveloping periosteum. Differentiated, mature osteoblasts secrete collagenous and non-collagenous proteins including collagen type I, bone-specific alkaline phosphatase, and the calcium and phosphate binding proteins—osteocalcin, osteopontin, and osteonectin—thus making bone matrix competent for mineralization.¹⁸⁰

After the transcription and translation of *COL1A1* and *COL1A2* encoding the subunits of collagen type I, the nascent proteins are modified in the rough endoplasmic reticulum of the osteoblast, further processed in the Golgi apparatus, and then secreted into the matrix surrounding the osteoblast where they undergo further modification. Collagen type I is a trimer of two strands of COL1 α 1 and one strand of COL1 α 2; the helical chains of COL1 α 1 and COL1 α 2 are composed of repeated amino acid trimers of ...Glycine-X-Y... (where X and Y are primarily proline and hydroxyproline, respectively).¹⁷⁷ After synthesis, COL1 α 1 and COL1 α 2 are modified in the osteoblast's endoplasmic reticulum; there they undergo hydroxylation of selected lysine and proline residues, the latter are later converted to pyridinolines. These molecules are

assembled into trimers of two COL1 α 1 fibers and one COL1 α 2 fiber and further processed in the Golgi apparatus of the osteoblast. After being secreted into the matrix, flanking amino- and carboxyl-propeptide domains are removed, thereby exposing amino (N)- and carboxyl (C) terminal telopeptides that then form cross-linked fibers. The carboxyl-propeptide sequence is cleaved by a specific proteinase encoded by *BMP1*. Procollagen type I C- and N-terminal telopeptides (PICP, PINP, respectively) are present and measurable in the circulation enabling assessment of collagen synthesis and processing.

Posttranslational modifications of COL1 α 1 and COL1 α 2 are essential for formation of normal collagen type I and its mineralization. Hydroxylation of proline at AA 986 of COL1 α 1 and at AA 707 of COL1 α 2 forming 3-hydroxyproline is accomplished by the coordinated interaction of the responsible enzyme prolyl 3-hydroxylase 1 (*P3H1*)—also termed *LEPRE1*—interacting with its essential stabilizing cofactors, cartilage associated protein (*CRTAP*) and peptidyl-prolyl isomerase B (cyclophilin B) (*PP1B*). In the absence of this functional tripartite complex, the helical domain of COL1 α 1 is modified by other enzymes leading to alterations in cross-linking that adversely affect the strength of collagen type I.¹⁷⁷ Hydroxylation of lysine residues forming hydroxylysine by procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 (*PLOD1*) at procollagen AA sequences of ...Gly-X-Lys ... in the helical regions of the three subunits that comprise collagen type I is essential for the stability of the pyridinoline cross-links of this molecule. In the telopeptide regions of collagen type I, *PLOD2* (*PLOD2*) is responsible for the formation of hydroxylysine.

Chaperone proteins play important roles as cofactors in the processing of COL1 α 1 and COL1 α 2 fibers.¹⁷⁷ Within the osteoblast's endoplasmic reticulum, stability of the three protein strands comprising collagen type I enabling its correct cross-linking is provided by heat shock protein (HSP) 47 encoded by *SERPINH1*. HSP47 binds to specified arginine residues within the collagen type I propeptides. The chaperone FK506-binding protein (*FKBP10*) may be involved in the hydroxylation of lysine residues of COL1 α 1 and COL1 α 2 and their subsequent cross-linking. *FKBP10* and HSP47 may also interact with one another during formation and maturation of collagen type I.

If the primary synthesis of the amino acid strands of the fibers of COL1 α 1 or COL1 α 2 or their initial glycosylation within the endoplasmic reticulum of the synthesizing osteoblast is incorrect, those strands are destroyed.^{177,181} However, if the corrective processes fail to occur or are overwhelmed, malformed and hence "misfolded" proteins accumulate within the endoplasmic reticulum, subjecting this organelle to "stress," leading to the "unfolded protein response," a process through which continued protein translation is attenuated and the abnormal product destroyed through the ubiquitin-proteasome pathway of protein degradation.¹⁸¹ When the destructive process becomes quantitatively overwhelmed, the endoplasmic reticulum is "stressed" and cellular activity declines eventuating in apoptosis of the stressed cell.¹⁸² Several factors are critical to the normal processing of newly translated subunits (COL1 α 1 and COL1 α 2) of collagen type I in the endoplasmic reticulum and their subsequent secretion into the bone matrix: (1) the cyclic AMP response element-binding protein 3-like 1 (*CREB3L1*) stimulates expression of *COL1A1* by binding to its 5' promoter and is also involved in the processing of osteoblast-synthesized bone matrix proteins and in its response to endoplasmic reticular "stress";¹⁷⁷ (2) membrane-bound transcription factor protease site 2 (*MBTPS2*) is an enzyme that alters several downstream factors that are then active in the endoplasmic reticular stress response; and (3) transmembrane protein 38B (*TMEM38B*), also designated *TRIC-B*, encodes a

homotrimeric intracellular cation (e.g., potassium) channel expressed on membranes of calcium storage sites that affects calcium transport from these organelles. Alteration in intracellular calcium levels affect function of the osteoblast, its endoplasmic reticulum, and the secretion of collagen type.¹⁸³

Osteoblasts, osteocytes, and osteoclasts also synthesize non-collagenous proteins and release them into the surrounding osteoid and into the circulation where they may act upon adjacent or distant tissues.¹⁸⁴ Among the secreted products of osteoblasts and osteocytes is FGF23, which decreases renal tubular reabsorption of phosphate, inhibits synthesis of PTH and calcitriol, and hastens degradation of calcitriol by enhancing synthesis of calcitriol-24 hydroxylase. Osteocalcin (encoded by *BGLAP*) is secreted by osteoblasts and exerts local effects upon bone and more distant effects upon energy metabolism and possibly reproductive function. Osteocalcin is a 49 AA peptide that is released into bone matrix but also into the circulation. It has three glutamic acid residues at AA sites 17, 21, and 24 that can be gamma carboxylated by a vitamin K-dependent gamma-carboxylase. The gamma-carboxylated residues of glutamic acid of osteocalcin bind to calcium on the surface of hydroxyapatite and facilitate formation of hydroxyapatite, growth of its crystals, and hence bone mineralization. During bone resorption many of the gamma carboxyl groups are removed, allowing osteocalcin to be released into the circulation where it may exert indirect and/or direct metabolic effects.^{184–187} Thus in vitro undercarboxylated osteocalcin enhances insulin release from pancreatic beta cells and systemically improves glucose tolerance and increases the synthesis and secretion of adiponectin (which augments insulin sensitivity) from white fat cells. Experimentally, osteocalcin also stimulates release of testosterone from testicular Leydig cells. Lipocalin-2 (*LCN2*) is secreted by osteoblasts and experimentally affects carbohydrate homeostasis by stimulating secretion of insulin and increasing insulin sensitivity; it reduces food intake through binding to hypothalamic melanocortin 4 receptors (*MC4R*) and activating an appetite-suppressing neural pathway.^{187,188}

Osteoblasts control mineralization of matrix, in part, by regulating local concentrations of phosphate through synthesis of cell membrane-bound alkaline phosphatase, which frees organically bound phosphate and by reducing levels of inhibitors of bone formation, such as pyrophosphates, the SIBLING proteins, and their intrinsic phosphorylated ASARM peptides. Calcium and phosphate can then precipitate in bone matrix as hydroxyapatite crystals. The hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂] crystal is the major inorganic component of bone; magnesium, sodium, potassium, and carbonate are also present in bone mineral. Crystalline hydroxyapatite is formed within but at the periphery of the osteoblast from a supersaturated solution of initially amorphous calcium and phosphate, encapsulated within vesicles derived from the interior surface of the osteoblast cell membrane and then extruded into the organic matrix (osteoid). After destruction of the membrane of the matrix vesicle and in concert with the calcium and phosphate-binding proteins bone alkaline phosphatase, osteocalcin, osteopontin, osteonectin, and bone sialoprotein, growth of the hydroxyapatite crystal upon collagen type I fibrils, the organic matrix of bone that serves as a template for the orderly growth of hydroxyapatite crystals, proceeds. This process is antagonized by pyrophosphate, which structurally is two molecules of inorganic phosphate linked by an oxygen bridge. Pyrophosphate is dephosphorylated and hence destroyed by bone alkaline phosphatase, thereby allowing bone mineralization to proceed. Formation of the hydroxyapatite crystal is antagonized by members of the SIBLING family of proteins.

Ectonucleotide pyrophosphatase/Phosphodiesterase 1 (*ENPP1*) is an enzyme that transforms inorganic phosphate to pyrophosphate, an inhibitor of bone matrix mineralization

(thus, ENPP1 has a function opposite to that of alkaline phosphatase). ENPP1 is required for synthesis of Klotho in the setting of high phosphate intake.¹⁸⁹ Mutations in *ENPP1* are associated with autosomal recessive hypophosphatemic rickets type 2 (ARHR2). Family with sequence similarity 20, Member C (*FAM20C*) is a constitutively active serine/threonine kinase that phosphorylates members of the SIBLING family of proteins including DMP1. Loss-of-function variants of *FAM20C* are related to ADHR3 with elevated levels of FGF23 and hypophosphatemia and are also linked to the osteosclerotic phenotype of Raine syndrome (OMIM 259775).

Within the extracellular matrix of bone are noncollagenous, multifunctional proteins that organize, regulate, and coordinate mineralization, serum proteins (e.g., albumin, α 2HS-glycoprotein, growth factors—for example, IGF-I), proteoglycans—proteins with acidic polysaccharide side chains (e.g., chondroitin sulfates, such as aggrecan, perlecan, glypican), glycosylated proteins with cell attachments (e.g., alkaline phosphatase, osteonectin), SIBLING proteins (e.g., osteopontin, BSP, DMP1, MEPE), fibronectin, fibrillins 1 and 2, and γ -carboxylated (Gla) proteins (e.g., osteocalcin, matrix Gla protein, protein S).

Osteoblasts mature into flat, relatively inactive bone lining cells on bone surfaces where neither bone formation nor bone reclamation is occurring and into osteocytes buried deeply within bone.¹⁹⁰ Some bone lining cells extend processes into canaliculi to meet those formed by osteocytes. Bone lining cells express both *TNFSF11* (encoding RANK-ligand) and *TNFRSF11B* (encoding osteoprotegerin) and thus participate in osteoclastogenesis, as well as in bone remodeling. Osteocytes are extremely long-lived cells (25 years) that have matured from osteoblasts embedded in lacunae within formed bone; they are connected to one another and to bone surface osteoblasts by long dendritic filaments (cytoplasmic processes) coursing within canaliculi that link with one another across gap junctions. Osteocytes play a crucial role in *bone remodeling*, the process through which injured sites in existing bone are repaired and reformed through the coordinated and sequential processes of osteolysis and osteogenesis within a bone remodeling unit. Osteocytes summon both osteoclasts and osteoblasts to the site of injury by sensing the mechanical load placed on the bone in which the osteocytes reside by monitoring movement of fluid and pressure within canaliculi, within a basic multicellular unit (BMU) of osteoblasts and osteoclasts. The osteocyte detects the small areas of damaged bone (microfractures) that result from the forces that act continually upon the skeleton.¹⁹¹ For this and other purposes, osteocytes synthesize and release a number of products including: sclerostin, DMP1, IGFBP, RANKL, DKK1, TGF β , PHEX, MEPE, and FGF23.

The “mechanostat” function of the osteocyte is indispensable for maintenance of optimal bone strength, mass, size, and shape. The stimulus for the functional adaptation of bone is mechanical strain.^{192,193} The magnitude of a strain is determined by its force, frequency, and distribution and is also dependent upon the site on which the strain is exerted and by genetic characteristics of the individual upon whom the mechanical load is placed. In response to mechanical deformation, fluid shifts in the lacunae and canaliculi provoke the osteocyte to release nitric acid and prostaglandin-E₂ that recruit osteoblasts to the strain site where new bone is formed beneath the periosteum of long bones and on existing trabeculae (*formation modeling*). When mechanical force on bone is reduced (e.g., immobilization, bed rest, space flight), the rate of osteocyte apoptosis increases. The dying osteocyte releases both macrophage colony-stimulating factor (M-CSF) and soluble RANK-ligand, leading to increase in osteoclastogenesis and bone resorption primarily on the endosteal surface of long bones without comparable increase in osteoblast-directed bone formation, resulting in diminished cortical thickness

and bone strength (*resorption modeling*). At sites of microfractures, signals (M-CSF, sRANK-ligand) from dying osteocytes recruit osteoclasts that, in turn, attract osteoblasts for removal and reformation of bone, respectively (*targeted remodeling*).¹⁹³ Bisphosphonates, sex steroids, and PTH impede osteocytic apoptosis, a property that partially underlies the positive physiological effects of these compounds on bone mass. The strain placed upon long bones by muscular exertion and other forces (e.g., jumping) exerts a positive anabolic effect through stimulation of osteocyte function. The “functional muscle-bone unit” confers the ability of bone to modify its strength, mass, and shape in response to muscular force.¹⁹⁴

Cortical or compact bone is present in the cranium, scapula, mandible, ilium, and shafts of the long bones; both its periosteal and endosteal surfaces are lined with layers of osteogenic cells. Cancellous (trabecular or spongy) bone is located in the vertebrae, basal skull, pelvis, and ends of the long bones. Because only 15% to 25% of trabecular bone volume is calcified (compared with 80%–90% of cortical bone volume) and thus has a far greater surface area, trabecular bone is metabolically quite active; it has a high turnover rate making it more vulnerable to disorders that adversely affect bone mineralization. In flat bones (skull, ilium, mandible), intramembranous ossification begins with the local condensation of mesenchymal cells that differentiate directly into preosteoblasts and osteoblasts and initiate the formation of irregularly calcified (woven) bone that is then replaced by mature lamellar bone. Membranous bones grow by apposition, a process supported by development of new blood vessels induced by VEGF, a protein that also enhances bone formation.¹⁹⁵ The external surface of bone is enveloped by periosteum (containing blood vessels, nerve terminals, osteoblasts, and osteoclasts), whereas the interior of bone next to marrow is lined by endosteum. The periosteum is a fibrous network in which osteoblasts synthesize peripheral compact bone; cortical bone reinforces bone strength and complements and extends that provided by trabecular and endosteal bone. Tendons and ligaments insert and are fixed into cortical bone.

Osteoclastogenesis

Osteoclasts are multinucleated giant cells that adhere to the surface of bone and form a subosteocytic lacuna into which the osteocyte secretes hydrochloric acid to dissolve the mineral phase of bone (hydroxyapatite) and proteolytic enzymes to digest organic matrix.¹⁹⁶ Osteoclasts are derived from hematopoietic progenitor cells and macrophages through signaling by M-CSF, CSF1 acting through its receptor (CSF1R—also designated c-fms) situated on the cell membrane of the osteoclast precursor cell (Fig. 9.11A).¹⁶⁰ M-CSF induces transcription of hematopoietic transcription factor PU.1 (*SPI1*) that furthers osteoclast differentiation. In response to PTH (or PTHrP), calcitriol, prostaglandins, interleukins-6 and 11, TNF- α , and other cytokines, osteoblasts and bone marrow stromal cells synthesize both M-CSF and RANK-ligand (*TNFSF11*) expressed on their plasma membrane surfaces.¹⁹⁷ M-CSF also induces expression of *TNFRSF11A* (encoding RANK) on the surface of osteoclast progenitor cells that then enhances their further differentiation and activation. RANK-ligand is a 317 AA protein composed of cytoplasmic (48 AA), transmembrane (21 AA), and extracellular (248 AA) domains with the binding site for RANK extending between AA 137 and 158. *TNFSF11* is also expressed in lymphoid tissue (where it is critical for development of the immune system), striated and cardiac muscle, lung, intestines, placenta, thyroid, prechondroblast mesenchymal cells, and hypertrophic chondrocytes. In addition to furthering osteoclast differentiation, RANK-ligand enhances function of the mature osteoclast and inhibits its apoptosis. RANK-ligand

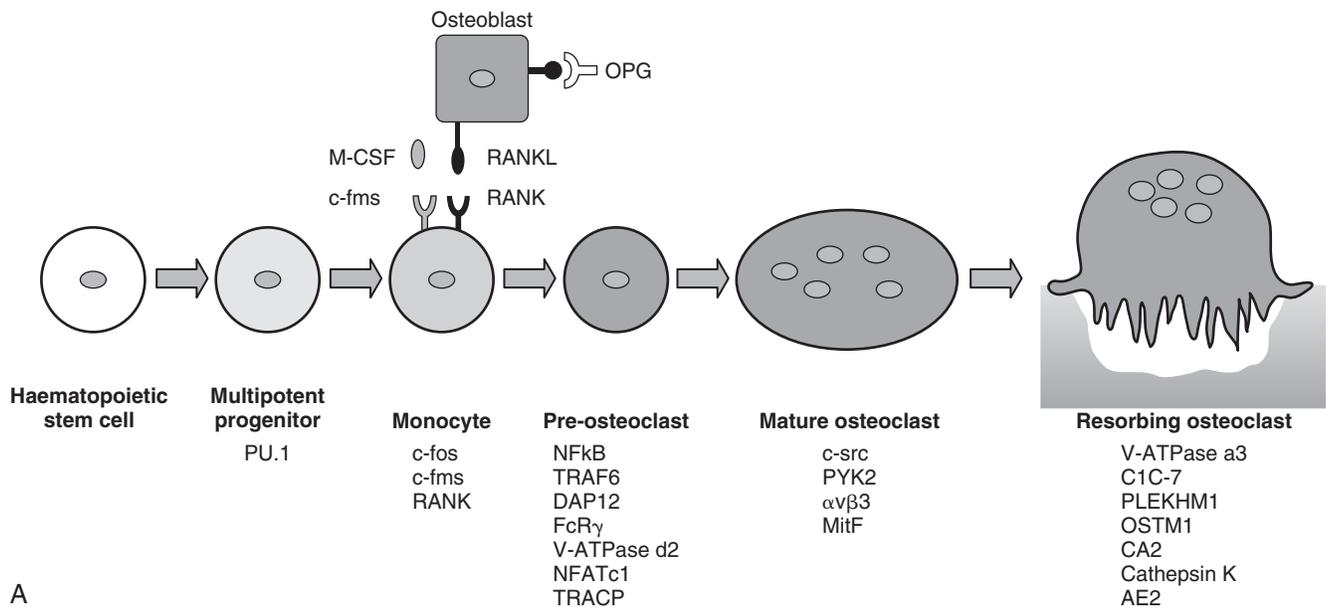


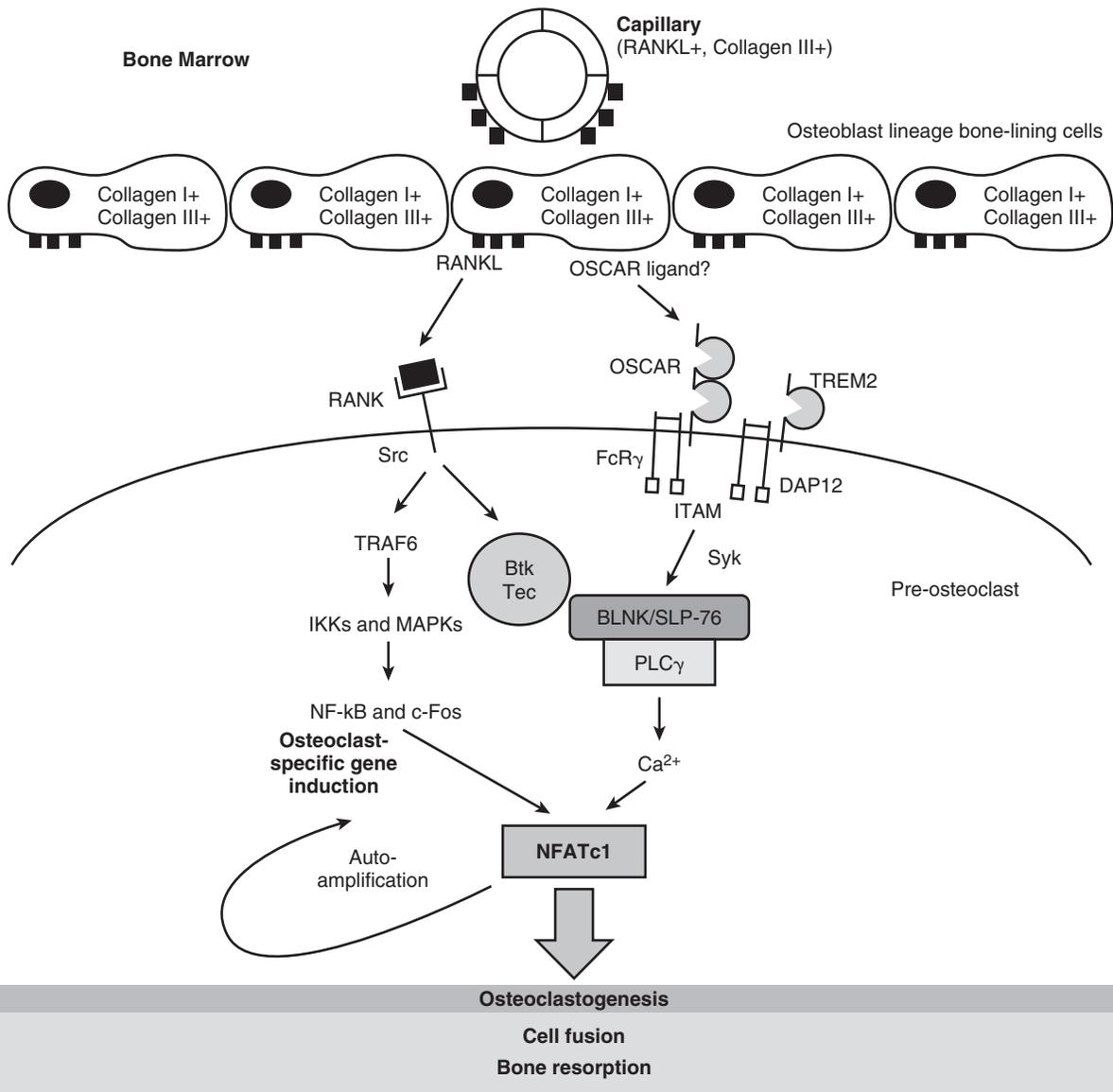
Fig. 9.11A Regulation of osteoclastogenesis. After initial differentiation following exposure of a mesenchymal stem cell to macrophage colony-stimulating factor (M-CSF), RANK-ligand synthesized by stromal cells and osteoblasts binds to RANK expressed on the plasma membrane of preosteoclasts, which signals through steroid receptor coactivator (SRC), tumor necrosis factor-associated factor 6 (TRAF6), and mitogen-activated protein kinase (MAPK) to enable nuclear factor (NF)-κB and c-Fos to translocate NFATc1 to the nucleus where it assists in the maturation of the preosteoclast into a mature osteoclast and serves as a transcription factor for osteoclast-specific proteins (tartrate-resistant acid phosphatase, cathepsin K, β_3 -integrin, calcitonin receptor). (From Henriksen, R., Bollerslev, J., Everts, V., Karsdal, M.A. (2011). Osteoclast activity and subtypes as a function of physiology and pathology—Implications for future treatments of osteoporosis. *Endocr Rev*, 32, 31–63. With permission.)

stimulates transcription of many osteoclast-specific genes and also enhances the development of calcium resorption lacunae and pits.¹⁹⁷

RANK is a 616 AA protein with a signal peptide (28 AA), cytoplasmic domain (383 AA), transmembrane domain (21 AA), and extracellular domain (184 AA) that is expressed in osteoclasts, fibroblasts, and B and T lymphocytes. Binding of RANK-ligand to RANK leads to transmission of an intracellular message through stimulation of the MAPK/ERK signal transduction pathway that activates NF-κB and c-Fos (encoded by *FOS*) (Fig. 9.11B).¹⁹⁸ *IKBKKG* encodes a phosphokinase that also activates NF-κB furthering osteoclastogenesis. The NF-κB family of five dimeric transcription factors includes NF-κB1 and NF-κB2 that are crucial for osteoclastogenesis; NF-κB is activated by degradation of the inhibitor of κB (IκB encoded by *NFKB1A*)—a protein that traps NF-κB in the cytoplasm and is itself destroyed through the ubiquitination/proteasomal pathway after phosphorylation of its serine residues. Free NF-κB then acts via c-Fos and the AP-1 complex of factors involved in movement of proteins to stimulate osteoclastogenesis in concert with the 827 AA master osteoclastogenic transcription factor encoded by *NFATc1*.^{196,198} Nuclear NFATc1 interacts within a transcriptional complex to stimulate expression of genes encoding osteoclast-specific proteins, such as tartrate-resistant acid phosphatase (TRAP, *ACP5*), matrix metalloproteinase 9 (*MMP9*), carbonic anhydrase II (*CA2*), cathepsin K (*CTSK*), the α_3 subunit of vacuolar $[H^+]$ -ATPase (encoded by *TCIRG1*), chloride channel 7 (encoded by *CLCN7*), osteopetrosis-associated transmembrane protein 1 (encoded by *OSTM1*), $\alpha_v\beta_3$ -integrin (*ITGB3*), and the calcitonin receptor (*CALCR*), leading to terminal differentiation of osteoclasts.¹⁹⁸ After binding of RANK-ligand to RANK and subsequent signal transduction, preosteoclasts fuse to become mature multinucleated osteoclasts. Osteoclastogenesis is also stimulated by cytokines, such as *TNF α* and interleukins-1-beta (*IL1B*) and -6 (*IL6*). *NOTCH1* encodes a cotranscription

regulating factor that inhibits initial commitment of the osteoclast precursor cell to further differentiation, but paradoxically stimulates further maturation of cells already committed to differentiation as osteoclasts.¹⁹⁹

The osteoblast also synthesizes, expresses on, and releases from its cell membrane osteoprotegerin (*TNFRSF11B*), a decoy protein that binds RANK-ligand and inhibits osteoclast differentiation. Mature B-lymphocytes also secrete osteoprotegerin.²⁰⁰ Osteoprotegerin is a member of the TNF receptor superfamily and is synthesized and secreted by the stromal cell/osteoblast; it acts as a decoy receptor by binding to RANK-ligand, thus inhibiting the interaction of RANK-ligand and RANK and thereby interfering with osteoclastogenesis.²⁰¹ The five exon gene (*TNFRSF11B*) encoding human osteoprotegerin is expressed also in the lung, liver, heart, kidney, intestinal cells, brain, thyroid, lymphocytes, and monocytes. Human osteoprotegerin is synthesized as a 401 AA propeptide; after cleavage of the 21 AA signal peptide, the mature protein of 380 AA contains four cysteine-rich amino-terminal domains and two carboxyl-terminal “death” domains; it is glycosylated and released into the paracellular space as a disulfide-linked homodimer. Osteoprotegerin synthesis is enhanced by interleukins-1 α and -1 β , TNF- α and - β , BMP-2, TGF β , and estrogen, and antagonized by calcitriol, glucocorticoids, and PGE₂. Through binding to RANK-ligand on the surface of the stromal cell/osteoblast or to its circulating secreted form, this decoy receptor inhibits the osteoclast activating and bone reabsorbing effects of calcitriol, PTH, and the interleukins. Overproduction of osteoprotegerin in transgenic mice leads to osteopetrosis whereas its knock-out is associated with loss of cortical and trabecular bone and osteoporosis, multiple fractures, and hypercalcemia; the latter model is the experimental counterpart of juvenile Paget disease (OMIM 239000).²⁰² The osteoblast and osteoclast also interact directly with one another; the osteoclast expresses on its cell surface the coupling factor ephrin-B1 (*EFNB1*) and secretes ephrin B2 (*EFNB2*), whereas the



B

Fig. 9.11B RANK-ligand produced by osteoblasts and stromal cells binds to RANK expressed on the surface of the osteoclast precursor cell; signal transduction through TRAF6 and NF-κB enables the translocation of NFATC1 into the nucleus and further maturation of the osteoclast. The costimulatory stimulus for osteoclastogenesis proceeds through OSCAR and TREM2, membrane proteins that recognize as ligands amino acid sequences within the structures of collagen types I and III. These receptors then signal through ITAM adaptors to stimulate phospholipase Cγ that converts membrane bound phosphatidylinositol to inositol trisphosphate (and DAG) resulting in mobilization of Ca²⁺ from storage sites in the endoplasmic reticulum and increase in cytosolic Ca²⁺ concentrations that also activate NFATC1 and stimulate osteoclastogenesis. (See text for details.) *BLNK*, B-cell linker protein; *ITAM*, immunoreceptor tyrosine-based activation motif; *NFATC1*, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1; *OSCAR*, osteoclast-associated receptor; *PLCγ*, phospholipase Cγ; *RANK*, receptor activator of nuclear factor κB; *RANKL*, RANK ligand; *SLP-76*, SH2 domain-containing leukocyte protein-76 kD; *TREM2*, triggering receptor expressed on myeloid cells-2). (From Barrow, A.D., Raynal, N., Andersen, A.L., et al. (2011). OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12- deficient humans and mice. *J Clin Invest*, 121, 3505–3516. With permission.)

osteoblast expresses the ephrin tyrosine kinase receptor (*EPHA1*); their interaction stimulates osteoblastogenesis.¹⁶⁰

Bone modeling, the initial process in bone formation, is accomplished by the independent action of osteoblasts and osteoclasts and is not dependent upon prior bone resorption.²⁰³ Bone remodeling is the process during which the strength, structure, and function of bone are renewed; bone resorption and deposition are sequentially linked. Bone remodeling is accomplished within the bone remodeling unit (BRU)

of designated osteoclasts and osteoblasts; it is a continuous process in which old cancellous and cortical bone is reabsorbed and replaced by new bone and takes place in the growing, as well as the mature skeleton. The bone remodeling unit is 1 to 2 mm in length, 0.2 to 0.4 mm in width, led by osteoclasts, and trailed by osteoblasts; in the adult skeleton, its life span is 6 to 9 months and 10% of the skeleton is “remodeled” each year. The site selected for remodeling is targeted by osteocytes sensing a mechanical or stress defect (microfracture), thereby

Schematic illustration of the differences between acid secretion and proteolysis during osteoclastic bone resorption, illustrating that the collagen matrix is removed by proteolysis after acidification

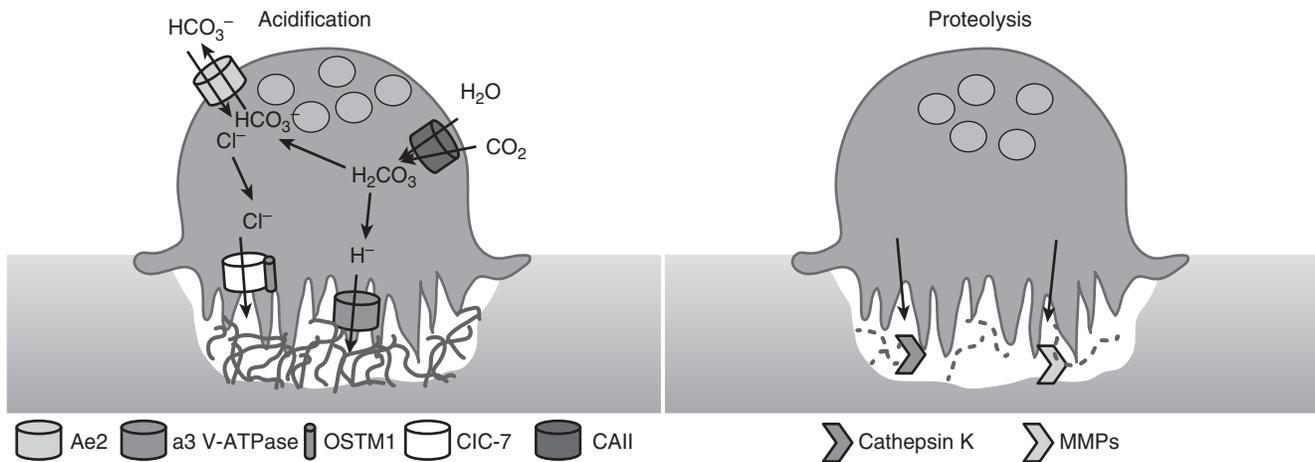


Fig. 9.12 Differentiated osteoclast form a ruffled border by adhering to bone surface through $\alpha\beta_3$ integrin receptors. A subosteoclast lacuna is formed by the dissolution of bone mineral by osteoclast secreted hydrochloric acid (*left panel*) and the resorption of organic bone matrix by cathepsin K and matrix metalloproteinases (*right panel*). Subsequently, osteoblasts are attracted to this pit (by the high local Ca^{2+} concentrations) as new bone is formed in the continuing process of bone remodeling. (From Henriksen, R., Bollerslev, J., Everts, V., Karsdal, M.A. (2011). Osteoclast activity and subtypes as a function of physiology and pathology - Implications for future treatments of osteoporosis. *Endocr Rev*, 32, 31–63. With permission.)

initiating bone remodeling. Osteoclasts are then summoned to the area of injured bone, bind to the insulated site, seal the microfracture using integrins present on the osteoclast's cell membrane, and develop a ruffled border below which a subosteocytic lacuna is formed by digestion of adjacent bone (Fig. 9.12). The mineral phase of bone (hydroxyapatite) is reabsorbed by acid (H^+) synthesized by *CAII* resulting in formation of rapidly dissociating carbonic acid (HCO_3^-) and the secretion of H^+ through the vacuolar H^+ -ATPase proton pump (encoded by *TCIRG1*) embedded within the ruffled border. To maintain neutrality in the lacuna, chloride (Cl^-) is also transported into this space through the H^+ - Cl^- -exchange channel encoded by *CLCN7*. The stability of *CLCN7* is maintained by osteopetrosis-associated transmembrane protein 1 (*OSTM1*). Osteoclasts also express *SLC4A2* encoding a $\text{Cl}^-/\text{HCO}_3^-$ exchanger that maintains normal acid/base balance within the cell.²⁰⁴ The osteoclast synthesizes and secretes into the lacuna several enzymes, including cathepsin K (*CTSK*) and zinc-dependent matrix metalloproteinases that digest and dissolve the organic matrix of bone. The digested debris within the lacuna is encapsulated within vesicles and transported across the osteoclast and released into the extracellular compartment. *SNX10* and *PLEKHMI* encode proteins that are necessary for vesicular maturation and transport within the osteoclast.²⁰⁵

RANK-ligand/RANK signaling also stimulates tyrosine kinases that form an osteoclastogenic signaling complex that activates PLC, leading to cleavage of membrane-associated phosphatidylinositol-4,5-bisphosphate to DAG and IP—the latter mobilizes Ca^{2+} from intracellular storage sites within the endoplasmic reticulum, resulting in increased cytosolic Ca^{2+} levels and activation of calcineurin and NFATC1.²⁰⁶ In the preosteoclast, there is an essential costimulatory signaling system of immunoglobulin-like receptors through which NFATC1 is also activated. The osteoclast-associated receptor (*OSCAR*) and the triggering receptor expressed on myeloid cells 2 (*TREM2*) are transmembrane c-stimulatory receptors expressed on the plasma membrane of preosteoclasts whose ligands are sequences of amino acids (...GPOGPX'GFX'...) derived from collagens types I, II, and III of extracellular matrix

to which preosteoclasts are exposed at sites of bone formation (see Fig. 9.11B).¹⁹⁸ The costimulatory receptors associate with intracellular adapter proteins that have been phosphorylated by RANK-ligand/RANK signaling. The complex of costimulatory receptors and other intracellular factors contribute to activation of *NFATC1*.^{196,198,206} *NFATC1* is capable of autoamplifying its own expression by linking to an NFAT-binding site within the promoter region of *NFATC1*, a site that is activated by epigenetic mechanisms that regulate histone acetylation and methylation.¹⁹⁶

During osteoclast differentiation, preosteoclast cell membranes fuse forming the mature multinucleated giant cell osteoclast. Maturing osteoclasts develop circumferential podosomes (adhesion structures with abundant actin that can degrade matrix) that are critical for cell-cell fusion.²⁰⁷ Syncytin-1 (encoded by *ERVW1*) is a plasma membrane protein that is involved in osteoclast fusion as are several adhesion factors and integrins.^{208, 209} In addition to the contribution of the endoplasmic stores of Ca^{2+} to cytosolic Ca^{2+} concentrations, extracellular Ca^{2+} enters the differentiating and mature osteoclast through TRPV4 and 5.¹⁹⁶ TRPV4 plays a role in osteoclast differentiation, whereas TRPV5 is important for the functional activity of the mature osteoclast. In the subosteoclast resorption lacuna, Ca^{2+} levels exceed 40 mM (serum Ca^{2+} values range between 1.1–1.3 mM); consequently, as intraosteoclast Ca^{2+} concentrations rise, bone resorbing activity is inhibited, and the osteoclast ages and dies. Concurrently, the high Ca^{2+} environment around the resorption lacuna is an attractant for osteoblasts that produce the bone needed to fill the lacuna. Thus, the processes of bone resorption and formation are coupled, in part, by lacunar levels of Ca^{2+} .

When mature osteoclasts attach to bone, the inferior surface of the osteoclast develops a ring structure composed of β -actin filaments and $\alpha_v\beta_3$ integrins that bind to matrix-embedded osteopontin and other components that contain the amino acid sequence – Arg-Gly-Asp (RGD) – thereby forming a sealed zone and creating an isolated microenvironment between the osteoclast's inferior cell membrane and the outer bone surface—the resorption lacuna (see Fig. 9.12).¹⁹⁷ The inferior

or apical surface of the osteoclast within the sealed zone develops an irregular ruffled border through which are secreted products of the osteoclast. Into the isolated sealed resorption lacuna the osteoclast pumps: (1) acid (H^+ or protons) generated from carbon dioxide by CA2 and actively transported via a vacuolar type ATPase proton pump (*TCIRG1*) and chloride ions passively transmitted through a chloride channel (*CLCN7*) to form a highly acidic (pH 4.5) milieu that dissolves hydroxyapatite, and (2) lysosomal proteolytic enzymes (such as the cysteine proteases—cathepsins K, B, and L—and collagenases, such as *MMP9*) that digest osteoid, the protein matrix of bone.^{197,210} Osteopetrosis-associated transmembrane protein 1 (encoded by *OSTM1*) is a 334 AA protein that is essential for normal processing of *CLCN7* and hence for normal acidification of the subosteocytic resorption lacuna and consequent dissolution of hydroxyapatite. Osteopetrosis associated with large numbers of osteoclasts develops in patients with loss-of-function mutations in the genes encoding CAII, the vacuolar ATPase proton pump, the *CLCN7* chloride channel, and associated proteins, whereas inactivating mutations in the gene encoding cathepsin K lead to pycnodysostosis.¹⁹⁷ After the osteoclast contacts bone, it functionally polarizes into two realms—the inferior portion of the osteoclast above the ruffled membrane transports protons, chloride ions, and enzymes from the interior of the cell into the subcellular space and reabsorbs the products degraded by these agents; the superior portion of the osteoclast processes and excretes the reabsorbed materials. TRPV5, the calcium channel required for intestinal and renal tubular absorption/reabsorption of calcium, is also found on the ruffled membrane of the osteoclast.²³ Multiple osteoclast subtypes have been described whose individual function depends on the genes the osteoclast expresses, the anatomic location of the osteoclast, and the functional response of the osteoclast to exogenous agents.¹⁹⁷ Thus there are subtle distinctions between endochondral and membranous bone osteoclasts, trabecular and cortical osteoclasts, and osteoclasts involved in targeted and stochastic bone remodeling. (Targeted bone remodeling occurs locally and involves replacement of damaged bone by new bone to maintain bone strength; stochastic bone remodeling occurs systemically in response to PTH secreted as the Ca^{2+} concentration falls.)

After digestion and resorption of injured bone have been accomplished, osteoblasts converge on the site of osteoclastic

activity. TGF β secreted by osteoblasts and stored in bone matrix is released by osteoclast-mediated bone resorption; it attracts mesenchymal precursor cells that differentiate into osteoblasts, thereby coupling bone resorption and formation and increasing osteocyte numbers.¹⁷⁷ IG-1 is a second osteoblast chemoattractant that is also stored in bone matrix and released by osteoclastic activity. In addition, osteoclasts secrete several “clastokines” that may be osteoblast differentiating factors, for example, WNT-Frizzled associated collagen triple helix repeat-containing protein 1 (encoded by *CTHRC1*) or chemoattractants that summon osteoblasts to the bone remodeling site—for example, sphingosine-1-phosphate (*S1PR1*).²¹¹ Thus the process of bone remodeling involves the sequential steps of activation (identification of the site of insulted bone by osteocytes), resorption (osteoblast-mediated recruitment and maturation of osteoclasts), reversal (removal of lacunar debris), formation (attraction of osteoblasts, leading to reformation of removed bone), and termination (conclusion of the remodeling cycle).²⁰⁰

Bone Extracellular Matrix

Organic matrix proteins comprise 35% of bone, and type I collagen makes up 90% of these proteins.¹⁸⁰ Type I collagen is composed of a coiled triple helix of two polypeptide chains of collagen type I(α 1) (*COL1A1*) and one of collagen type I(α 2) (*COL1A2*) that are cross-linked intramolecularly by disulfide bonds and intermolecularly at the amino (N) and carboxyl (C) telopeptides by pyridinium compounds that permit bundling of collagen molecules into fibrils and fibers (Figs. 9.13 and 9.14). The helical chains of COL1 α 1 and COL1 α 2 are composed of repeated amino acid trimers of ...Glycine-X-Y... (where X and Y are primarily proline and hydroxyproline, respectively).¹⁷⁷ Glycine permits the chains to coil. After their synthesis, the components of collagen type I (COL1 α 1/COL1 α 2) are modified in the osteoblast's endoplasmic reticulum; there they undergo hydroxylation of selected lysine and proline residues, the latter are later converted to pyridinolines. After the assembly of two molecules of COL1 α 1 and one of COL1 α 2, they are further processed in the Golgi apparatus of the osteoblast. After being secreted into the matrix, flanking amino- and carboxyl-propeptide domains are removed, thereby exposing amino- and carboxyl terminal telopeptides

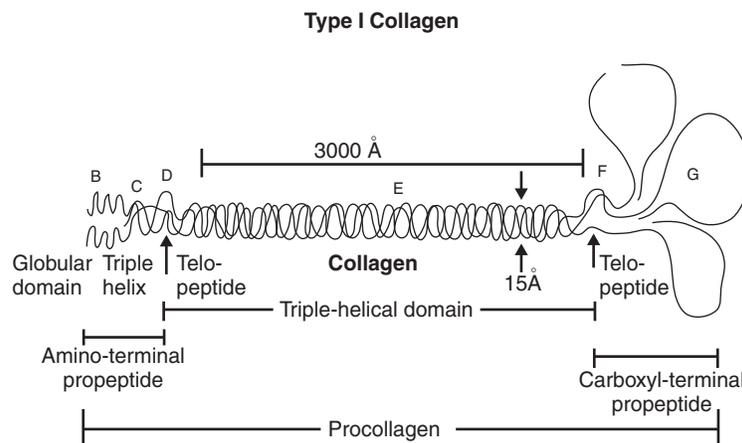


Fig. 9.13 Type I collagen is synthesized in the endoplasmic reticulum of osteoblasts as a larger procollagen molecule with amino (N) and carboxyl (C)-terminal extensions that are cleaved into carboxyl (PICP)- and amino (PINP)- terminal propeptides after secretion of procollagen type I into extracellular matrix. Mature type I collagen is a coiled triple helix of two polypeptide chains of collagen α 1(I) and one of α 2(I) that are cross-linked intramolecularly by disulfide bonds and intermolecularly at the N and C terminals by nonhelical pyridinium telopeptides. (From Byers, P.H. (1995). Disorders of collagen biosynthesis and structure. In: Scriver, C.R., Beaudet, A.L., Sly, W.S., Vale, D. *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed. McGraw-Hill Inc, New York, p. 4029–4077. With permission.)

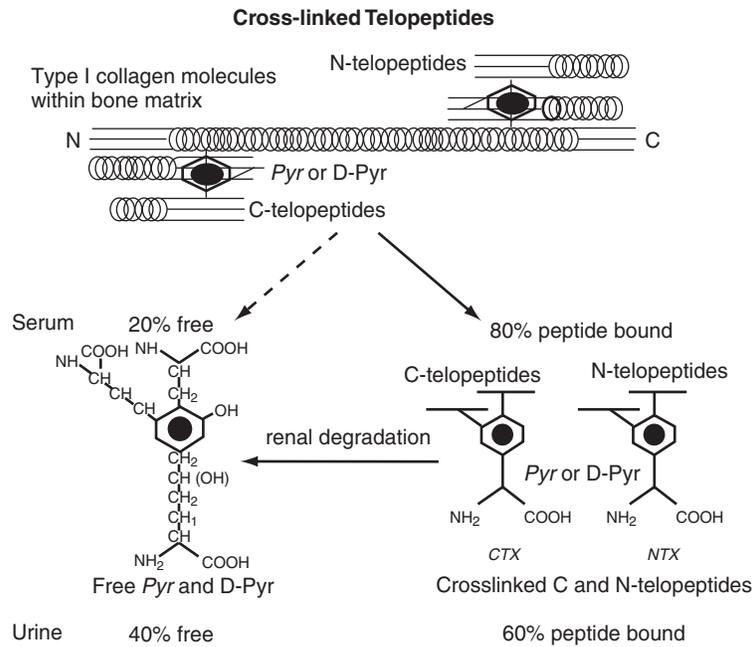


Fig. 9.14 Pyridinium and telopeptides of collagen. Pyridinoline (Pyr, hydroxylysyl-pyridinoline) and deoxypyridinoline (D-Pyr or Dpd, lysyl-pyridinoline) form nonreducible pyridinium cross-links between mature collagen fibers rendering them insoluble. Amino (–) and carboxyl (C) terminal regions (NTX, CTX) of collagen are proteolytically removed during degradation of mature collagen and secreted into extracellular space and serum. The carboxyl-terminal telopeptide of type I collagen (ICTP) is a marker of collagen type I degradation. (From Garnero, P., Delmas, P.D. (1998). Biochemical markers of bone turnover. Applications for osteoporosis. *Endocrinol Metab Clin NA*, 27, 303–323. With permission.)

that then form cross-linked fibers. Procollagen type I C- and N-terminal telopeptides (PICP, PINP respectively) are present and measurable in the circulation enabling assessment of collagen synthesis and processing.

Hydroxylation of proline at AA 986 of COL1 α 1 and at AA 707 of COL1 α 2 forming 3-hydroxyproline residues is accomplished by the coordinated interaction of the responsible enzyme prolyl 3-hydroxylase 1 (P3HI—also termed *LEPRE1*) interacting with its cofactors—cartilage associated protein (*CRTAP*) and cyclophilin B (encoded by *PP1B*). *CRTAP* and cyclophilin B are essential for the stability of P3HI activity. In the absence of this functional tripartite complex, the helical domain of COL1 α 1 is modified by other enzymes, leading to alterations in cross-linking that adversely affect the strength of collagen type I.¹⁷⁷ *CRTAP* is expressed in the proliferative zone of developing cartilage and at the chondroosseous junction; cyclophilin B is a peptidyl-prolyl cis-trans isomerase. That posttranslational modifications of COL1 α 1 are essential for normal bone formation is evidenced by the association of inactivating mutations of P3HI with osteogenesis imperfecta type VIII (OMIM 610915), of *CRTAP* with osteogenesis imperfecta type VII (OMIM 610682), and of *PP1B* with osteogenesis imperfecta type IX (OMIM 259440).^{212–214} Hydroxylation of lysine residues forming hydroxylysine by procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (encoded by *PLOD1*), at procollagen AA sequences of ...Gly-X-Lys ... in the helical regions of the three subunits that comprise collagen type 1, is essential for the stability of the pyridinoline cross-links of this molecule. In the telopeptide regions of collagen type 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (*PLOD2*) catalyzes the formation of hydroxylysine.

Chaperone proteins play important roles as cofactors in the processing of COL1 α 1 and COL1 α 2 fibers.¹⁷⁷ Within the osteoblast's endoplasmic reticulum, stability of the three protein strands comprising collagen type 1 enabling its correct cross-linking is provided by HSP 47 (encoded by *SERP1NH1*). HSP47 binds to specified arginine residues within the collagen type I propeptides. The chaperone FK506-binding protein

(*FKBP10*) may also be involved in the hydroxylation of lysine residues of COL1 α 1 and COL1 α 2 and their subsequent cross-linking. *FKBP10* and *HSP47* may also interact with one another during formation and maturation of collagen type I.

After secretion of procollagen types I α 1 and α 2 subunits into bone matrix, their amino- and carboxyl-terminal propeptide domains are removed and secreted into extracellular space and serum. The carboxyl-propeptide sequence is cleaved by the proteinase activity of BMP1 (*BMP1*). Pyridinoline (PYR—hydroxylysyl-pyridinoline) and deoxypyridinoline (DPD—lysyl-pyridinoline) form nonreducible pyridinium cross-links between mature collagen fibers thus making them insoluble (see Fig. 9.14). Type I collagen predominates in bone but is also present in ligaments, tendons, fascia, and skin. Type II cartilage is composed of three procollagen type II(α 1) chains and is primarily deposited in cartilage. Type III collagen (three procollagen type III[α 1] chains) is present in bone, tendons, arteries, and intestine, and type IV (three procollagen type IV[α 1]) cartilage is a component of cell basement membranes.

Measurement of serum (and urine) concentrations of markers of bone formation and bone resorption provide information about bone turnover in adults and children (Fig. 9.15).²¹⁵ Serum levels of soluble (s)RANK-ligand and osteoprotegerin reflect osteoblast function as related to osteoclastogenesis.²¹⁶ Serum levels of sRANK-ligand are higher in male children and adolescents and increase with age in both genders; the median concentration in males is 0.27 pmol/L (range nondetectable–0.94) and in females 0.08 pmol/L (range nondetectable–1.42). Serum concentrations of osteoprotegerin do not vary with age or gender in children and adolescents; the median concentration of this analyte is 3.7 pmol/L (range 1.02–6.63). Determinations of the procollagen I extension peptides—PICP and PINP—reflect collagen synthesis and thus osteoblast function as does determination of the osteoblast products, osteocalcin and bone-specific alkaline phosphatase. Degradation of mature bone matrix collagen type I by osteoclast-secreted cathepsin K and matrix metalloproteinases releases PYR, DPD, and the amino-terminal (NTX) and

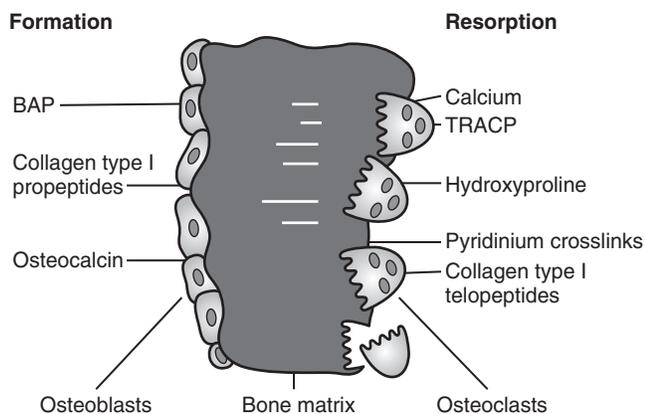


Fig. 9.15 Biochemical markers of bone formation and resorption. (From Jurimae, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr*, 22, 494–500. With permission.)

carboxyl-terminal (ICTP) telopeptides of type I collagen. The urinary excretion of hydroxyproline and hydroxylysine and measurements of PYR, DPD, NTX, CTX, and ICTP in urine or serum reflect catabolism of collagen type I and thus bone resorption. Osteoclast activity is also reflected by measuring serum concentrations of TRAP5b.²¹⁵ In the normal pregnant woman, serum concentrations of sRANKL, osteoprotegerin, and ICTP are highest in the first trimester, whereas osteocalcin values are maximal in the first trimester—suggesting that early in normal pregnancy the rate of bone formation is increased whereas in the second trimester, the rate of bone resorption is amplified.²¹⁷ Serum levels of markers of both bone formation and resorption are higher in the fetus than mother (Table 9.4). Fetal umbilical cord plasma PICP concentrations are highest in mid-gestation and decline in the last trimester to term values; after birth, PICP values fall in preterm neonates during the first 3 days of life and then increase steadily to peak values at 36 weeks

postconceptual age; PICP levels in cord plasma are higher in males than females and correlate with gestational age and birth weight.²¹⁸ In general, values of bone formation and resorption markers are highest in infants, fall during childhood, increase slightly during adolescence, and then decline to adult levels (Tables 9.5A and 9.5B).^{215,219–224} In children and adolescents, serum concentrations of markers of bone turnover are unrelated to the BMI; however, age and gender significantly influence values of serum bone markers with higher levels in younger subjects and earlier decline in values in females than in males.

Serum concentrations of sclerostin reflect the function of osteocytes but vary with the assay used.^{225,226} Values of serum sclerostin are generally higher in healthy adult males than females (50 vs. 37 pmol/L) and rise two- to fourfold with aging; depending on age and gender, serum concentrations of sclerostin may correlate positively with bone mineral content and density (BMC, BMD) and negatively with serum levels of calcium, bone alkaline phosphatase, PINP, osteocalcin, and ICTP. Sclerostin values are decreased by physical activity and increased by immobilization. They are elevated in patients with hypoparathyroidism and decline after intermittent administration of PTH^{1–34}.^{227–230} Serum sclerostin concentrations are higher in boys (median ~23 pmol/L) than girls (median ~19 pmol/L), peak at 10 years of age in girls and 14 years in boys, and fall during puberty in both sexes.²³¹

The adult skeleton is composed of mineral (50%–70%), organic matrix (20%–40%), water (5%–10%), and lipids (<3%). Some 10% to 15% of bone matrix is composed of noncollagenous peptides secreted by the osteoblast, including proteoglycans (chondroitin sulfate, heparan sulfate), glycoproteins, growth stimulating proteins (BMP, TGF β , IGF-I), cell attachment peptides (integrin ligands—osteopontin, osteonectin, fibronectin), and Gla proteins or proteins derived from serum, for example, albumin.¹⁸⁷ Macromolecular proteoglycans are composed of glycosaminoglycans (acidic polysaccharide side chain) linked to a core protein and are important for normal synthesis of collagen and bone development. Osteonectin (encoded by SPARC) is a phosphorylated, 32-kDa glycoprotein that binds to Ca²⁺ in hydroxyapatite and to collagen fibrils enabling calcification of bone matrix. Bone alkaline

TABLE 9.4 Markers of Bone Formation and Resorption in Pregnant Women and Neonates

Trimester	BONE FORMATION			Full-Term Cord	
	1	2	3	M	F
Osteocalcin ng/mL \pm SD	4.47 2.75	2.02	1.56	3.3 0.3	
Bone alkaline Phosphatase IU/L	273 11	69 12			
PICP ng/mL (range)				822 475–1420	824 246–1450
BONE RESORPTION					
sRANKL pmol/L (< SD)	0.59 0.38	1.17 0.54	0.76 0.41		
OPG pmol/L (<SD)	3.50 1.51	6.64 0.12	3.52 0.36		
ICTP ng/mL (<SD)	1.23 0.41	1.71 0.56	0.89 0.29	2.7 0.2	4.6 0.1

ICTP, Carboxyl-terminal cross-link telopeptide of collagen type I generated by matrix metalloproteinase; OPG, osteoprotegerin; PICP, procollagen type I carboxyl-terminal propeptide; RANKL, receptor activator of NF- κ B ligand.

(Modified from Jurimae, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr*, 22, 494–500; Wasilewska, A., Rybi-Szuminska, A.A., Zoch-Zwierz, W. (2009). Serum osteoprotegerin (OPG) and receptor activator of nuclear factor κ B (RANKL) in healthy children and adolescents. *J Pediatr Endocrinol Metab*, 22, 1099–1104; Dorota, D., Bogdan, K.G., Mieczyslaw, G., et al. (2012). The concentrations of markers of bone turnover in normal pregnancy and preeclampsia. *Hypertens Pregnancy*, 31, 166–176; Seibold-Weiger, K., Wollmann, H.A., Ranke, M. B., Speer, C.P. (2000). Plasma concentrations of the carboxyterminal propeptide of type I procollagen (PICP) in preterm neonates from birth to term. *Pediatr Res*, 48, 104–108; Yamaga, A., Taga, M., Hashimoto, S., Ota, C. (1999). Comparison of bone metabolic markers between maternal and cord blood. *Horm Res*, 51, 277–279.)

TABLE 9.5A Markers of Bone Formation and Resorption in Male Children and Adolescents (2.5–97.5 Percentiles)

BONE FORMATION					
Age (years)	6–15	>15			
Alk Ptase (U/L)	136–414	50–251			
Age (years)	6–9	>9–11	>11–15	>15	
BAP (U/L)	51–164.3	65.6–138.2	45.5–208.4	13.1–80	
Age (years)	6–9	>9–15	>15		
OC (ng/mL)	56.5–152.1	48.2	226.4		
Age (years)	6–11	>11–14	>14–15	>15	
TPINP (ng/mL)	407–1079	339–1399	>1217	61–718	
BONE RESORPTION					
Age (years)	6–9	>9–15	>15		
β-CTX (ng/mL)	2.05–2.38	1–2.9	0.50–2.43		
Bone age (years)	6–8	9–11	12–14	15–17	18–21
PINP (ng/mL)	295	311	319	251	124
(± = SE)	13	10	11	14	10
β-CTX (ng/mL)	1.9	1.8	2.4	1.8	1.2
(± = SE)	0.2	0.2	0.2	0.2	0.1
PTH (pg/mL)	37.2	26.7	36.8	36.4	24.7
(± = SE)	2.9	3.3	4.3	6.2	3.5
25OHD	31.2	30.8	26.8	27.9	29.2
(± = SE)	4	1.3	1.2	2	2.8

25OHD, 25-Hydroxyvitamin D; Alk Ptase, alkaline phosphatase; BAP, bone alkaline phosphatase; β-CTX, carboxyl-terminal cross-link telopeptide of type I collagen; OC, osteocalcin; PTH, parathyroid hormone; TPINP, total procollagen N-terminal peptide.

(Modified from Huang, Y., Eapen, E., Steele, S., Grey, V. (2011). Establishment of reference intervals for bone markers in children and adolescents.

Clin Biochem, 44, 771–778; Kirmani, S., Christen, D., van Lenthe, G.H., et al. (2009). Bone structure at the distal radius during adolescent growth. *J Bone Miner Res*, 24, 1033–1042.)

TABLE 9.5B Markers of Bone Formation and Resorption in Female Children and Adolescents (2.5–97.5 Percentiles)

BONE FORMATION					
Age (years)	6–11	>11–14	>14–15	>15	
Alk Ptase (U/L)	157–359	60–401	50–232	26–110	
Age (y)	6–11	>11–14	>14–15	>15	
BAP (U/L)	23.5–151.1	20.8–172.3	12.6–105.8	8.1–43.9	
Age (years)	6–10	>10–14	>14–15	>15	
OC (ng/mL)	61.4–136.2	24.1–232.1	17.8–119.6	21.1–76.7	
Age (years)	6–10	>10–11	>11–14	>14–15	>15
TPINP (ng/mL)	411–1022	–1451	109–1346	38–510	49–277
BONE RESORPTION					
Age (years)	6–10	>10–14	>14–15	>15	
ICTP (ng/mL)	0.82–2.06	0.49–2.76	0.12–1.73	>1.59	
Bone age (years)	6–8	9–11	12–14	15–17	18–21
PINP (ng/mL)	273	275	263	143	89.1
(± = SE)	5	9	8	15	8
β-CTX (ng/mL)	2.3	2.2	2.3	1.1	0.8
(± = SE)	0.1	0.1	0.2	0.1	0.1
PTH (pg/mL)	30.8	24.6	38.8	29.4	28.4
(± = SE)	3.5	2	4.2	4.6	3.5
25OHD	25.5	23.9	19.9	22.4	22.1
(± = SE)	1.8	1.4	0.9	1.6	1.9

25OHD, 25-Hydroxyvitamin D; Alk Ptase, alkaline phosphatase; BAP, bone alkaline phosphatase; β-CTX, carboxyl-terminal cross-link telopeptide of type I collagen; OC, osteocalcin; PTH, parathyroid hormone; TPINP, total procollagen N-terminal peptide.

(Modified from Huang, Y., Eapen, E., Steele, S., Grey, V. (2011). Establishment of reference intervals for bone markers in children and adolescents.

Clin Biochem, 44, 771–778; Kirmani, S., Christen, D., van Lenthe, G.H., et al. (2009). Bone structure at the distal radius during adolescent growth. *J Bone Miner Res*, 24, 1033–1042.)

phosphatase is an isoform of tissue nonspecific alkaline phosphatase (encoded by *ALPL*), an 80-kDa glycoprotein essential for bone mineralization. Osteopontin (also termed *bone sialoprotein* or *secreted phosphoprotein 1* and encoded by *SPPI1*) is a 75-kDa sulfated and phosphorylated glycoprotein that contains the amino acid sequence — Arg-Gly-Asp (RGD) — necessary for linkage to integrins and hence for attachment of osteoclasts to bone; it also binds Ca^{2+} and hydroxyapatite and may play a role in the initiation of bone matrix mineralization. Osteopontin is secreted by osteoblasts in response to

calcitriol. Osteocalcin (encoded by *BGLAP*) is a 49 AA, Gla-containing 6-kDa peptide that plays an essential role in bone mineralization; it is produced only by osteoblasts in response to BMP-7 and calcitriol acting through RUNX2, whereas its posttranslational synthesis is dependent upon vitamin K.

Bone Formation and Mineralization

Bone is composed primarily of solid mineral [hydroxyapatite = $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] that has been deposited on collagen fibrils

within extracellular matrix.²³² Bone extracellular matrix is comprised of the secreted products of the osteoblasts and is composed of collagenous fibrils (collagen types—I, III, V) upon which the mineral phase of bone is deposited. Type I collagen and alkaline phosphatase promote bone mineralization, a process modulated by vitamin K-dependent osteocalcin (encoded by *BGLAP*) and matrix Gla protein (encoded by *MGP*). Long bones consist of a central hollow shaft (diaphysis) distal to which are the metaphyses, cartilaginous growth plates, and epiphyses. The diaphysis is composed of cortical bone, and the metaphysis/epiphysis consists of trabecular bone enveloped by cortical bone. Some 80% of the adult skeleton is dense cortical bone; 20% is cancellous and composed of a network of trabeculae.

The skeleton is essential for posture and movement, shielding of vital organs, formation of all blood cells, and reservoir of the bone minerals (calcium, phosphate) quintessential for cellular function.¹⁹⁰ Very early in the first trimester the outlines of the future skeleton are present as patterns of cartilaginous anlagen that later form the long bones and skull base and undifferentiated mesenchymal fibers that condense to become membranous bone (face, skull, pelvis, clavicles). Bone modeling or ossification of these anlagen begins approximately 5 to 6 weeks after conception. Initially, mesenchymal stem cells differentiate into chondroblasts (and evolve into chondrocytes) or osteoblasts, some of which develop into osteocytes and bone lining cells, whereas osteoclasts derive from hematopoietic stem cells. The bulk of the skeleton forms from cartilage. Mesenchymal stem cells that differentiate into prechondroblasts then become chondroblasts that further develop into prehypertrophic and later into hypertrophic chondrocytes. The chondrocyte produces cartilage—an avascular matrix encased in a perichondrial membrane that is composed of collagen types II and X, hyaluronic acid, and chondroitin sulfate. When the chondrocytes in the midshaft of the long bone outgrow their available nutrients (supplied by diffusion through the cartilage matrix), they undergo apoptosis and necrosis permitting vascular invasion of this site from the periphery and inward movement of chondroclasts and osteoblasts. While the debris of apoptotic chondrocytes and cartilage matrix is being cleared, osteoblasts form a primary ossification center and begin to secrete collagen type I and associated bone matrix proteins, express alkaline phosphatase on their surface, and initiate deposition of calcium and phosphate later reconstituted into hydroxyapatite crystals. A periosteum forms and secondary ossification centers develop at the distal ends of the long bones (the epiphyses) separated from the primary ossification center by replicating chondrocytes in the epiphyseal growth plate (see Fig. 9.9). Ossification of membranous bones begins with gathering of pluripotential mesenchymal stem cells into an ossification center and their differentiation into preosteoblasts and then osteoblasts that secrete bone matrix—primarily composed of collagen type 1 into which calcium and phosphate are deposited as hydroxyapatite. Further differentiation leads to development of a periosteum beneath which develops a thin veneer of compact bone deep to which is cancellous or trabecular bone.

Although calcium and phosphate are co-stored in extracellular and intracellular fluid compartments, they are prevented from precipitating by inhibitors of their association such as polyphosphates (polymeric groups of phosphate anions linked together by oxygen bridges in linear chain or cyclic ring structures the first member of which is pyrophosphate composed of two phosphate groups linked by an oxygen bridge), organic ion chelators (oxalate, citrate), and skeletal proteins (e.g., osteocalcin and osteopontin, a SIBLING protein with the ASARM sequence).⁷ Pyrophosphate is generated by ectonucleotide pyrophosphatase/phosphodiesterase 1 (encoded by

ENPP1) mediated cleavage of nucleotides. Controlled formation and deposition of calcium and phosphate into the extracellular matrix of cartilage (composed of collagens type II and X) and then into the hydroxyapatite lattice in bone matrix (collagen type I) occurs as the inhibitors of biomineralization are degraded locally. In the first phase of bone formation, hypertrophic chondrocytes and osteoblasts initiate bone crystal formation by forming 100-nm subsurface matrix vesicles containing calcium, phosphate, TNSALP, calbindin-D_{9k}, CA, pyrophosphatases, osteocalcin, and osteopontin. After initial precipitation of calcium and phosphate as amorphous calcium phosphate within the matrix vesicles themselves and fusion of the vesicular and cell membranes, the precipitate is extruded into the extracellular matrix where further increases in local concentrations of phosphate and calcium, together with the structural properties of collagen type I, direct the formation of the hydroxyapatite crystal. TNSALP, synthesized by chondrocytes and osteoblasts, is tethered both to the interior of the matrix vesicle and to the exterior of the osteoblast plasma membrane. After pyrophosphate and other polyphosphates have been transported into the extracellular matrix, TNSALP removes their inorganic phosphate moieties, thereby increasing local phosphate concentrations and promoting hydroxyapatite formation and deposition in bone. Another phosphatase (encoded by *PPP1R1B*) also raises local levels of phosphate by releasing this anion from phosphoethanolamine and phosphocholine.

Inhibitors of bone mineral formation include osteopontin (*SPP1*) and matrix Gla (*MGP*). The function of osteopontin (a SIBLING protein with an ASARM sequence that binds to hydroxyapatite and inhibits deposition of mineral) is partially dependent upon the extent of its phosphorylation. Thus when 40% of the phosphorylation sites of osteopontin are phosphorylated bone mineralization is inhibited; however, when 95% of its sites are phosphorylated hydroxyapatite formation is promoted.^{187,233} Matrix Gla is an 84 AA, vitamin K-dependent peptide containing Gla (related to but distinct from osteocalcin) that has great affinity for Ca²⁺ and inhibits precipitation of calcium and phosphate; *MGP* is expressed in arteries and chondrocytes but not in osteoblasts; thus a normal function of *MGP* is to prevent calcification of cartilage; accordingly, in patients with biallelic loss-of-function mutations in *MGP*, there is extensive calcification of cartilage (Keutel syndrome, OMIM 245150).

Bone strength is determined by its size (height, width, depth), mineral mass, macro- and microarchitecture, and material properties (e.g., elasticity) of collagen that in turn are regulated not only by endocrine hormones and paracrine growth factors but also by mechanical forces exerted upon the skeleton by the environment (gravity), erect stance, and movement using the muscular system.^{194,234,235} Bone mass and strength are determined in part by the loads placed on bone by biomechanical forces exerted by muscles—the “mechanostat” model. In this model, osteocytes monitor the stresses and strains (deformations) that are the result of mechanical forces placed upon it. In response to mechanical force: (1) the osteocyte rate of apoptosis declines, (2) the osteocyte synthesizes less sclerostin, (3) the rate of WNT1-stimulated bone formation increases, and (4) stromal cells decrease their expression of RANK-ligand, thereby (5) slowing the rate of osteoclastogenesis. In addition, in response to mechanical stress, multiple growth factors generated by the osteoblast (FGFs, IGF-1, TGFβ) act in autocrine/paracrine manners upon their respective tyrosine kinase receptors expressed in the cell membrane of the osteoblast to activate multiple intracellular signals, including the adenylyl cyclase, PKA, PKB, PI3K, and MAPK signal transduction pathways. Generation of PLC leads to increase in cytosolic Ca²⁺ and osteoblast function as does influx of Ca²⁺ through transmembrane L-type calcium

channels.²³⁶ One of the target genes affected by mechanical stimulation is *RUNX2* whose transcript is essential for osteoblast differentiation and expression and synthesis of osteoblast-specific proteins. Repetitive bone strain (an applied deforming force that might be compressive, lengthening, or angulating) leads to enhanced quantity and quality of bone (bone strength). The mechanisms that lead to increased bone mass are inactivated by decreased weight bearing, such as immobilization or decreased gravity (e.g., space flight) and lead to bone loss (“disuse osteoporosis”). Although bone strength is in part dependent on bone mineralization, it is the size and integrity of bone that primarily determine its strength.²³⁷ Clinically, this paradox is illustrated by the increased rate of fractures in children with osteopetrosis or “marble bone disease” despite extremely dense bones with thick cortices and trabeculae.

Bone mineralization begins in utero with most calcium being deposited in the third trimester and progresses through childhood and puberty.³⁰ The skeleton accumulates 25 to 30 g of calcium in utero and accrues approximately 2500 g by adulthood. Bone mass increases throughout childhood and as puberty progresses and continues in the third decade of life. By 7 years of age, children have achieved approximately 70% of adult stature but have accumulated only approximately 35% of maximal whole-body bone mineral content.²³⁸ Approximately one-half of the skeleton mass is formed during puberty, and differences in bone mass between teenagers of similar genders and races is attributable to the velocity of sexual maturation as influenced by changes in secretion of GH, estrogens, and androgens during this phase of life.²³⁹ The rate of gain of bone mass is greatest 6 months after the peak height velocity of puberty has been achieved; the older the chronological age that the pubertal peak growth velocity is realized, the lower is peak adult bone mass. Females have lower peak bone mass than do males because of the smaller size of their bones, accounting in part for the greater number of vertebral fractures in adult females than in adult males. The average total body BMC of the adult male approximates 2800 g and of the adult female 2200 g with greater values in black than white individuals. Approximately 60% of total adult bone calcium is acquired during adolescence—25% in the 2 years before and after the peak velocity of BMC accrual; 30% of the mean BMC of the adult female lumbar spine (60 g) is deposited during this interval. In both males and females, peak bone mass achieved in young adulthood is inversely related to the age of pubertal onset.^{240,241}

Optimal bone mineralization requires good health and adequate nutrition—particularly intake of calcium and vitamin D, normal linear growth and weight gain, and frequent weight-bearing exercise as these increase periosteal bone mass.²³⁹ Recommended dietary intakes of calcium and vitamin D for children and adolescents are presented in Table 9.2. Calcium supplementation is usually unnecessary in the well child/adolescent; high intake of carbonated beverages should be discouraged as they lower intake of milk and calcium. Vitamin D supplementation has been recommended for breastfeeding infants, but older well children are usually able to consume sufficient vitamin D in a healthy diet and synthesize cholecalciferol endogenously if exposed to sufficient sunlight; vitamin D supplementation during winter months is appropriate for all children if exposure to sunlight is curtailed, as it is for children with malabsorption syndromes, those receiving anticonvulsant drugs or antiretroviral agents.³⁰

As evidenced by the close concordance of bone mineral status between mothers and daughters, identical and fraternal twins and siblings, 60% to 80% of the peak or maximum adult bone mass is determined by genetic factors. Thus an individual's expressed genetic composition exerts a substantial effect

on accrual of bone and the peak BMD achieved.²⁴¹ Genome-wide association studies have identified many genes that influence bone mineralization.^{241–243} Those that are related to the WNT- β -catenin osteoblast differentiation pathway are among the most prominent in this regard. Although many of these genes are involved in bone metabolism, others have not been known heretofore to be related to skeletal homeostasis. In addition to intrafamilial factors, ethnicity, sex, and body size and composition are important determinants of bone density. In black male and female youths, there is higher whole body less head BMC and areal (a) BMD and lumbar spine, one-third radius, total hip, and femoral neck BMC and areal BMD than in white, Asian, or Hispanic youth (Tables 9.6 and 9.7).^{239,244,245} Asian female children and adolescents have lower whole-body and femoral neck BMD than do white and Hispanic subjects. Hispanic males have lower lumbar spine BMD than do white and Asian youth. Radial and femoral neck (peripheral) and vertebral (axial) BMDs correlate with sex, age, height, weight, BMI, pubertal and postpubertal hormonal status, calcium intake, and exercise in children, adolescents, and adults. In young women only 16% to 21% of peak vertebral and femoral bone mass can be accounted for by weight, height, physical activity as an adolescent, and the VDR genotype emphasizing the essential roles of multiple factors in this process. Because adult peak bone mass is inversely related to the risk of osteopenia and osteoporosis in later adulthood (in adults, a 10% increase in BMD reduces the risk of femoral neck fracture by 50%), it is essential that bone mass be maximized during childhood and adolescence.

The rate of accumulation of bone mass increases during puberty, and peak bone mass is achieved early in the third decade of life; calcium intake accounts for perhaps 5% of accrued peak bone mass whereas exercise may contribute 10% to 22% of peak bone mass and is, therefore, one of the factors that can be modified in the pursuit of the goals of attaining and maintaining maximum peak bone mass.²⁴⁶ Thirty minutes of programmed weight-bearing exercise thrice weekly increases BMC of the femoral neck and lumbar spine in prepubertal boys and girls as may resistance training of moderate intensity performed 20 to 40 minutes once or twice per day 5 days per week; high-impact, weight-bearing exercises (ballet, tennis, volleyball, gymnastics, jumping, running, soccer, rugby, ice hockey) increase mass of weight-bearing bones, particularly in children and adolescents, an effect that may long outlast the period of exercise itself.²⁴⁶ Weight-bearing and high-impact exercises do so in part by augmenting periosteal bone acquisition and increasing the cortical thickness of long bones, particularly of the legs at their most distal extremes closest to the ground where weight bearing is maximal. At peak BMC velocity, the annual gain and total accumulation (>2 years) of total body bone mineral in active boys and girls are 80 g/year and 120 g/year, respectively, greater than in inactive adolescents. One year after peak BMC velocity, the total body, femoral neck, and lumbar spine BMCs are 9% to 17% greater in active than in relatively inactive subjects. Even greater normal activity (such as walking longer distances to school) convey long-lasting salutary effects on bone mineralization and later fracture risks.²⁴⁷ The effects of diminished physical activity on bone growth and strength are clearly illustrated by children with neurological insults that prevent normal motion (Erb paresis, hemiplegia, spinal cord insults) and thus restrain limb growth. An abrupt decrease in the mechanical load on bone (e.g., bed rest, space flight) evokes rapid loss of bone mineralization as the rate of bone formation declines whereas that of bone resorption is maintained. In patients with limited physical activity because of neurological or muscular insults, bone density may be increased by high frequency, low amplitude mechanical stimulation.²⁴⁶

TABLE 9.6 Bone Mineral Content (BMC g) and Areal Bone Mineral Density (aBMD g/cm²) by Dual Energy X-Ray Absorptiometry (DXA) in Prematurely Born and Full-Term Appropriate or Small for Gestational Age Infants (AGA, SGA)

GA	BMC		BMD	
	AGA	SD	SGA	SD
32–33	21.9	(9.7)		
34–35	32	(11.3)		
36–37	39.4	(15.9)	24.9	(7.4)
38–39	45.5	(18.4)	27	(11.1)
BIRTH WEIGHT	MEAN	(95% RANGE)		
1001–1500	22.9	(21.7–24.1)		0.146 (0.141–0.150)
1501–2000	31.8	(30.9–32.8)		0.162 (0.158–0.165)
2001–2500	42.2	(41.7–43.2)		0.178 (0.175–0.181)
2501–3000	54.6	(53.9–55.3)		0.199 (0.196–0.202)
3001–3500	66.9	(66–67.9)		0.220 (0.217–0.224)
3501–4000	77.6	(76.3–78.8)		0.234 (0.229–0.238)
POSTNATAL AGE		SD		SD
Term			6 months	12 months
BMC				
WB	76	(14)	17	(29)
WB-H	44	(8)	88	(16)
Femur	2.94	(0.54)	5.58	(1.46)
L1-L4	2.35	(0.42)	3.59	(0.63)
BMD				
L1-L4	0.266	(0.044)	0.252	(0.031)
				0.304 (0.044)

GA, Gestational age—weeks, birth weight (g), postnatal age (days); WB, whole body; WB-H, whole body-head.
 (Modified from Gallo, J., et al. (2012). *J Osteoporosis*, 2012, 672403; Koo, W.W.K., et al. (1996). Dual-energy x-ray absorptiometry studies of bone mineral status in newborn infants. *J Bone Miner Res*, 11, 1997–1002; Lapillone, A., et al. (1997). Body composition in appropriate and in small for gestational age infants. *Acta Paediatr*, 86, 196–200; Koo, W.W.K., et al. (1998). Postnatal development of bone mineral status during infancy. *J Am Coll Nutr*, 17, 65–70.)

TABLE 9.7A Total Body Less Head DXA Bone Mineral Content (BMC - g) and Areal Bone Mineral Density (aBMD - g/cm²) in Non-Black and Black Male Children and Adolescents

	Non-Black		Black	
TOTAL BODY LESS HEAD	BMC	aBMD	BMC	aBMD
5	416 (337–516)	0.503 (0.436–0.577)	460 (364–581)	0.551 (0.466–0.639)
7	550 (440–692)	0.585 (0.506–0.674)	620 (482–796)	0.641 (0.544–0.743)
9	706 (554–904)	0.669 (0.577–0.774)	786 (599–1028)	0.722 (0.616–0.837)
11	880 (673–1160)	0.740 (0.637–0.860)	1001 (741–1348)	0.803 (0.685–0.933)
13	1195 (870–1661)	0.832 (0.716–0.975)	1374 (977–1927)	0.905 (0.764–1.063)
15	1692 (1244–2323)	0.957 (0.822–1.131)	1876 (1353–2594)	1.024 (0.859–1.215)
17	2026 (1537–2691)	1.047 (0.898–1.247)	2226 (1675–2950)	1.113 (0.939–1.316)
20	2196 (1670–2909)	1.088 (0.933–1.301)	2424 (1887–3109)	1.172 (0.997–1.378)

Age in Years; 50th percentile (3rd – 97th percentiles).
 (Modified from Zemel, B.S., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, 96, 3160–3169; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)

TABLE 9.7B Total Body Less Head DXA Bone Mineral Content (BMC - g) and Areal Bone Mineral Density (aBMD - g/cm²) in Black and Non-Black Female Children and Adolescents

	Non-Black		Black	
TOTAL BODY LESS HEAD	BMC	aBMD	BMC	aBMD
5	424 (365–537)	0.497 (0.428–0.575)	469 (369–590)	0.540 (0.469–0.622)
7	549 (437–703)	0.576 (0.493–0.671)	599 (467–759)	0.619 (0.534–0.710)
9	689 (540–894)	0.652 (0.556–0.763)	765 (590–980)	0.705 (0.604–0.805)
11	908 (674–1237)	0.745 (0.631–0.877)	1051 (800–1362)	0.814 (0.696–0.928)
13	1265 (903–1758)	0.861 (0.730–1.012)	1404 (1072–1816)	0.917 (0.792–1.055)
15	1511 (1128–1988)	0.933 (0.802–1.083)	1630 (1251–2098)	0.977 (0.850–1.145)
17	1606 (1233–2053)	0.960 (0.830–1.107)	1738 (1335–2235)	1.003 (0.875–1.190)
20	1636 (1269–2071)	0.968 (0.839–1.114)	1823 (1400–2345)	1.021 (0.893–1.225)

Age in Years; 50th percentile (3rd–97th percentiles).
 (Modified from Zemel, B.S., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, 96, 3160–3169; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)

Hormonal Regulation of Bone Mineralization

PTH; PTHrP; calcitriol; thyroid hormone; interleukins-1 β , -3, -6, and -11; TNF- α ; PGE₂; and glucocorticoids stimulate expression of RANK-ligand and M-CSF and depress that of osteoprotegerin and hence favor osteoclast development and bone resorption. Calcitonin; estrogens; androgens; interferon- γ ; interleukins-4, -10, and -18; TGF β ; and bisphosphonates antagonize these processes. Calcitonin acting through its GPCR dissociates the processes of bone resorption and bone formation by transiently depressing the bone-resorbing activity of osteoclasts; it does so by impeding formation of the ruffled membrane. Although osteoclasts do not express *PTH1R*, PTH¹⁻⁸⁴ enhances osteoclastogenesis by stimulating osteoblast and stromal cell production of RANK-ligand. However, when PTH¹⁻³⁴ is administered intermittently, it increases osteoblast activity and the rate of bone formation and slows the rate of osteoblast apoptosis, functioning in part through IGF-I. Carboxyl-terminal fragments of PTH¹⁻⁸⁴ can inhibit osteoclast formation and function and antagonize the osteoclast stimulating effects of PTH¹⁻⁸⁴, calcitriol, prostaglandins, and interleukins.⁸⁶

After binding to its receptor, pituitary GH enhances the differentiation and proliferation of chondrocytes and osteoblasts and extends the life of osteoblasts, thereby increasing bone mass and bone size.¹⁶⁰ GH increases the synthesis of BMPs, directly enhances differentiation of prechondrocytes within the resting zone of the cartilaginous growth plate, supports proliferation of chondrocytes in the reserve or resting zone, and increases local expression of *IGF1*. (In osteoblasts, the synthesis of IGF-I is also stimulated by PTH.) GHRs are expressed in chondrocytes in the reserve, proliferative, and hypertrophic zones and mediate chondrocyte proliferation, maturation, and synthesis of IGF-I.²⁴⁸ IGF-I is synthesized predominantly by proliferating chondrocytes; functions through the IGF type 1 receptor expressed in chondrocytes in the reserve, proliferative, and hypertrophic zones; and stimulates the clonal expansion of committed chondrocytes.²⁴⁹ IGF-I coordinates chondrocyte proliferation and inhibits their apoptosis; it modulates their differentiation and maturation and their synthesis of matrix heparan sulfate proteoglycan, a matrix component that is necessary for efficient signaling of FGF and their receptors.²⁵⁰ IGF-binding proteins (IGFBP)-1 through -6 are synthesized by growth plate chondrocytes; they regulate levels of bioactive IGF-I, as well as exert direct stimulatory/inhibitory effects on chondrocyte proliferation depending on the stage of chondrocyte differentiation.²⁵¹ In utero, both IGF-I and -II are essential for normal fetal growth as denoted by the in utero growth retardation experienced by the fetus with a loss-of-function mutation in *IGF1*, *IGF2*, or *IGF1R*.²⁵⁰ Neither GH nor IGF-I is necessary for patterning of the skeleton, however. Systemic loss of GH secretion or IGF-I synthesis or inactivation of their receptors impairs linear growth of long bones postnatally. Experimentally, selective loss of hepatic IGF-I production lowers its total circulating levels to 25% of normal but does not adversely affect growth in transgenic mice, indicating that it is the somatomedin synthesized by the cartilage growth plate that affects chondrocyte division by a paracrine mechanism. In patients with inactivating mutations of *GHR* encoding the GHR or deletion of *IGF1*, administration of IGF-I enhances linear growth, indicating that this growth factor is able to stimulate cartilage proliferation without the initial differentiating effect of GH but does so to a lesser extent than does GH in the GH-deficient subject, indicating that sufficient numbers of differentiated prechondrocytes are necessary for optimal somatomedin effect.²⁵² The GH secretagogue, ghrelin, is also synthesized and secreted by chondrocytes and affects their intracellular metabolism.²⁵³

Through expression of *GHR* by osteoblasts, GH stimulates their differentiation, proliferation and function-enhancing

synthesis and secretion of osteocalcin, bone-specific alkaline phosphatase, and type I collagen. GH also increases synthesis of IGF-I by the osteoblast as do estrogen, PTH, cortisol, and calcitriol. IGF-I is essential for GH-induced osteoblast proliferation in vitro; however, it also decreases expression of *GHR*, whereas estrogen enhances *GHR* expression. IGFBPs modulate activity of both IGF-I and -II; thus, IGFBP-5 binds to bone cells, matrix, and hydroxyapatite and enhances the actions of IGF-I on bone. Expression of *GHR* by the osteoblast is upregulated by IGFBP inhibition of somatomedin activity. GH increases synthesis of RANK-ligand and consequently osteoclastogenesis, osteoclast proliferation, and activity; human osteoclasts also have receptors for IGF-I, which stimulates osteoclast formation and function. IGF-I enhances differentiation of osteoblasts, as well as their function by stimulating the transcription of *COL1A1* and *COL1A2* encoding collagen type I, thereby increasing bone formation. GH also stimulates intestinal calcium absorption (through activation of 25-hydroxyvitamin D-1 α hydroxylase and synthesis of calcitriol) and renal phosphate retention. Interestingly, cortical bone structure is maintained by systemic (hepatic) IGF-I, whereas that synthesized by the skeleton stabilizes trabecular bone. Prolactin enhances bone formation, as well as osteoclastogenesis and bone resorption, the latter by increasing osteoblast production of RANK-ligand.

In children with GH deficiency and adults with childhood-onset or adult-onset GH deficiency, BMC and areal and volumetric BMDs are decreased and increase when GH is administered.²⁵⁴ Administration of GH to the hypsomatotropic patient increases serum and urine levels of markers of bone formation and resorption (e.g., osteocalcin, bone-specific alkaline phosphatase, procollagen type I amino and carboxyl propeptides, pyridinoline, deoxypyridinoline) with maximal values achieved 3 to 6 months after beginning treatment. There is a biphasic response of bone mass during GH treatment of deficient subjects; during approximately the first 6 months of GH administration BMD declines as bone resorption exceeds formation; over the next 6 to 12 months; this value increases steadily. In subjects with inactivating variants of *GHR* or *IGF1*, areal BMD and BMC are decreased, but volumetric BMD is not, suggesting that bone size but not bone mineral acquisition is impaired by isolated somatomedin deficiency. In acromegalic patients there is increased bone turnover, and variably increased lumbar spine and femoral neck BMD and iliac crest cortical and trabecular bone mass.

The thyroid hormones secreted under the control of pituitary thyroid-stimulating hormone (thyrotropin) and hypothalamic thyrotropin-releasing hormone exert major effects upon the chondrocyte, osteoblast, osteocyte, and osteoclast.^{148,160} Thyroxine is the primary product of the thyroid gland, but triiodothyronine is the biologically active thyroid hormone after conversion of thyroxine to triiodothyronine by types 1 and 2 monodeiodinase. The skeletal action of triiodothyronine is mediated by its binding to the cytoplasmic thyroid hormone receptors alpha 1 and beta (TR α 1/ β), transcription factors that translocate to the nucleus, pair with the RXR, bind to the thyroid response element in the 5' region of the target gene, link to corepressor or coactivating proteins, and stimulate or inhibit expression of selected genes. TR α and β are expressed in the reserve and proliferative zones of the growth cartilage. Triiodothyronine acting primarily through TR α 1 enables the differentiation of resting chondrocytes and their entrance into the proliferative phase, but there thyroid hormone inhibits further chondrocyte proliferation and promotes differentiation to terminal hypertrophic chondrocytes and secretion of collagen type X. They do so in part by disrupting the reciprocal interaction between IHH and PTHrP, thereby accelerating chondrocyte maturation, effects mediated by FGFR3 and by

downregulation of *IGF1* expression in chondrocytes.²⁵⁵ Thyroid hormone is essential also for vascular invasion of the growth plate's hypertrophic zone and induction of metaphyseal trabecular bone formation. Triiodothyronine stimulates osteoblast differentiation and proliferation and synthesis of osteocalcin, type I collagen, and alkaline phosphatase. In osteoblasts, TR β mediates intracellular nongenomic, rapid responses to triiodothyronine through activation of the PI3K signal transduction pathway and other routes. Thyroid hormone enhances bone resorption, but it is unclear if this a direct or indirect effect upon osteoclasts.^{148,160}

Thyroid hormones are necessary for fusion of the epiphyseal cartilage plate, although fusion may occur in the absence of thyroid hormone through estrogenic action. Through osteoblast-expressed receptors for thyroid hormone, triiodothyronine increases osteoblast production of osteocalcin, bone-specific alkaline phosphatase, and IGF-I. Thyroid hormones increase the rate of bone remodeling by expanding the number of osteoclasts and sites of bone resorption and the amount of bone resorptive surface; urinary calcium excretion is increased by thyroid hormones; in excess, thyroid hormones lead to net bone loss. Thyroid-stimulating hormone may directly impede osteoclast formation and bone resorption by reducing osteoblast expression of RANK-ligand and advance bone formation by enhancing WNT-mediated osteoblastogenesis.²⁵⁶

Estrogen and androgens promote chondrocyte maturation.²⁵⁷ Although many of the effects of androgens are mediated by their conversion to estrogens, nuclear androgen receptors are expressed by chondrocytes, and nonaromatizable androgens stimulate chondrocyte proliferation and long bone growth. Estrogens acting through estrogen receptors α and β (ER α , ER β) expressed in chondrocytes exert a biphasic effect on chondrocyte proliferation—increasing its rate at low doses and reducing it at higher levels; by decreasing chondrocyte proliferation and accelerating the rates of chondrocyte maturation, senescence, and apoptosis, estrogens lead to epiphyseal fusion.²⁵⁸ Complete maturation and fusion of the growth plate are mediated primarily by estrogens, as evidenced by the failure of growth plate fusion in young adult males with inactivating mutations of the genes encoding aromatase (the enzyme that converts androgens to estrogens) or ER α despite adult levels of testosterone and estrogen. Chondrocytes may also be capable of synthesizing estrogens from androgens as aromatase activity has been found in growth plate chondrocytes.²⁵⁷ This observation suggests that locally produced, as well as systemic estrogens, may contribute to chondrocyte maturation and growth plate fusion. Estrogens acting directly and indirectly upon osteoclasts through ER α maintain and augment bone mass, by inhibiting its dissolution, by suppressing T-cell production of osteoclast activating cytokines, such as interleukins-1 and -6, and TNF- α and by suppressing expression of RANK-ligand and increasing that of TGF- β . In addition, estrogens interfere with RANK-ligand/RANK stimulated intracellular signal transduction through the MAPK pathway and downregulate expression of genes encoding integrins thus impairing bone resorption.¹⁹⁷ Both estrogens and androgens depress the rates of apoptosis of osteoblasts and osteocytes. Androgens primarily promote mineralization by conversion to estrogens, as evidenced by the osteopenia noted in adult males with aromatase deficiency or loss-of-function mutations in the gene encoding ER α .²⁵⁹ Androgens also have a direct effect on bone mineralization; acting through the androgen receptor, they increase periosteal bone growth during puberty in both males and females; however, the stronger bones of men compared with women reflect not increased volumetric BMD but rather increased bone size because of greatly expanded periosteal bone width.²⁶⁰ Increased periosteal bone width in males is caused in part by the androgen-induced effect of increased

muscle mass, strain, and mechanical loading on bones.²⁶¹ Nevertheless, estrogens too are necessary for normal periosteal bone growth as despite normal testosterone secretion the aromatase-deficient adult male has decreased periosteal bone width, a situation that can be reversed by administration of estrogen. Thus gonadal sex hormones increase bone growth, maturation, and mineralization. At moderately high (menopausal) concentrations, follicle-stimulating hormone enhances osteoclast formation and decreases its rate of apoptosis, thereby increasing the rate of bone resorption.²⁵⁶

That sex hormones play major roles in the accretion of bone mineral in both females and males is evidenced by the increase in bone mineralization that occurs during puberty. The bulk of adult bone calcium stores are deposited during puberty when the peak rate of accrual of total body BMC occurs in boys and girls 0.7 years after attainment of peak height velocity (PHV) and 0.4 to 0.5 years after peak accrual of lean body mass (a surrogate measurement of muscle mass).²⁶² After controlling for size, the rate of total body and femoral neck accrual of bone mineral over the 2 years encompassing the PHV are greater in males than females; there is no gender effect on accrual of BMC of the lumbar spine, however. Both androgens and estrogens markedly influence rates of bone formation and resorption, although it is the effect of estrogen that predominates as evidenced by: (1) the marked osteopenia of adult males with androgen sufficiency but estrogen deficiency related to loss-of-function mutations in the genes encoding aromatase and ER α , (2) the beneficial effects of estrogen but not of testosterone upon bone mineralization in males with aromatase deficiency, (3) the very close association of BMD and serum levels of bioavailable estrogen in elderly men, and (4) the significant correlation in adult men treated with testosterone between changes in bone mineralization and increases in serum concentrations of estradiol but not of testosterone.²⁵⁸ Nevertheless, the osteopenia of adult (46XY) females with complete androgen insensitivity because of loss-of-function mutations of the X-linked androgen receptor, despite elevated serum testosterone and (endogenous or exogenous) estradiol concentrations, indicates that androgens, too, increase bone mineralization. Furthermore, nonaromatizable dihydrotestosterone has a direct anabolic effect on bone, as it stimulates the proliferation and maturation of osteoblasts, increases the production of procollagen I(α 1), and prevents bone loss in orchidectomized rats.

Estrogens increase bone mass primarily by suppressing bone resorption; they do so through inhibition of osteoclastogenesis and downregulation of osteoblast production of osteoclast activating factors, such as interleukin-6 (and its receptor), TNF- α , and M-CSF, increasing production of osteoprotegerin, and accelerating apoptosis of mature osteoclasts. Estrogens also prolong the life span of osteoblasts and osteocytes. During adolescence in the female, not only does the rate of bone deposition increase but that of bone resorption declines. There is maturation-related increase in BMC and areal and volumetric BMD and in metacarpal length, width, and cortical thickness. Data suggest that the pubertal increase in production of GH and IGF-I mediates, in part, longitudinal and periosteal skeletal growth and mineral acquisition during puberty.

Cortisol acts directly on chondrocytes, osteoblasts, osteocytes, and osteoclasts to affect cartilage and bone formation, remodeling, and dissolution.^{160,263} Glucocorticoids act through cytoplasmic receptors that translocate to the nucleus, form homodimers, and bind to specific 5' response elements in designated genes.²⁶⁴ The nuclear glucocorticoid receptor is expressed in proliferative and hypertrophic chondrocytes where cortisol inhibits chondrocyte proliferation, growth, synthesis of cartilage matrix, and maturation, but it also delays senescence of the growth plate, thereby permitting "catch-up"

growth when excess glucocorticoid exposure is temporary.^{265,266} Glucocorticoids depress expression of *GHR*, *IGF1*, and *IGF1R* in growth plate chondrocytes and, by regulating synthesis of IGF-BPs, they indirectly decrease the biological function of IGF-I. Paradoxically, glucocorticoids also increase expression of *SOX9* and the earliest phase of chondrocyte differentiation. Thus glucocorticoids exert dual but opposing effects on skeletal biology.²⁶⁴ In physiological amounts, glucocorticoids stimulate differentiation of osteoblasts from mesenchymal stem cells in part by inhibiting their differentiation into adipocytes. However, they also increase the rates of autophagy and apoptosis of osteoblasts and osteocytes, thereby decreasing their life span. Glucocorticoids stimulate osteoblast and osteocyte synthesis of osteocalcin, alkaline phosphatase, and sclerostin, the latter an antagonist of WNT/ β -catenin signaling and osteoblast differentiation. Excessive amounts of glucocorticoids impede osteoblastogenesis and accelerate osteoblast apoptosis by reducing expression and accelerating proteasomal degradation of *CTNNB1* (encoding β -catenin) and other osteoblast differentiating genes and increasing endoplasmic reticulum stress, thereby reducing the rate of bone formation, a process that may be impeded by calcitonin and bisphosphonates.

Glucocorticoids accelerate osteoclastogenesis by increasing expression of *TNFSF11* (RANK-ligand), which enhances osteoclastogenesis and osteoclast activation and by depressing expression of *TNFRSF11B* and hence the synthesis of osteoprotegerin, the endogenous antagonist of RANK-ligand.¹⁹⁷ They also prolong the life span of the osteoclast, further enhancing bone resorption. Physiological quantities of glucocorticoids have no substantial effect on osteoclast differentiation, function, or longevity. In excess, glucocorticoids also prolong the life span of the osteoclast further enhancing bone resorption. The overall effect of glucocorticoids on bone is to reduce the rate of trabecular bone formation and to increase the tempo of endocortical bone resorption. Other effects of glucocorticoids relative to bone formation include inhibition of calcium absorption by the intestine and enhancement of calcium excretion by the kidney, suppression of GH secretion and synthesis of IGF-I, decrease in muscular strength and hence of the mechanical forces exerted on the skeleton; and alteration of metabolic pathways that impinge on bone metabolism (e.g., obesity, glucose intolerance).

Transcripts of C-type natriuretic peptide (CNP, encoded by *NPPC*) and natriuretic peptide receptor 2 (encoded by *NPR2*), a guanylyl cyclase B, are expressed by chondrocytes. This ligand-receptor complex stimulates the growth of proliferative and hypertrophic chondrocytes, enhances osteoblast function, and induces endochondral ossification by generation of intracellular cyclic guanosine monophosphate (cGMP) and subsequent activation of the MAPK signal transduction pathway.²⁶⁷ CNP increases the thickness of the growth plate by enlarging chondrocyte size, but it does not affect differentiation of chondrocytes. Natriuretic peptide receptor C/3 (*NPR3*) lacks an intracellular domain but links to CNP and increases its clearance and that of other circulating natriuretic peptides. Osteocrin (*OSTN*) is a 133 AA peptide secreted by bone cells that competes with CNP for binding to NPR C/3, thus slowing its rate of clearance and prolonging its biological activity.²⁶⁸ Plasma concentrations of the amino-terminal pro-C-type natriuretic peptide are positively related to growth velocity in normal children and adolescents.²⁶⁹ Biallelic loss-of-function mutations in *NPR2* have been identified in patients with acromesomelic dysplasia—Maroteaux type (OMIM 602875), manifested by shortening and deformation of the forearms, forelegs, and vertebrae, resulting in severely compromised adult stature. Heterozygous inactivating mutations of *NPR2* have been recorded in subjects with short stature and nonspecific skeletal abnormalities (OMIM 616255).

Monoallelic gain-of-function mutations in *NPR2* have been associated with Miura type epiphyseal chondrodysplasia (OMIM 615923) manifested by tall stature, scoliosis, and enlargement of the great toes.

Another factor to which bone mass is closely related is body weight. Using data of 8348 children and adolescents who underwent studies of areal BMD and body composition by dual energy x-ray absorptiometry (DXA), a decline in whole-body and lumbar spine areal BMD with increasing percent body fat in both males and females of diverse ethnicities (white/non-Hispanic, African American/non-Hispanic, Hispanic, other ethnicities) has been recorded, whereas the effect of weight on pelvic areal BMD was more variable.²⁷⁰ Chondroblasts, osteoblasts, and adipocytes arise from a common mesenchymal stem cell and can be interconverted depending upon the transcription factor that is expressed in the stem cell (e.g., PPAR γ promotes adipogenesis); consequently, the fat cell synthesizes and secretes several adipokines that may affect bone development. Among these products is leptin (encoded by *LEP*), a 16-kDa protein with anorexigenic properties that acts within the ventromedial hypothalamic nucleus to depress appetite, increase the rate of energy utilization, enable fertility by enhancing gonadotropin secretion, and regulate thyrotropin secretion. Leptin acting through leptin receptors expressed by mesenchymal stem cells stimulates their differentiation into osteoblasts by inhibiting their transformation into adipocytes; leptin increases osteoblast proliferation, prolongs osteoblast life span, and assists their maturation into preosteocytes; leptin enhances synthesis of collagen type 1 and mineralization.^{271–273} Leptin also impairs osteoclast development by enhancing the expression of *TNFRSF11B* and synthesis of osteoprotegerin while decreasing that of *TNFSF11* encoding RANK-ligand.²⁷¹ However, leptin also acts indirectly upon the skeleton. Within the central nervous system, leptin is linked to osteoblasts through the sympathetic nervous system; mediated by the β_2 -adrenergic receptor expressed on the osteoblast cell membrane, leptin-stimulated increase in sympathetic input both inhibits osteoblast proliferation, hence decreasing bone formation, and increases osteoblast expression of *TNFSF11*, thereby favoring osteoclastogenesis.^{272,273} The dual central actions of leptin upon appetite and bone accrual may thus link the processes of energy metabolism and bone accretion.

Adiponectin (encoded by *ADIPOQ*), a 28-kDa product of the white adipocyte whose plasma values are inversely related to visceral fat mass, stimulates osteoclastogenesis by inducing osteoblast expression of *TNFSF11* and inhibiting that of its antagonist *TNFRSF11B*.^{271,274} However, acting through the sympathetic nervous system, adiponectin exerts the opposite effects, for example, suppression of *TNFSF11* expression and osteoclastogenesis and enhancement of osteoblastogenesis and bone mineralization.²⁷¹ Indeed, experimental loss-of-function mutations in *ADIPOQ* are associated with increase in bone mass.²⁷⁵ The storage site of fat within the body—subcutaneous, intraabdominal (visceral), bone marrow—also appears to influence bone mineralization. Thus subcutaneous adipose tissue has a positive effect and visceral adipose tissue a negative influence upon bone formation and size, cortical and trabecular bone mineral densities, and strength. Within bone marrow, the relative rates of differentiation of mesenchymal stem cells into either adipocytes or osteoblasts appears to influence bone mass.

Assessment of Bone Quality, Mass, and Strength

Evaluation of bone mineralization is indicated in children and adolescents with nontraumatic (low-impact—e.g., a fall from her/his standing height or less) or frequent fractures (two or more fractures before age 10 years, three or more fractures

between 11–19 years of age) or whenever a fracture of a vertebra occurs without local disease or high-energy trauma. Indeed, development of a vertebral fracture without local disease or high-energy trauma are criteria for the diagnosis of osteoporosis in a child or adolescent.²⁷⁶ Prospective evaluation of bone mineralization is also appropriate in patients with primary (e.g., osteogenesis imperfecta) or secondary bone disease, such as suboptimal nutrition (anorexia nervosa, cystic fibrosis, celiac disease or other malabsorptive illness) or disease-related risk factors (e.g., inflammatory bowel or joint disease, longer than 3 months exposure to therapeutic doses of glucocorticoids, GH deficiency, hypogonadism, immobility, e.g., cerebral palsy or myopathy, systemically treated childhood cancer survivors).²⁷⁷ Evaluation commences with historical review, documentation of physical findings (height, limb length, presence/absence of skeletal deformities) and appropriate laboratory studies (measurement of serum and urinary levels of calcium, phosphate, alkaline phosphatase, creatinine, PTH, and calcidiol together with other clinically appropriate analytes), and skeletal roentgenograms (radiogrammetry). If skeletal survey suggests abnormal mineralization, further evaluation is warranted. There are several invasive/noninvasive methods that may be used to evaluate bone mineralization, including direct assessment by bone biopsy and histomorphometric analysis of bone formation and resorption.^{278,279} Undecalcified transiliac biopsies permit limited assessment of bone modeling (changes in bone size, shape, mass) but detailed analysis of remodeling (bone renewal) as the iliac crest biopsy is primarily composed of trabecular bone with a circumscribed amount of cortical bone. During bone modeling, osteoblasts and osteoclasts are active on opposite bone surfaces across from one another. Thus the bone surface may change position, size, or mass during the modeling process. Usually modeling is associated with gain in bone mass as the rate of osteoblastic deposition of bone is more rapid than is the rate of osteoclastic resorption. During bone remodeling, osteoclastic resorption of bone is followed by osteoblastic replacement of the reabsorbed bone at the same surface with a net change of zero in bone mineral at the remodeling site under normal circumstances. Histomorphometry enables quantitation of structural parameters of bone size and amount (cortical width, trabecular number and thickness); static bone formation (thickness and surface of osteoid or unmineralized bone matrix, osteoblast surface); dynamic bone formation after labeling with a fluorochrome, such as tetracycline (mineral apposition and bone formation rates); and static resorption (osteoclast number and appearance and extent of eroded surfaces). Iliac trabecular thickness but not trabecular number increases substantially between 2 and 20 years of age, whereas remodeling activity peaks in young children, declines, and then increases again during puberty.²⁷⁸ Compact or cortical bone (present in the shafts of long bones and in the outer layer of vertebral bone) has a complex system of osteons (layers or lamellae of bone surrounding Haversian canals through which pass the intraosseous vasculature), lacunae in which the osteocytes reside, and canaliculi—passageways in bone through which extensions of osteocytes interconnect with one another, as well as osteoblasts and bone lining cells.^{280,281} Using small sections of excised bone examined by x-ray microcomputed tomography, it is possible to quantify several cortical bone microstructural components, including cortical porosity (the volume of the intracortical canals) and the diameter, separation, connectivity density, and canal surface to tissue volume of the intracortical canals.²⁸⁰ In normal children, there is a negative correlation between cortical porosity and BMD. “Noninvasive bone biopsy” is also achievable with the use of high-resolution peripheral quantitative computed tomography (HRpQCT) that permits construction of microfinite element models of bone strength, thus enabling quantitation of both cortical and trabecular bone dimensions.^{223,282} Micromagnetic

resonance imaging (μ MRI) is also a noninvasive technique that allows “virtual bone biopsy.”²⁸³

Noninvasive methods of assessment of skeletal mineralization besides skeletal roentgenograms include radiographic absorptiometry or photodensitometry, single photon or single x-ray absorptiometry, dual photon or DXA, spinal and peripheral quantitative computed tomography (pQCT), HRpQCT, quantitative ultrasonography (QUS), high-resolution quantitative MRI, quantitative μ MRI, and magnetic resonance microscopy.^{237,284} Automated radiogrammetry uses a standard radiograph of the hand measuring the dimensions of its metacarpals enabling calculation of cortical thickness, bone length, and bone width and predictions of bone fragility.

The most commonly used method for two-dimensional assessment of bone mineralization is DXA (Tables 9.6 and 9.7). The ratios of attenuation of x-rays of two energies (70 kV, 140 kV) traversing the same pathway through the patient reflect the “density” and mass of the tissue through which the x-rays have crossed; computer analysis of these captured energies then reconstructs the boundaries, density, and mass of the tissue. Because of variability between DXA instruments and analytical software programs (pediatric, adult) used, the report of the DXA scan should include not only the recorded data but also the type of DXA instrument and the software version used for analysis. This procedure provides two-dimensional densitometric analysis of cortical plus trabecular BMC (g) and areal BMC (g/cm^2). The microarchitecture of bone is a determinant of bone strength that is measured in part by assessment of the trabecular bone score that can be determined by differential analysis of the DXA pattern of radiation absorbed/transmitted by/through cancellous bone sites, primarily composed of trabeculae and their interconnected processes, and intertrabecular spaces (e.g., vertebrae, distal ends of the long bones, skull, ribs). The most useful DXA analyses in children are those that determine whole body minus (–) head (primarily cortical bone) and anterior/posterior lumbar spine vertebrae 1 to 4 (mostly trabecular bone).^{276,277,285} DXA is the most widely used densitometric technique because it is easily accessible, there are many standard data (related to gender, age, ethnicity), the procedure is rapid, and radiation exposure (5–6 μSv) is reasonably low.²⁷⁶

However, DXA does not measure “true” BMD—the mass within a volume of uniform composition that is expressed as grams of hydroxyapatite/ cm^3 (g/cm^3); rather DXA measures the two-dimensional areal or surface mass of mineral within a region of bone of nonuniform composition (cortex, trabeculae, osteoid, marrow); it is expressed as grams of hydroxyapatite/ cm^2 (g/cm^2). Because DXA does not take into account the depth of a bone, it may underestimate BMD in small children and overestimates it in large subjects.²⁸⁶ In children and adolescents, bone size (volume) increases with growth and maturation; the larger the three-dimensional structure of bone, the greater is the recorded areal BMD, even though the actual or volumetric (v) BMD may not change substantially. Therefore calculated or “apparent” (v) BMDs (BMAD) (g/cm^3) data have been generated in an attempt to correct this problem. Volumetric BMD increases as the cortex thickens, the number and width of trabeculae per unit volume rises, and the amount of hydroxyapatite per unit of trabecular volume accrues. Although during childhood and adolescence areal BMD of the femoral shaft increases with age, its vBMD remains relatively constant. Volumetric BMD of the lumbar spine increases in late puberty-early adulthood because of increasing thickness of trabeculae that is not gender specific but is greater in blacks than in whites after puberty.²⁸⁷ Cross-sectional areas of cortical and trabecular bone increase with age, with males achieving greater increase in periosteal apposition than do females during puberty. Cortical cross-sectional area is similar in white and black subjects.²⁸⁸ Heretofore the skull has been included in whole-body

DXA-generated BMD data; however, the skull has twice the BMD of that of the rest of the skeleton; whole-body BMD correlates better with height when the skull is excluded. Consequently, there are reference data for DXA whole-body BMC and BMD with inclusion or exclusion of the skull.^{244,245}

Inasmuch as DXA BMC and BMD values increase with height in both boys and girls, DXA-derived bone mass and density data should be adjusted for height (height age) when interpreting study results in children and adolescents.^{289,290} In the adolescent with markedly delayed skeletal maturation, it is helpful to consider DXA data in relation to skeletal maturation/bone age.²⁷⁷ The BMC and BMD of the total body—head, lumbar spine, hip, femoral neck, and distal one-third of the radius—are the most commonly measured indices of bone mass in childhood by DXA and recorded as g/cm². DXA data are reported as the standard deviation score (SDS) about the mean (SDS = 0) for gender, age, and ethnicity and interpreted individually for height SDS and/or bone age and/or Tanner stage(s) of pubertal maturation and/or body composition.²⁸⁵ Thus data derived from DXA may be “low, normal, or high” relative to the patient’s race, gender, and/or chronological age. These data are reported as a “Z score” using standard deviations about the mean, where Z=0 indicates that the value is at the mean for race, gender, and chronological age. To establish the presence of osteoporosis in a child/adolescence, it has been recommended that there be a history of a low-impact traumatic vertebral fracture in the absence of vertebral disease or two or more long bone fractures and that the total body (minus head) BMC/lumbar spine vertebrae 1-4 by DXA be equal to or below

2 SDs for gender, age, ethnicity, and height ($Z \leq 2.0$). The diagnosis of osteoporosis in a child/adolescent cannot be made on the basis of bone mineralization data alone; use of the term *osteopenia* in children is invalid.^{277,285,291}

In neonates and infants, whole/total body BMC is the primary measurement used to assess bone mineralization by DXA, although data for the lumbar spine and femur are also available (see Table 9.6).^{292,293} In neonates with appropriate weight for gestational age (AGA), whole-body BMC by DXA doubles between 32 and 40 weeks and increases 3.5-fold between birth weights of 1000 and 4000 grams; small for gestational age (SGA) neonates have lower whole-body BMC than do AGA neonates of comparable gestational age but they are similar to those of AGA infants with the same birth weights.²⁹⁴ In term infants, whole-body BMC, lumbar spine, and femur increase 200%, 130%, and 190% by their first birthday.²⁹² Total body—head BMC by DXA increases approximately four-fold between 7 and 17 years of age and is greater in males than females and in black relative to non-black youth (see Tables 9.7A and 9.7B).^{244,245} Lumbar vertebral areal BMD doubles between 7 and 17 years of age in both boys and girls (Tables 9.8A and 9.8B). The BMD of the femoral neck increases 1.5-fold between 7 and 17 years of age in both genders. The BMC of the distal one-third radius approximately doubles and its BMC increases 1.5-fold between 7 and 17 years.^{223,244,245} At all skeletal sites, BMC and BMD assessed by DXA are higher in males than females and in black relative to non-black children and adolescents (Tables 9.8C and 9.8D).^{244,245} In females, the rate of maximal increase in

TABLE 9.8A Lumbar Spine 1-4 DXA Bone Mineral Content (g) and Lumbar Spine, Total Hip, and One-Third Radius Bone Mineral Density (BMD - g/cm²) in Black and Non-Black Male Children and Adolescents

Non-Black			Black	
LUMBAR SPINE				
CA	BMC	BMD	BMC	BMD
5	14.06 (10.47–18.10)	0.484 (0.393–0.586)	15.16 (11.33–20.15)	0.506 (0.386–0.603)
7	18.68 (13.99–24.36)	0.531 (0.427–0.647)	19.45 (14.39–26.10)	0.555 (0.422–0.669)
9	22.70 (17.00–30.07)	0.573 (0.457–0.703)	23.45 (17.17–31.78)	0.602 (0.456–0.733)
11	26.70 (19.82–36.19)	0.619 (0.488–0.767)	28.70 (20.59–39.65)	0.662 (0.501–0.816)
13	35.23 (24.85–50.56)	0.710 (0.548–0.891)	38.96 (26.72–56.13)	0.770 (0.590–0.973)
15	51.97 (35.46–73.10)	0.873 (0.674–1.094)	54.76 (37.36–79.34)	0.923 (0.719–1.174)
17	64.38 (46.29–88.31)	0.991 (0.786–1.236)	65.88 (46.00–93.35)	1.035 (0.818–1.303)
20	70.66 (51.73–98.89)	1.055 (0.847–1.320)	72.48 (51.14–101.70)	1.105 (0.882–1.375)

CA, Age in years; 50th percentile (3rd–97th percentiles).

(Modified from Zemel, B.S., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, 96, 3160–3169; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)

TABLE 9.8B Lumbar Spine 1-4 DXA Bone Mineral Content (g) and Lumbar Spine, Total Hip, and One-Third Radius Bone Mineral Density (BMD - g/cm²) in Black and Non-Black Female Children and Adolescents

Non-Black			Black	
LUMBAR SPINE 1-4				
CA	BMC	BMD	BMC	BMD
5	14.38 (11.12–18.87)	0.499 (0.407–0.630)	15.33 (11.66–20.82)	0.539 (0.410–0.675)
7	17.87 (13.62–23.81)	0.535 (0.432–0.676)	18.75 (14.11–25.76)	0.573 (0.438–0.717)
9	21.42 (16.02–29.10)	0.578 (0.460–0.734)	22.53 (16.67–31.49)	0.622 (0.479–0.778)
11	27.90 (19.59–40.17)	0.664 (0.507–0.859)	31.18 (21.70–45.94)	0.737 (0.575–0.919)
13	41.27 (27.31–60.49)	0.831 (0.623–1.066)	45.04 (31.26–63.46)	0.905 (0.717–1.124)
15	51.85 (36.67–71.07)	0.954 (0.759–1.171)	54.93 (39.24–72.85)	1.018 (0.816–1.262)
17	56.20 (41.39–74.61)	1.002 (0.820–1.209)	59.70 (41.30–78.89)	1.074 (0.866–1.329)
20	58.26 (43.90–75.93)	1.023 (0.847–1.222)	62.83 (40.85–83.86)	1.106 (0.895–1.367)

CA, Age in years; 50th percentile (3rd–97th percentiles).

(Modified from Zemel BS et al. (2001). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: Results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab* 96:3160-3169,2011; <https://doi.org/10.1210/jc.2011-1111>; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)

TABLE 9.8C Total Hip Areal Bone Mineral Density (BMD, g/cm²) and Femoral Neck Bone Mineral Content (g) and Areal BMD by DXA in Male and Female Non-Black and Black Children and Adolescents

TOTAL HIP AREAL BONE MINERAL DENSITY					
	Males			Females	
	Non-Black	Black		Non-Black	Black
5	0.573 (0.473–0.669)	0.610 (0.509–0.686)		0.559 (0.475–0.662)	0.588 (0.493–0.706)
7	0.646 (0.523–0.764)	0.708 (0.571–0.815)		0.603 (0.505–0.721)	0.657 (0.546–0.795)
9	0.705 (0.563–0.844)	0.778 (0.613–0.911)		0.650 (0.536–0.788)	0.720 (0.594–0.880)
11	0.755 (0.597–0.912)	0.835 (0.648–0.993)		0.727 (0.579–0.905)	0.811 (0.662–1.003)
13	0.838 (0.653–1.027)	0.936 (0.716–1.142)		0.856 (0.664–1.079)	0.930 (0.750–1.167)
15	0.977 (0.754–1.214)	1.068 (0.818–1.339)		0.940 (0.735–1.167)	1.003 (0.801–1.271)
17	1.064 (0.820–1.331)	1.154 (0.892–1.470)		0.969 (0.763–1.194)	1.030 (0.819–1.311)
20	1.098 (0.846–1.377)	1.179 (0.914–1.510)		0.975 (0.769–1.200)	1.040 (0.826–1.326)
FEMORAL NECK BONE MINERAL CONTENT					
	Males			Females	
	Non-Black	Black		Non-Black	Black
5	1.714 (1.224–2.191)	1.890 (1.358–2.372)		1.713 (1.274–2.173)	1.784 (1.335–2.325)
7	2.158 (1.541–2.777)	2.408 (1.746–3.044)		2.014 (1.494–2.567)	2.118 (1.588–2.757)
9	2.579 (1.844–3.339)	2.861 (2.090–3.391)		2.334 (1.720–3.000)	2.526 (1.889–3.293)
11	2.985 (2.141–3.885)	3.289 (2.421–4.218)		2.827 (2.034–3.717)	3.128 (2.336–4.082)
13	3.631 (2.624–4.757)	4.008 (2.981–5.208)		3.575 (2.561–4.761)	3.780 (2.865–4.872)
15	4.611 (3.381–6.071)	4.967 (3.737–6.574)		4.022 (2.962–5.279)	4.171 (3.091–5.478)
17	5.243 (3.879–6.936)	5.620 (4.261–7.555)		4.149 (3.099–5.396)	4.313 (3.136–5.756)
20	5.552 (4.123–7.373)	5.821 (4.424–7.871)		4.189 (3.144–5.430)	4.387 (3.155–5.906)
FEMORAL NECK BONE MINERAL DENSITY					
	Males			Females	
	Non-Black	Black		Non-black	Black
5	0.544 (0.448–0.648)	0.575 (0.460–0.663)		0.541 (0.462–0.641)	0.559 (0.470–0.685)
7	0.611 (0.497–0.735)	0.672 (0.526–0.788)		0.574 (0.484–0.687)	0.613 (0.509–0.757)
9	0.665 (0.536–0.807)	0.740 (0.569–0.878)		0.614 (0.510–0.744)	0.677 (0.554–0.843)
11	0.711 (0.567–0.870)	0.794 (0.604–0.953)		0.681 (0.549–0.844)	0.764 (0.612–0.962)
13	0.778 (0.612–0.962)	0.877 (0.657–1.071)		0.786 (0.611–0.991)	0.867 (0.674–1.108)
15	0.885 (0.683–1.111)	0.984 (0.726–1.230)		0.860 (0.660–1.081)	0.937 (0.707–1.214)
17	0.965 (0.732–1.226)	1.067 (0.779–1.359)		0.888 (0.680–1.112)	0.964 (0.716–1.257)
20	0.998 (0.751–1.275)	1.093 (0.796–1.403)		0.894 (0.685–1.119)	0.977 (0.719–1.277)

CA, Age in years; 50th percentile (3rd–97th percentiles).(Modified from Zemel, B.S., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, 96, 3160–3169; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)**TABLE 9.8D** Distal One-Third Radius Bone Mineral Content (g) and Areal Bone Mineral Density (g/cm²) by DXA in Male and Female Non-Black and Black Children and Adolescents

DISTAL ONE-THIRD RADIUS BONE MINERAL CONTENT					
	Males			Females	
	Non-Black	Black		Non-Black	Black
5	0.722 (0.568–0.877)	0.825 (0.655–1.025)		0.718 (0.541–0.896)	0.806 (0.636–0.999)
7	0.891 (0.691–1.091)	0.999 (0.788–1.250)		0.845 (0.633–1.057)	0.940 (0.741–1.171)
9	1.036 (0.794–1.278)	1.174 (0.918–1.481)		0.979 (0.729–1.229)	1.096 (0.864–1.373)
11	1.182 (0.897–1.468)	1.353 (1.048–1.720)		1.163 (0.863–1.463)	1.318 (1.046–1.655)
13	1.424 (1.069–1.779)	1.626 (1.250–2.080)		1.421 (1.073–1.770)	1.589 (1.279–1.980)
15	1.797 (1.360–2.234)	1.974 (1.533–2.503)		1.596 (1.240–1.952)	1.769 (1.448–2.179)
17	2.077 (1.604–2.550)	2.230 (1.773–2.770)		1.682 (1.330–2.034)	1.856 (1.533–2.269)
20	2.262 (1.777–2.746)	2.419 (1.976–2.761)		1.749 (1.402–2.097)	1.928 (1.605–2.343)
DISTAL ONE-THIRD RADIUS BONE MINERAL DENSITY					
	Males			Females	
	Non-Black	Black		Non-Black	Black
5	0.411 (0.358–0.466)	0.435 (0.378–0.491)		0.406 (0.352–0.466)	0.439 (0.378–0.506)
7	0.454 (0.394–0.518)	0.489 (0.419–0.559)		0.448 (0.386–0.513)	0.476 (0.411–0.547)
9	0.492 (0.425–0.563)	0.534 (0.451–0.618)		0.487 (0.416–0.559)	0.516 (0.447–0.592)
11	0.526 (0.453–0.606)	0.576 (0.479–0.672)		0.537 (0.453–0.618)	0.571 (0.496–0.653)
13	0.577 (0.493–0.672)	0.627 (0.515–0.739)		0.605 (0.511–0.693)	0.637 (0.556–0.726)
15	0.658 (0.564–0.767)	0.694 (0.572–0.817)		0.653 (0.564–0.740)	0.682 (0.596–0.774)
17	0.728 (0.633–0.838)	0.752 (0.633–0.871)		0.678 (0.592–0.765)	0.705 (0.618–0.800)
20	0.778 (0.684–0.889)	0.801 (0.696–0.905)		0.693 (0.609–0.780)	0.730 (0.641–0.826)

CA, Age in years; 50th percentile (3rd–97th percentiles).(Modified from Zemel, B.S., et al. (2011). Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab*, 96, 3160–3169; Revised 2012; <https://doi.org/10.1210/jc.2012-3878>.)

whole-body BMC occurs in the year of menarche and follows the year of peak height velocity (Fig. 9.16).²⁹⁵ In girls with menarche before 12 years, there is greater peak BMD (at age 18–19 years) than in females with menarche after 14 years. BMD is greater in children with premature adrenarche than in preadrenarchal peers, but appropriate for the tall stature of the adrenarchal subjects.²⁸⁹ In adult males with a history of delayed puberty, there is lower lumbar spine BMD than in those with pubertal onset at 11 to 12 years. DXA measurements of bone mineralization are affected by the composition of soft tissues that surround the axial skeleton; variations in fat about the bone may significantly influence the recorded DXA measurements, thus limiting the use of DXA in extremely thin or obese children.²⁹⁶ In general, BMC, areal BMD, and/or BMAD below -2 SD for age and gender are considered abnormally

low. However, DXA measurements must always be interpreted in relationship to the patient's clinical status, for example, height, pubertal stage.²⁸⁶

There are several other techniques for assessment of bone mineralization.²⁹⁷ Quantitative ultrasonography of the heel, fingers, radius, and ulna avoids radiation exposure and enables measurement of the speed of sound, bone transmission time, and related data.²⁹⁸ Quantitative computed tomography (QCT) and peripheral (p)QCT separately measure the volume of cortical (cancellous) and trabecular bone of the lumbar vertebrae, midshaft of the femur, distal radius, and ulna enabling determination of volumetric BMD (g/cm^3), cross-sectional area of the bone, including cortex and vertebrae, and its periosteal and endosteal surfaces. HRpQCT can provide even finer detail.^{299,300} MRI of the vertebrae and long bones also permits

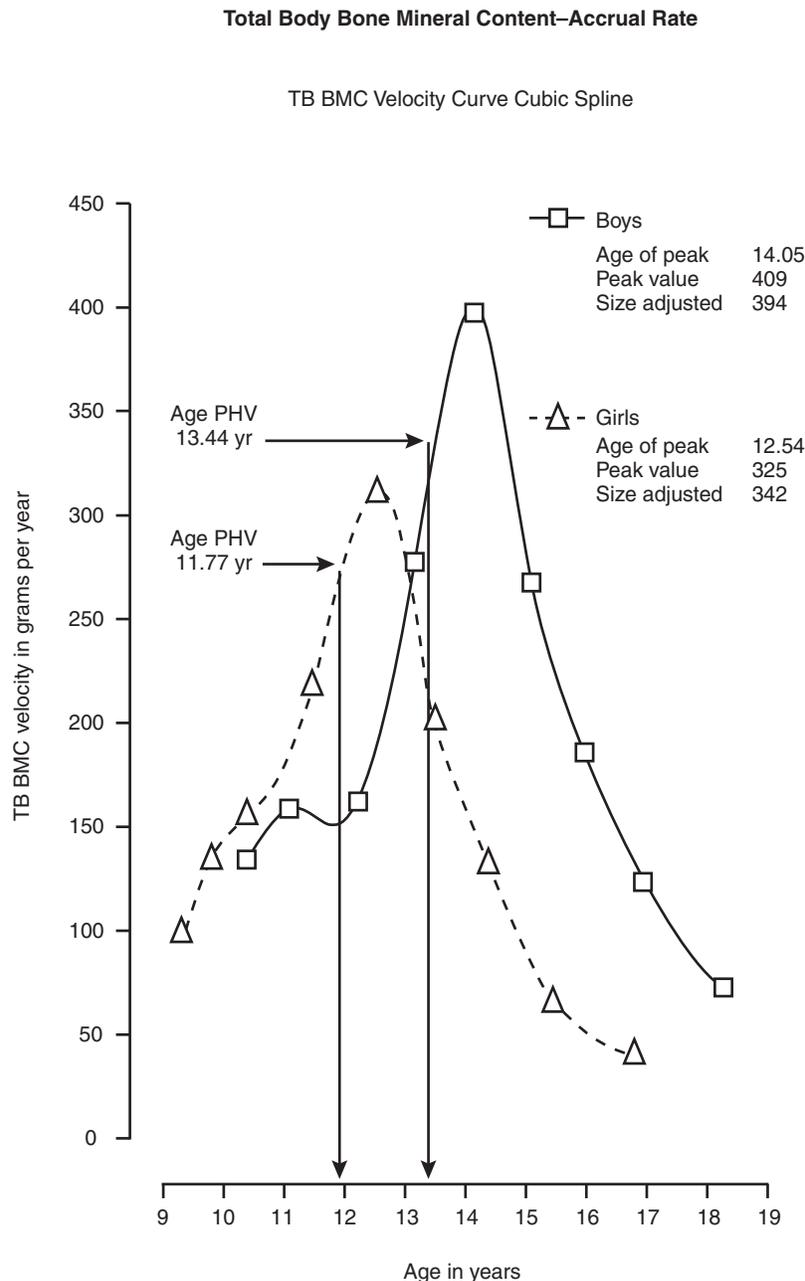


Fig. 9.16 During adolescence the peak rate of accrual of total body bone mineral content (BMC) occurs in boys and girls 0.7 years after attainment of peak height velocity (PHV). (From Bailey, D.A., McKay, H.A., Mirwald, R.L. et al. (1999). A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: The University of Saskatchewan bone mineral accrual study. *J Bone Miner Res*, 14, 1672–1679. With permission.)

separate measurements of cortical and trabecular bone volume and mineral content and concentration, and estimation of apparent trabecular number, thickness, and spacing without exposure to ionizing radiation. Vertebral size and the presence of compression fractures may also be assessed by MRI, as well as by DXA.²⁹⁷

QCT of the spine, radius, and tibia provides three-dimensional measurements of cortical and trabecular bone mineralization and quantitation of cortical area and thickness and periosteal and endosteal circumference. QCT measures volumetric BMD of both trabecular and cortical bone at any site but has been commonly applied to the lumbar spine.²⁸⁷ However, the radiation dose delivered to the spine by this method is high (~30 μSv). During childhood, lumbar spine volumetric BMD measured by QCT is similar in black and white youth; during puberty, black males and females gain twice the volumetric BMD recorded in whites with no gender difference. Although BMD values of the lumbar spine acquired by DXA and QCT are reasonably well related, low bone density is reported in children far more often by DXA than by QCT, unless DXA measurements take into account body height and bone size.²⁹⁶ pQCT enables quantitation of bone mineralization in distal and/or proximal radial, femoral, or tibial sites (regions of both cortical and trabecular bone); the delivered radiation dose (10 μSv) is low. Forearm pQCT is assessed at 4% and 66% of the length of the nondominant forearm with a reference line being drawn through the most distal aspect of the cartilage growth plate when it is open, or through the

middle of the ulnar border of the articular cartilage when the growth plate is closed.^{299,301} pQCT permits measurement of total, cortical, and trabecular volumetric BMC and BMD, total and cortical cross-sectional bone area, cortical thickness, periosteal and endosteal circumferences, marrow area, and fat and muscle cross-sectional area (Table 9.9).

HRpQCT measurements may be obtained at the ultradistal tibia and radius in the interval between 1 and 10 mm above the proximal limit of the epiphyseal growth plate of the non-dominant limb and deliver a local absorbed radiation dose of 0.065 cGy and a total radiation exposure of <0.01 mSv.^{223,300} HRpQCT enables quantitative measurement of cortical and trabecular bone including: cortical bone volumetric BMD, cortical bone area, cortical bone thickness (Ct.Th— μm), periosteal and endosteal (endocortical) circumferences (μm), cortical pore volume (cortical areas of inverse density) and the “cortical porosity index” (cortical pore volume/cortical bone volume ratio), trabecular bone volumetric BMD, trabecular bone volumetric BMD/total bone volume ratio (BV/TV—%), trabecular number (Tb.N— mm^{-1}), trabecular thickness (Tb.Th— μm), and trabecular spacing (Tb.SP— μm) (Table 9.10). Determination of the “cortical porosity index” affords an estimate of strength of the distal wrist. HRpQCT also permits construction of microfinite element (μFE) models of bone strength enabling calculation of strain energies and loads supported by cortical and trabecular bone and “failure loads.”^{223,302} Fig. 9.17 depicts visually three-dimensional reconstructions of cortical and trabecular bone as derived by HRpQCT examinations.²²³

TABLE 9.9 Peripheral Quantitative Computed Tomography—Nondominant Distal Radius (Mean \pm SD)

Age	vBMD-tot ^a	vBMD-Trab ^a	vBMD-cort ^a	CSA ^b
MALES				
6–7	306 (34)	206 (32)	388 (42)	174 (31)
8–9	294 (34)	189 (34)	380 (41)	211 (31)
10–11	290 (33)	194 (32)	368 (41)	245 (37)
12–13	292 (38)	201 (36)	366 (47)	289 (47)
14–15	293 (35)	201 (33)	369 (47)	351 (70)
16–17	349 (56)	217 (30)	458 (86)	358 (49)
18–23	401 (60)	220 (42)	549 (83)	377 (64)
Adults	438 (56)	224 (46)	594 (81)	374 (45)
PUBERTAL STAGE				
I	299 (32)	198 (31)	381 (41)	212 (47)
II	288 (40)	186 (31)	372 (52)	269 (43)
III	286 (33)	197 (36)	359 (37)	293 (44)
IV	296 (42)	210 (35)	367 (50)	334 (65)
V	361 (72)	215 (40)	481 (109)	377 (59)
FEMALES				
6–7	290 (36)	191 (31)	370 (45)	164 (30)
8–9	283 (22)	186 (23)	362 (32)	185 (25)
10–11	281 (36)	191 (36)	355 (44)	237 (39)
12–13	295 (39)	197 (32)	376 (54)	260 (55)
14–15	303 (37)	179 (25)	407 (53)	297 (32)
16–17	350 (57)	186 (26)	483 (95)	300 (45)
18–23	371 (50)	195 (35)	516 (74)	295 (42)
Adults	395 (46)	182 (34)	569 (69)	281 (37)
PUBERTAL STAGE				
I	284 (30)	187 (29)	363 (39)	188 (38)
II	277 (34)	190 (34)	348 (37)	239 (57)
III	288 (44)	204 (44)	356 (50)	250 (47)
IV	291 (43)	197 (32)	375 (56)	282 (30)
V	347 (54)	190 (28)	476 (87)	295 (43)

CSA, Cross-sectional area. vBMD, volumetric bone mineral density.

^amg/cm³.

^bmm².

(Modified from Neu, C.M., et al. (2001). Bone densities and bone size at the distal radius in healthy children and adolescents: a study using peripheral quantitative computed tomography. *Bone*, 28, 227–232; Rauch, F., Schonau, E. (2005). Peripheral quantitative computed tomography of the distal radius in young subjects - new reference data and interpretation of results. *J Musculoskelet Neuronal Interact*, 5, 119–126.)

TABLE 9.10 High-Resolution Peripheral Quantitative Computed Tomography (HRpQCT) in Midadolescent Males and Young Adult Females

MALES (n = 89)		FEMALES (n = 15)	
Age (y)	15.2 ± 0.5	Age (y)	19.4 ± 1.2
DEXA		DEXA	
Radial metaphysis BMD (mg/cm ²)	383 ± 54	Femoral neck BMD (mg/cm ²)	830 ± 110
Radial diaphysis BMD (mg/cm ²)	660 ± 67	Total hip BMD (mg/cm ²)	940 ± 70
Femoral neck BMD (mg/cm ²)	901 ± 133	L2-L4 BMD (mg/cm ²)	1010 ± 130
Total hip BMD (mg/cm ²)	992 ± 139	HRpQCT	
Femoral diaphysis BMD (mg/cm ²)	1682 ± 171	Ultradistal Radius	
L2-L4 BMD (mg/cm ²)	918 ± 135	Total area (mm ²)	265.4 ± 54.1
HRpQCT		Total vBMD (mm ³)	298.2 ± 52.6
Ultradistal Radius		Cortical vBMD (mm ³)	825.8 ± 64.6
Total vBMD (mg/cm ³)	257 ± 38	Trabecular vBMD (mm ³)	158.1 ± 26.6
Cortical vBMD (mg/cm ³)	637 ± 73	Trabecular area (mm ²)	212.3 ± 53.3
Trabecular vBMD (mg/cm ³)	195 ± 27	Trabecular area (%)	79.4 ± 5.0
BV/TV (%)	16.2 ± 2.3	Tb.N (mm ⁻¹)	1.96 ± 0.26
Tb.N (mm ⁻¹)	2.23 ± 0.20	Tb.Th (μm)	70.0 ± 10.0
Tb.Th (μm)	72.6 ± 8.4	Tb.Sp (μm)	450.0 ± 70.0
Tb.Sp (μm)	379 ± 41	Cortical area (mm ²)	47.9 ± 13.2
Ct.Th (μm)	388 ± 219	Cortical area (%)	18.7 ± 5.8
CSA (mm ²)	333 ± 61	Ct.Th (μm)	710.0 ± 20.0
Stiffness (kN/mm)	87.2 ± 21.6	Cortical perimeter (mm)	67.6 ± 6.6
Failure load (N)	4239 ± 996	Distal Tibia	
Distal Tibia		Total area (mm ²)	700.6 ± 104.6
Total vBMD (mg/cm ³)	272 ± 45	Total vBMD (mm ³)	308.1 ± 39.6
Cortical vBMD (mg/cm ³)	730 ± 56	Cortical vBMD (mm ³)	870.7 ± 31.3
Trabecular vBMD (mg/cm ³)	205 ± 27	Trabecular vBMD (mm ³)	192.3 ± 24.7
BV/TV (%)	17.1 ± 2.3	Trabecular area (mm ²)	583.0 ± 106.0
Tb.N (mm ⁻¹)	2.13 ± 0.31	Trabecular area (%)	83.0 ± 3.7
Tb.Th (μm)	81.1 ± 10.6	Tb.N (mm ⁻¹)	1.77 ± 0.26
Tb.Sp (μm)	398 ± 62	Tb.Th (μm)	90.0 ± 20.0
Ct.Th (μm)	851 ± 336	Tb.Sp (μm)	480.0 ± 70.0
CSA (mm ²)	888 ± 151	Cortical area (mm ²)	116.1 ± 20.5
Stiffness (kN/mm)	259.6 ± 54.7	Cortical area (%)	16.9 ± 4.0
Failure load (N)	12430 ± 2559	Ct.Th (μm)	1.14 ± 0.22
		Cortical perimeter (mm)	102.8 ± 7.6

(Mean ± SD)

CSA, Cross-sectional area; DEXA, dual energy x-ray absorptiometry; vBMD, volumetric bone mineral density.

(Modified from Ackerman, K.E., Nazem, T., Chapko, D., et al. (2011). Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. *J Clin Endocrinol Metab*, 96, 3123–3133; Chevalley, T., Bonjour, J.P., van Rietbergen, B., et al. (2011). Fractures during childhood and adolescence in healthy boys: Relation with bone mass, microstructure, and strength. *J Clin Endocrinol Metab*, 96, 3134–3142.)

In boys, cortical bone volumetric BMD, Ct.Th, BV/TV, and Tb.Th increase in late puberty. In girls, cortical bone volumetric BMD and Ct.Th decline in midpuberty and then increase. Total bone strength increases in males and females through puberty. The percent of load borne by cortical bone decreases and the cortical bone porosity index increases in midpuberty, coincident with the point of peak incidence of adolescent radial fractures.

QUS enables evaluation of “bone quality” by measuring the speed of a longitudinal sound (SOS) wave as it is propagated along a bone; “speed of sound” and “sound wave attenuation” assess bone elasticity and connectivity and some aspects of bone architecture.²³⁷ This methodology has been applied to long bones (tibia, radius), to phalanges, the patella, and the os calcis. The rate of movement of sound through bone is dependent on its microstructural and macrostructural characteristics, mineral density, and elasticity and is thought to be a measure of bone strength. It is an attractive method for assessment of bone because it does not use radiation, is low in cost, and the measuring equipment is portable. Because of its safety, it is a measurement used in newborns and children. Transmitter and receiver ultrasound transducers placed on either side of the examination site (os calcis, patella, tibia, radius, phalange) quantitate transmission velocity or signal attenuation and convert these observations to SOS. In a study of 1085 children and

adolescents, SOS increased steeply at the tibia and radius during the first 5 years of life, more slowly between 6 and 11 years of age, and then again more rapidly during pubertal development.³⁰³ In 3044 healthy subjects ages 2 to 21 years, phalangeal QUS SOS and bone transmission time increased over time and with advancing adolescent development and were related to gender, age, height, and weight.³⁰⁴ QUS SOS is higher in postmenarchal than in premenarchal girls; adiposity (BMI) and serum leptin levels are inversely related to SOS in pubertal girls.³⁰⁵ The overlap of QUS data between various ages makes interpretation of a single SOS measurement problematic; serial assessment may be useful. Thus in a cohort of 29 preterm infants, tibial SOS values declined over time in neonates whose gestational ages were less than 29 weeks suggesting progressive loss of bone strength in this population and consistent with the development of “osteopenia of prematurity.”²⁹⁸ Although there is marginal correlation between vBMD determined by pQCT and SOS measurements in children and adolescents, QUS may complement radiographic methods.

MRI measures bone volume and cortical and trabecular bone microarchitecture; peripheral MRI has enabled assessment of bone structure and mineralization in the radius, ulna, femur, and calcaneus; MRI of the skeleton may also be used to assess bone geometry and estimate bone strength.³⁰⁶ Bone

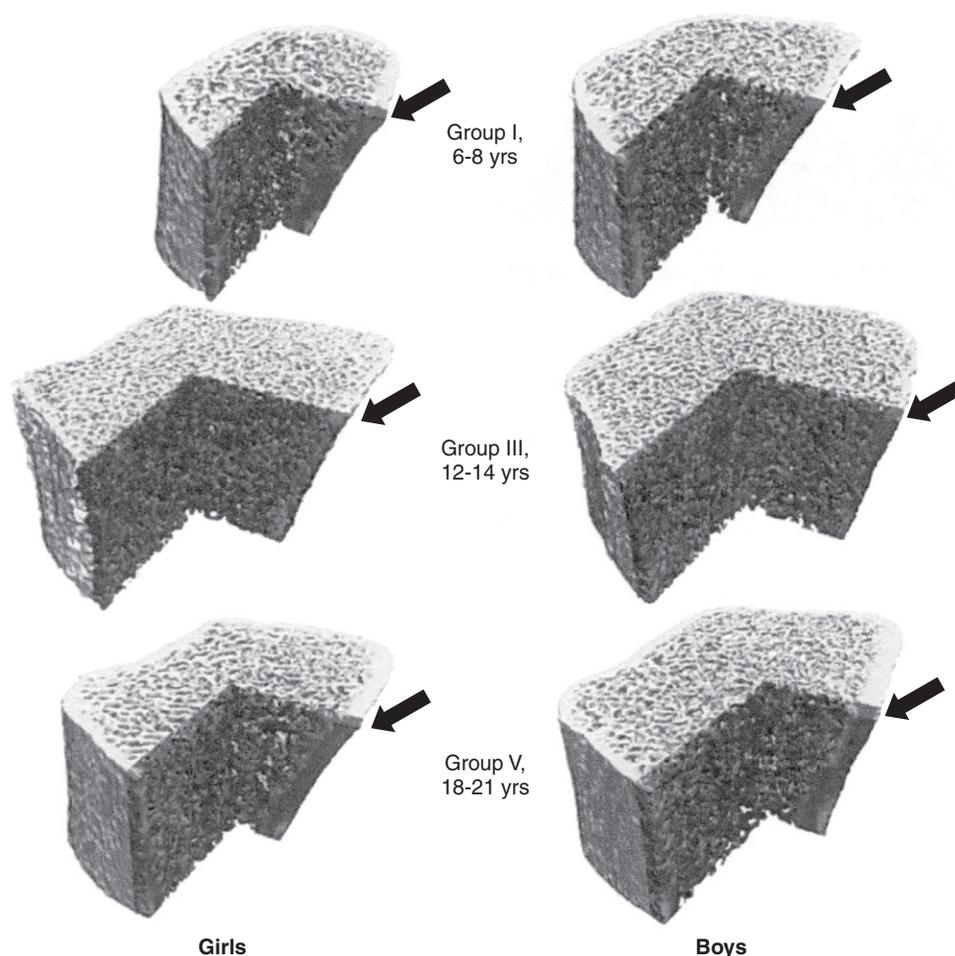


Fig. 9.17 Three-dimensional (3D) reconstructions of cortical and trabecular bone of ultradistal radius using high-resolution peripheral quantitative computed tomography (HRpQCT). Arrows point to cortical bone. (From Kirmani, S., Christen, D., van Lenthe, G.H., et al. (2009). Bone structure at the distal radius during adolescent growth. *J Bone Miner Res*, 24, 1033–1042. With permission.)

high-resolution MRI of the wrist and tibia measures cortical and trabecular bone structure including: trabecular number (mm^{-1}), thickness (mm), bone fraction (%), and separation (mm).³⁰⁷ Further, micro-MRI can be used to measure whole bone and trabecular bone stiffness, an indicator of whole bone mechanical competence, quality, and strength, and to obtain “virtual bone biopsies.”^{283,308} MRI of the vertebrae and long bones also permits separate measurements of cortical and trabecular bone volume and mineral content and concentration, and estimation of apparent trabecular number, thickness, and spacing without exposure to ionizing radiation. Vertebral size and the presence of compression fractures may also be assessed by MRI, as well as by DXA.²⁹⁷

Dedicated to the memory of Frank B. Diamond Jr, MD

REFERENCES

- Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clin J Am Soc Nephrol*. 2015;10:1257–1272.
- Favus MJ, Goltzman D. Regulation of calcium and magnesium. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:104–108.
- Heaney RP. The nutrient problem, as seen through the lens of calcium. *J Clin Endocrinol Metab*. 2011;96:2035–2037.
- Bringham FR, Demay MB, Kronenberg HM. Hormones and disorders of mineral metabolism. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, eds. *Williams Textbook of Endocrinology*. 11th ed. Philadelphia: Saunders/Elsevier; 2008:1203–1268.
- Cahalan MD. How to STIMulate calcium channels. *Science*. 2010;330:43–44.
- Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers*. 2017;3:17055.
- Kornak U. Animal models with pathologic mineralization phenotypes. *Joint Bone Spine*. 2011;78:561–567.
- Gill DL, Spassova MA, Soboloff J. Calcium entry signals - trickles and torrents. *Science*. 2006;313:183–184.
- Halling DB, Aracena-Parks P, Hamilton SL. Regulation of voltage-gated Ca^{2+} channels by calmodulin. *Sci STKE*. 2005. 2005, re15.
- Nwokonko RM, Cai X, Laktionova NA, et al. The STIM-Orai pathway: conformational coupling between STIM and Orai in the activation of store-operated Ca^{2+} entry. *Adv Exp Biol Med*. 2017;993:83–98.
- Vig M, Peinelt C, Beck A, et al. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science*. 2006;312:1220–1223.
- Park CY, Shcheglovitov A, Dolmetsch R. The CRAC channel activator STIM1 binds and inhibits L-type voltage-gated calcium channels. *Science*. 2010;330:101–105.
- Wang Y, Deng X, Mancarella S, et al. The calcium store sensor, STIM1, reciprocally controls Orai and $\text{CaV}1.2$ channels. *Science*. 2010;330:105–109.
- Feske S, Gwack Y, Prakriya M, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006;441:179–185.
- Byun M, Abhyankar A, Lelarge V, et al. Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *Exp Med*. 2010;207:2307–2312.

16. Dellis O, Dedos SG, Tovey SC, et al. Ca²⁺ entry through plasma membrane IP₃ receptors. *Science*. 2006;313:229–233.
17. Abramson J, Paz A, Philipson KD. It's all in the symmetry. *Science*. 2012;335:669–670.
18. Liao J, Li H, Zeng W, et al. Structural insight into the ion-exchange mechanism of the sodium/calcium exchanger. *Science*. 2012;335:686–688.
19. McMurray JJV, Smith GL. Calcium handling in the failing heart and SUMO - Weighing the evidence. *N Engl J Med*. 2011;365:1738–1739.
20. Thiele S, Mantovani G, Barlier A, et al. From pseudohypoparathyroidism to inactivating PTH/PTHrP signalling disorder (iPPSD), a novel classification proposed by the UroPHP network. *Eur J Endocrinol*. 2016;175:P1–P17.
21. Bronner F. Recent developments in intestinal calcium absorption. *Nutr Rev*. 2008;67:109–113.
22. Christakos S, Dhawan P, Verstuyf A, et al. Vitamin D: metabolism, molecular mechanisms of action, and pleiotropic effects. *Physiol Rev*. 2016;96:365–408.
23. Dimke H, Hoenderop JGJ, Bindels RJM. Molecular basis of epithelial Ca²⁺ and Mg²⁺ transport: insights from the TRP channel family. *J Physiol*. 2011;5897:1535–1542.
24. Tiosano D, Hadad S, Chen Z, et al. Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. *J Clin Endocrinol Metab*. 2011;96:3701–3709.
25. Ajibade DV, Dhawan P, Fechner AJ, et al. Evidence for a role of prolactin in calcium homeostasis: regulation of intestinal transient receptor potential vanilloid type 6, intestinal calcium absorption, and the 25-hydroxyvitamin D₃ gene by prolactin. *Endocrinology*. 2010;151:2974–2984.
26. Stigler J, Ziegler F, Gieske A, et al. The complex folding network of single calmodulin molecules. *Science*. 2012;334:512–516.
27. Fujita H, Sugimoto K, Inatomi S, et al. Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca²⁺ absorption between enterocytes. *Mol Biol Cell*. 2008;19:1912–1919.
28. Lanou AJ, Berkow SE, Barnard ND. Calcium, dairy products, and bone health in children and young adults: A reevaluation of the evidence. *Pediatrics*. 2005;115:736–743.
29. Greer FR, Krebs NF. Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics*. 2006;117:578–585.
30. Golden NH, Abrams SA, Committee on Nutrition. Optimizing bone health in children and adolescents. *Pediatrics*. 2014;134:e1229–e1243.
31. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab*. 2011;96:53–58.
32. Hill KM, Braun MM, Egan KA, et al. Obesity augments calcium-induced increases in skeletal calcium retention in adolescents. *J Clin Endocrinol Metab*. 2011;96:2171–2177.
33. Greer FR. Bone health: it's more than calcium intake. *Pediatrics*. 2005;115:792–794.
34. Hannan FM, Babinsky VN, Thakker RV. Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Molec Endocrinol*. 2016;57:R127–R142.
35. Alexander RT, Woudenberg-Vrenken TE, Buurman J, et al. Klotho prevents renal calcium loss. *J Am Soc Nephrol*. 2009;20:2371–2379.
36. Chang Q, Hoefs S, van der Kemp AW, et al. The beta glucuronidase klotho hydrolyzes and activates the TRPV5 channel. *Science*. 2005;310:490–493.
37. De Groot T, Kovalevskaya NV, Verkaar S, et al. Molecular mechanisms of calmodulin action on TRPV5 and modulation by parathyroid hormone. *Molec Cell Biol*. 2011;31:2845–2852.
38. Muto S. Physiological roles of claudins in kidney tubule paracellular transport. *Am J Renal Physiol*. 2017;312:F9–F24.
39. Brown EM. Ca²⁺-sensing receptor. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:134–141.
40. Magno AL, Ward BK, Ratajczak T. The calcium-sensing receptor: a molecular perspective. *Endocr Rev*. 2011;32:3–30.
41. Pidasheva S, Grant M, Canaff L, et al. Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of the CASR mutants retained intracellularly. *Hum Molec Genet*. 2006;15:2200–2209.
42. Yamaguchi T, Chattopadhyay N, Brown EM. G protein-coupled extracellular Ca²⁺ (Ca₂₊o)-sensing receptor (CaR): roles in cell signaling and control of diverse cellular functions. In: O'Malley BW, ed. *Hormones and Signaling*. New York: Academic Press; 2000:209–253. 2000.
43. Mizobuchi M, Ritter CS, Krits I, et al. Calcium-sensing receptor expression is regulated by glial cells missing-2 in human parathyroid cells. *J Bone Miner Res*. 2009;24:1173–1179.
44. Breitwieser BE. The calcium sensing receptor life cycle: trafficking, cell surface expression, and degradation. *Best Pract Res Clin Endocrinol Metab*. 2013;27:303–313.
45. Scillitani A, Guarnieri V, de Geronimo S, et al. Blood ionized calcium is associated with clustered polymorphisms in the carboxyl-terminal tail of the calcium-sensing receptor. *J Clin Endocrinol Metab*. 2004;89:5634–5638.
46. Renkema KY, Bindels RJM, Hoenderop JGJ. Role of the calcium-sensing receptor in reducing the risk for calcium stones. *Clin J Am Soc Nephrol*. 2011;6:2076–2082.
47. Steddon SJ, Cunningham J. Calcimimetics and calcilytics - fooling the calcium receptor. *Lancet*. 2005;365:2237–2239.
48. Dong BJ. Cinacalcet: An oral calcimimetic agent for the management of hyperparathyroidism. *Clin Ther*. 2005;27:1725–1751.
49. Kinoshita Y, Fukumoto S. X-linked hypophosphatemia and FGF23-related hypophosphatemic diseases: prospect for new treatment. *Endocr Rev*. 2018;39:274–291.
50. Minisola S, Peacock M, Fukumoto S, et al. Tumour-induced osteomalacia. *Nat Rev*. 2017;3:17044.
51. Farrow EG, White KE. Recent advances in renal phosphate handling. *Nat Rev Nephrol*. 2010;6:207–215.
52. Bergwitz C, Collins MT, Kamath RS, Rosenberg AE. Case 33-2011: a 56 year-old man with hypophosphatemia. *N Engl J Med*. 2011;365:1625–1635.
53. Marks J, Debnam ES, Unwin RJ. The role of the gastrointestinal tract in phosphate homeostasis in health and chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2013;22:482–487.
54. White KE, Larsson TE, Econs MJ. The roles of specific genes implicated as circulating factors in normal and disordered phosphate homeostasis: Frizzled related protein-4, matric extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev*. 2006;27:221–241.
55. Prie D, Friedlander G. Genetic disorders of renal phosphate transport. *N Engl J Med*. 2010;362:2399–2409.
56. Clinkenbeard EL, White KE. Heritable and acquired disorders of phosphate metabolism: etiologies involving FGF23 and current therapeutics. *Bone*. 2017;102:31–39.
57. Kolek OL, Hines ER, Jones MD, et al. 1 α ,25-Dihydroxyvitamin D₃ up-regulates FGF23 gene expression in bone: the final link in a renal-gastrointestinal-skeletal axis that controls phosphate transport. *Am J Physiol Gastrointest Liver Physiol*. 2005;289:G1036–G1042.
58. Masuyama R, Stockmans I, Torreks S, et al. Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest*. 2006;116:3150–3159.
59. Chen G, Liu Y, Goetz R, et al. α -Klotho is a non-enzymatic molecular scaffold for FGF23 hormone signalling. *Nature*. 2018;553:461–466.
60. Covic A, Vervloet M, Massy ZA, et al. Bone and mineral disorders in chronic kidney disease: implications for cardiovascular health and ageing in the general population. *Lancet Diab Endocrinol*. 2018;6:315–331.
61. Yu X, Ibrahim OA, Goetz R, et al. Analysis of the biochemical mechanisms for the endocrine actions of fibroblast growth factor-23. *Endocrinology*. 2005;146:4647–4656.
62. Haussler MR, Whitfield GK, Haussler CA, et al. 1,25-Dihydroxyvitamin D and klotho: A tale of two renal hormones coming of age. *Vitam Horm*. 2016;100:165–230.
63. Dalton GD, Xie J, An S-W, Huang C-L. New insights into the mechanism of action of soluble klotho. *Front Endocrinol*. 2017;8:323.
64. Rowe PS. Regulation of bone-renal mineral and energy metabolism: the PHEX, FGF23, DMP1, MEPE ASARM pathway. *Crit Rev Eukaryotic Gene Express*. 2012;22:61–86.

65. Hebert JM. FGFs: neurodevelopment's Jack-of-all-trades - how do they do it? *Front Neurosci.* 2011;5:133.
66. Georges GT, Najera O, Sowers K, Sowers JR. Fibroblast growth factor 23 and hypophosphatemia: A case of hypophosphatemia along the rickets-osteomalacia spectrum. *Cardiorenal Med.* 2017;7:60–65.
67. Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B. An FGF23 missense mutation causes familial tumor calcinosis with hyperphosphatemia. *Hum Molec Genet.* 2005;14:385–390.
68. Carpenter TO, Ellis TK, Insogna RL, et al. Fibroblast growth factor 7: an inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors. *J Clin Endocrinol Metab.* 2004;90:1012–1020.
69. Acar S, Demir K, Shi Y. Genetic causes of rickets. *J Clin Res Pediatr Endocrinol.* 2017;9(Suppl 2):88–105.
70. Addison WN, Nakano Y, Loisel T, et al. MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: an inhibition regulated by PHEX cleavage of ASARM. *J Bone Miner Res.* 2008;23:1638–1649.
71. Jan de Beur SM. Tumor-induced osteomalacia. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:345–351.
72. Shimizu Y, Fukumoto S, Fujita T. Evaluation of a new automated chemoluminescence immunoassay for FGF23. *J Bone Miner Met.* 2012;30:217–221.
73. Smith ER, Cai MM, McMahon LP, Holt SG. Biological variability of plasma intact and C-terminal FGF23 measurements. *J Clin Endocrinol Metab.* 2012;97:3357–3365.
74. White KE, Econs MJ. Fibroblast growth factor-23. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:112–117.
75. San-Cristobal P, Dimke H, Hoenderop JGJ, Bindels RJM. Novel molecular pathways in renal Mg²⁺ transport: a guided tour. *Curr Opin Nephrol Hypertens.* 2010;19:456–462.
76. Chubanov V, Waldegger S, Maderos y Schnitzler M, et al. Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc Nat Acad Sci.* 2004;101:2894–2899.
77. Kausalya RJ, Amasheh S, Gunzel D, et al. Disease associated mutations affect intracellular traffic and paracellular Mg²⁺ transport function of Claudin16. *J Clin Invest.* 2006;116:878–891.
78. Ferre S, Hoenderop JGJ, Bindels RJM. Insight into renal Mg²⁺ transporters. *Curr Opin Nephrol Hypertens.* 2011;20:169–176.
79. Whyte MP. Hypophosphatasia: an overview for 2017. *Bone.* 2017;102:15–25.
80. Nissenson RA, Juppner H. Parathyroid hormone. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:123–127.
81. Navah-Many T, Silver J. Transcription factors that determine parathyroid development power PTH expression. *Kidney Int.* 2018;93:7–9.
82. Pillar N, Pleniceanu O, Fang M, et al. A rare variant in the FHL1 gene associated with X-linked recessive hypoparathyroidism. *Hum Genet.* 2017;136:835–845.
83. Teti A, Econs MJ. Osteopetroses, emphasizing potential approach to treatment. *Bone.* 2017;102:50–59.
84. Drechsler N, Frobel J, Jahreis G, et al. Binding specificity of the parathyroid hormone receptor. *Biophys Chem.* 2011;154:66–72.
85. D'Amour P, Brossard J, Rousseau L, et al. Structure of non-(1-84) PTH fragments secreted by parathyroid glands in primary and secondary hyperparathyroidism. *Kidney Int.* 2005;68:998–1007.
86. Murray TM, Rao LG, Divieti P, Bringham PR. Parathyroid hormone secretion and action: Evidence for discrete receptors for the carboxyl-terminal region and related biological actions of carboxyl-terminal ligands. *Endocr Rev.* 2005;26:78–113.
87. Lombardi G, Di Somma C, Rubino M, et al. The roles of parathyroid hormone in bone remodeling: prospects for novel therapeutics. *J Endocrinol Invest.* 2011;34(7 Suppl):18–22.
88. Chelola RW, Gellman SH, Vilardaga J-P, Gardella TJ. PTH receptor-1 signalling-mechanistic insights and therapeutic prospects. *Nat Rev Endocrinol.* 2015;11:712–724.
89. Bohine BN, Gesty-Palmer D. β -arrestin-biased agonism at the parathyroid hormone receptor uncouples bone formation from bone resorption. *Endocr Metab Immune Disord Drug Targets.* 2011;11:112–119.
90. Vieira JGH. PTH assays: understanding what we have and forecasting what we will have. *J Osteopor.* 2012;2012:523246.
91. Zimmerman MK. Discrepant intact parathyroid hormone result by immunoassay. *Clin Chim Acta.* 2012;413:344–345.
92. Vietri MT, Sessa M, Pilla P, et al. Serum osteocalcin and parathyroid hormone in healthy children assessed with two new automated assays. *J Pediatr Endocrinol Metab.* 2006;19:1413–1419.
93. Cavalier E, Cartisi A, Bekaert AC, et al. New insights on the stability of parathyroid hormone as assayed by an automated 3rd generation PTH assay. *Clin Chim Acta.* 2012;413:253–254.
94. Terry AH, Orrock J, Meikle AW. Comparison of two third-generation parathyroid hormone assays. *Clin Chem.* 2003;49:336–337.
95. Dempster DW, Zhou H, Recker RR, et al. Skeletal histomorphometry in subjects on teriparatide or zoledronic acid therapy (SHOTZ) study: a randomized controlled trial. *J Clin Endocrinol Metab.* 2012;97:2799–2808.
96. Lopez I, Rodriguez-Ortiz ME, Almaden Y, et al. Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 in vivo. *Kidney Int.* 2011;80:475–482.
97. Wyslowski JJ. Parathyroid hormone-related protein: an update. *J Clin Endocrinol Metab.* 2012;97:2947–2956.
98. Chau M, Forcinito P, Andrade AC, et al. Organization of the Indian hedgehog - parathyroid hormone-related protein system in the postnatal growth plate. *J Molec Endocrinol.* 2011;47:99–107.
99. Broadus AE, Nissenson RA. Parathyroid hormone-related protein. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:99–106.
100. Buhimschi CS. Endocrinology of lactation. *Obstet Gynecol Clin North Am.* 2004;31:963–979.
101. Miao D, He B, Karaplis AC, Goltzman D. Parathyroid hormone is essential for normal fetal bone formation. *J Clin Invest.* 2002;109:1173–1182.
102. Pioszak AA, Harikimar KG, Parker NR, et al. Dimeric arrangement of the parathyroid hormone receptor and a structural mechanism for ligand-induced dissociation. *J Biol Chem.* 2010;285:12435–12444.
103. Pickard BW, Hodsman AB, Fraher LJ, Watson PH. Type 1 parathyroid hormone receptor (PTH1R) nuclear trafficking: association of PTH1R with importin α 1 and β . *Endocrinology.* 2006;147:3326–3332.
104. Gough NR. Focus Issue: cracking the G protein-coupled receptor code. *Sci Signal.* 2011;4. eg7.
105. Mahon MJ, Donowitz M, Yun CC, Segre GV. Na⁺/H⁺ exchanger regulatory factor 2 directs parathyroid hormone 1 receptor signaling. *Nature.* 2002;417:858–861.
106. Felsenfeld AJ, Levine BS. Calcitonin, the forgotten hormone: does it deserve to be forgotten? *Clin Kidney J.* 2015;8:180–187.
107. Adam S. Calcitonin. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:250–251.
108. Holick MF. Vitamin D: evolutionary, physiological and health perspectives. *Curr Drug Targets.* 2011;12:4–18.
109. Schuster I. Cytochromes P450 are essential players in the vitamin D signaling system. *Biochim Biophys Acta.* 2011;1814:186–199.
110. Bickle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol.* 2014;21:319–329.
111. Autier P, Gandini S, Mullie P. A systematic review: Influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. *J Clin Endocrinol Metab.* 2012;97:2606–2613.
112. Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: Implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr.* 2005;135:317–322.
113. Bickle D, Adams J, Christakos S. Vitamin D: Production, metabolism, mechanism of action, and clinical requirements. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders*

- of *Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:141–149.
114. Chiellini G, DeLuca HF. The importance of stereochemistry on the actions of vitamin D. *Curr Top Med Chem*. 2011;11:840–859.
 115. Christakos S, Ajibade DV, Dhawan P, et al. Vitamin D: metabolism. *Endocrinol Metab Clin North Am*. 2010;39:243–253.
 116. de Oliveira RB, Moyses RMA. FGF-23: state of the art. *J Bras Nefrol*. 2010;32:316–323.
 117. Petersen RA, Larsen LH, Damsgaard CT, et al. Common genetic variants are associated with serum 25-hydroxyvitamin D concentrations across the year among children at northern latitudes. *Brit J Nutr*. 2017;117:829–833.
 118. Antonucci R, Locci C, Clemente MG, et al. Vitamin D deficiency in childhood: old lessons and current challenges. *J Pediatr Endocrinol Metab*. 2018;31:247–260.
 119. Giustina A, Adler RA, Binkley N, et al. Controversies in vitamin D: summary statement from an international conference. *J Clin Endocrinol Metab*. 2019;104(2):234–240.
 120. Saggese G, Vierucci F, Prodam F, et al. Vitamin D in pediatric age: consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians. *Italian J Pediatr*. 2018;44:51–90.
 121. Rostami M, Tehrani FR, Simbar M, et al. Effectiveness of prenatal vitamin D deficiency screening and treatment program: a stratified randomized field trial. *J Clin Endocrinol Metab*. 2018;103:2936–2948.
 122. Autier P, Mullie P, Macacu M, et al. Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomized trials. *Lancet/Diabetes-Endocrinol*. 2017;5:986–1004.
 123. Zhou C, Assem M, Tay JC, et al. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. *J Clin Invest*. 2006;116:1703–1712.
 124. Schlingmann KP, Kaufmann M, Weber S, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med*. 2011;365:410–421.
 125. Holick MF. The D-batable Institute of Medicine report: a D-lightful perspective. *Endocrine Pract*. 2011;17:143–149.
 126. Rosen CJ, Adams JS, Bikle DD, et al. The non-skeletal effects of vitamin D: An Endocrine Society scientific statement. *Endocr Rev*. 2012;33:456–492.
 127. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology*. 2006;147:5542–5548.
 128. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med*. 2010;235:1034–1045.
 129. Xue Y, Karaplis AC, Hendry GN, et al. Exogenous 1,25-dihydroxyvitamin D₃ exerts a skeletal anabolic effect and improves mineral ion homeostasis in mice that are homozygous for both the 1 α -hydroxylase and parathyroid hormone null alleles. *Endocrinology*. 2006;147:4801–4810.
 130. Holick MF, Garabedian M. Vitamin D photobiology, metabolism, mechanism of action, and clinical application. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:106–114.
 131. Christakos S, DeLuca HF. Minireview: Vitamin D: Is there a role in extraskelatal health? *J Clin Endocrinol Metab*. 2011;152:2930–2936.
 132. Pike JW, Meyer MB, Lee SM, et al. The vitamin D receptor: contemporary genomic approaches reveal new basic and translational insights. *J Clin Invest*. 2017;127:1146–1154.
 133. Gilad LA, Bresler T, Gnainsky J, et al. Regulation of vitamin D receptor expression via estrogen-induced activation of the ERK 1/2 signaling pathway in colon and breast cancer cells. *J Endocrinol*. 2005;185:577–592.
 134. Barnett C, Krebs JE. WSTF does it all: a multifunctional protein in transcription, repair and replication. *Biochem Cell Biol*. 2011;89:12–23.
 135. Kouzmenko A, Ohtake F, Fujiki R, Kato S. Hormonal gene regulation through DNA methylation and demethylation. *Epigenomics*. 2010;2:765–744.
 136. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev*. 2005;26:662–687.
 137. Dawson MA, Kouzarides T, Huntly BJP. Targeting epigenetic readers in cancer. *N Engl J Med*. 2012;367:647–657.
 138. Carlberg C, Seuter S, Heikkinen S. The first genome-wide view of vitamin D receptor locations and their mechanistic implications. *Anticancer Res*. 2012;32:271–282.
 139. Amling M, Priemel M, Holzmann T, et al. Rescue of skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology*. 1999;140:4982–4987.
 140. Demay MB. The hair cycle and vitamin D receptor. *Arch Biochem Biophys*. 2012;523:18–21.
 141. Hii CS, Ferrante A. The non-genomic actions of vitamin D. *Nutrients*. 2016;8:135.
 142. Boyan BD, Schwartz Z. Rapid vitamin D-dependent PKC signaling shares features with estrogen-dependent PKC signaling in cartilage and bone. *Steroids*. 2004;69:591–597.
 143. Fleet JC. Rapid, membrane-initiated actions of 1,25 dihydroxyvitamin D: what are they and what do they mean? *J Nutr*. 2004;134:3215–3218.
 144. Huhtakangas JA, Olivera CJ, Bishop JE, et al. The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 α ,25(OH)₂-vitamin D₃ in vivo and in vitro. *Mol Endocrinol*. 2004;18:2660–2671.
 145. Cohen Jr MM. The new bone biology: pathologic, molecular, and clinical studies. *Am J Med Genet*. 2006;140A:26462706.
 146. Krause C, de Gorter DJJ, Karperien M, ten Dijke P. Signal transduction cascades controlling osteoblast differentiation. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington, DC: American Society for Bone and Mineral Research; 2008:10–16.
 147. Rosello-Diez A, Joyner AL. Regulation of long bone growth in vertebrates; it is time to catch up. *Endocr Rev*. 2015;36:646–680.
 148. Bassett JHD, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. *Endocr Rev*. 2016;37:135–187.
 149. Mak KK, Kronenberg HM, Chuang P-T, et al. Indian hedgehog signals independently of PTHrP to promote chondrocyte hypertrophy. *Development*. 2008;135:1947–1956.
 150. Marino R. Growth plate biology: new insights. *Curr Opin Endocrinol Diab Obes*. 2011;18:9–13.
 151. Spath S-S, Andrade AC, Chau M, Nilsson O. Local regulation of growth plate cartilage. In: Camacho-Hubner C, Nilsson O, Savendahl L, eds. *Cartilage and Bone Development and Its Disorders*: 2011:12–22. Basal, Karger.
 152. Mackie EJ, Tatarczuch L, Mirams M. The skeleton: a multifunctional complex organ. The growth plate chondrocyte and endochondral ossification. *J Endocrinol*. 2011;211:109–121.
 153. Cinque L, Forrester A, Bartolomeo R, et al. FGF signalling regulates bone growth through autophagy. *Nature*. 2015;528:272–275.
 154. Lorenzo JA, Canalis E, Raisz LG. Metabolic bone disease. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, eds. *Williams Textbook of Endocrinology*. 11th ed. Philadelphia: Saunders/Elsevier; 2008:1269–1310.
 155. Rauch F. Fetal and neonatal bone development. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:72–74.
 156. Yang Y. Skeletal morphogenesis and embryonic development. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:2–10.
 157. Olsen BR. Bone embryology. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:2–6.
 158. Komori T. Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell Tissue Res*. 2010;339:189–195.
 159. Zuscik MJ, Hilton MJ, Zhang X, et al. Regulation of chondrogenesis and chondrocyte differentiation by stress. *J Clin Invest*. 2008;118:429–438.
 160. Mazziotti G, Frara S, Giustina A. Pituitary diseases and bone. *Endocr Rev*. 2018;39:440–488.
 161. Kaplan FS, Fiori J, Serrano de la Pena L, et al. Dysregulation of the BMP-4 signaling pathway in fibrodysplasia ossificans progressiva. *Ann NY Acad Sci*. 2006;1068:54–65.

162. Regan J, Long F. Notch signaling and bone remodeling. *Curr Osteoporos Rep.* 2013;11:126–129.
163. Cui Y, Niziolek PJ, MacDonald BT, et al. Lrp5 functions in bone to regulate bone mass. *Nat Med.* 2011;17:684–691.
164. Johnson ML. LRP5 and bone mass regulations: where are we now? *BoneKey Reports.* 2012;1:1.
165. Ramakrishnan A-b, Cadigan KM. Wnt target genes and where to find them. *F1000Res.* 2017;6:746.
166. Williams BO. LRP5: from bedside to bench to bone. *Bone.* 2017;102:26–30.
167. McCarthy TL, Centrella M. Novel links among Wnt and TGF- β signaling and Runx2. *Molec Endocrinol.* 2010;24:587–597.
168. Han Y, Kim YM, Kim HS, Lee KY. Melatonin promotes osteoblast differentiation by regulating osterix protein stability and expression. *Sci Rep.* 2017;7:5716.
169. James MJ, Jarvinen E, Wang X-P, Thesleff I. Different roles of Runx2 during early neural crest-derived bone and tooth development. *J Bone Miner Res.* 2006;21:1034–12044.
170. Reinhold MI, Naski MC. Direct interactions of Runx2 and canonical Wnt signaling induce FGF18. *J Biol Chem.* 2007;282:3653–3663.
171. Eubelen M, Bostaille N, Vabochette P, et al. A molecular mechanism for Wnt ligand-specific signaling. *Science.* 2018;361:11178.
172. Aubin JE, et al. Bone formation: maturation and functional activities of osteoblast lineage cells. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:20–29.
173. Backesjo C-M, Li Y, Lindgren U, Haldosen L-A. Activation of Sirt1 decreases adipocyte formation during osteoblast differentiation of mesenchymal stem cells. *J Bone Miner Res.* 2006;21:993–1002.
174. Nelson WJ, Nusse R. Convergence of Wnt, β -catenin, and cadherin pathways. *Science.* 2004;303:1483–1487.
175. Krishnan V, Bryant HU, MacDougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest.* 2006;116:1202–1209.
176. Broadhead ML, Akiyama T, Chooa PF, Dass R. The pathophysiological role of PEDF in bone disease. *Curr Mol Med.* 2010;10:296–301.
177. Lim J, Grafe I, Alexander S, Lee B. Genetic causes and mechanisms of osteogenesis imperfecta. *Bone.* 2017;102:40–49.
178. Rubin J, Rubin C. Stand UP! *J Clin Endocrinol Metab.* 2010;95:2050–2053.
179. Sims NA, Chia LY. Regulation of sclerostin expression by paracrine and endocrine factors. *Clin Rev Bone Miner Metab.* 2012;10:98–107.
180. Robey PG, Boskey AL. Extracellular matrix and biomineralization of bone. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:12–19.
181. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature.* 2003;426:895–899.
182. Hetz C, Papa FR. The unfolded protein response and cell fate control. *Mol Cell.* 2018;69:169–181.
183. Ichimura A, Takeshima H. TRIC-B mutations causing osteogenesis imperfecta. *Biol Pharm Bull.* 2016;39:1743–1747.
184. Han Y, You X, Xing W, et al. Paracrine and endocrine actions of bone - the function of secretory proteins from osteoblasts, osteocytes, and osteoclasts. *Bone Res.* 2018;6:16.
185. Kanazawa I. Osteocalcin as a hormone regulating glucose metabolism. *World J Diabetes.* 2015;25:1345–1354.
186. Levinger I, Brennan-Speranza TC, Zulli A, et al. Multifaceted interaction of bone, muscle, lifestyle interventions and metabolic and cardiovascular disease: role of osteocalcin. *Osteoporos Int.* 2017;28:2265–2273.
187. Bilotta FL, Arcidiacono B, Messineo S, et al. Insulin and osteocalcin: further evidence for a mutual cross-talk. *Endocrine.* 2018;59:622–632.
188. Mosialou I, Shikhel S, Liu J-M, et al. MC4R-dependent suppression of appetite by bone-derived lipocalin 2. *Nature.* 2017;543:385–390.
189. Watanabe R, Fujita N, Sato Y, et al. Enpp1 is an anti-aging factor that regulates Klotho under phosphate overload conditions. *Sci Rep.* 2017;7(1):7786.
190. Florencio-Silva R, Sasso GRS, Sasso-Cerri E, et al. Biology of bone tissue: Structure, function, and factors that influence bone cells. *BioMed Res Intern.* 2015;2015:421746.
191. Teti A, Zallone A. Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited. *Bone.* 2009;44:11–16.
192. Chen J-H, Liu C, You L, Simmons CA. Boning up on Wolff's Law: mechanical regulation of the cells that make and maintain bone. *J Biomech.* 2010;43:108–118.
193. Hughes JM, Petit MA. Biological underpinnings of Frost's mechanostat thresholds: The important role of osteocytes. *Musculoskelet Neuronal Interact.* 2010;10:128–135.
194. Fricke O, Beccard R, Semler O, Schoenau E. Analysis of muscular mass and function: the impact on bone mineral density and peak muscle mass. *Pediatr Nephrol.* 2010;25:2393–2400.
195. Zelzer E, Olsen BR. Multiple roles of vascular endothelial growth factor (VEGF) in skeletal development, growth, and repair. *Curr Topics Dev Biol.* 2005;65:169–187.
196. Negishi-Koga T, Takayangi H. Ca $^{2+}$ -NFATc1 signaling is an essential axis of osteoclast differentiation. *Immunol Rev.* 2009;231:241–256.
197. Henricksen R, Bollerslev J, Everts V, Karsdal MA. Osteoclast activity and subtypes as a function of physiology and pathology - Implications for future treatments of osteoporosis. *Endocr Rev.* 2011;32:31–63.
198. Barrow AD, Raynal N, Andersen AL, et al. OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. *J Clin Invest.* 2011;121:3505–3516.
199. Ashley JW, Ahn J, Hankenson KD. Notch signaling promotes osteoclast maturation and resorptive activity. *J Cell Biochem.* 2015;116:2598–2609.
200. Raggatt LJ, Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem.* 2010;285:25103–25108.
201. Fili S, Karalaki M, Schaller B. Therapeutic implications of osteoprotegerin. *Cancer Cell Intern.* 2009;9:26–33.
202. Whyte MP, Obrecht SE, Finnegan PM, et al. Osteoprotegerin deficiency and juvenile Paget's disease. *N Engl J Med.* 2002;347:175–184.
203. Dempster DW. Anatomy and function of the adult skeleton. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:7–11.
204. Wu J, Glimcher LH, Aliprantis AO. HCO $_3^-$ /Cl $^-$ anion exchanger is required for proper osteoclast differentiation and function. *PNAS.* 2008;105:16934–16939.
205. McEwan DG, Popovic D, Gubas A, et al. PLEKHM1 regulates autophagosome-lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol Cell.* 2015;57:39–54.
206. Shinora M, Koga T, Okamoto K, et al. Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell.* 2008;132:794–806.
207. Oikawa T, Oyama M, Kozuka-Hata H, et al. Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell-cell fusion. *J Cell Biol.* 2012;197:553–568.
208. Helming L, Gordon S. Molecular mediators of macrophage fusion. *Trends Cell Biol.* 2009;19:514–522.
209. Soe K, Andersen TL, Hobolt-Pedersen AS, et al. Involvement of human endogenous retroviral syncytin-1 in human osteoclast fusion. *Bone.* 2011;48:837–846.
210. Weinert S, Jabs S, Supancharit C, et al. Lysosomal pathology and osteopetrosis upon loss of H $^+$ -driven lysosomal Cl $^-$ accumulation. *Science.* 2010;328:1401–1403.
211. Ikeda K, Takeshita S. The role of osteoclast differentiation and function in skeletal homeostasis. *J Biochem.* 2016;159:1–8.
212. Barnes AM, Chang W, Morello R, et al. Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta. *N Engl J Med.* 2006;355:2757–2764.
213. Cabral WA, Chang W, Barnes AM, et al. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nat Genet.* 2007;39:359–365.
214. Morello R, Bertin TK, Chen Y, et al. CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell.* 2006;127:291–304.
215. Jurimae J. Interpretation and application of bone turnover markers in children and adolescents. *Curr Opin Pediatr.* 2010;22:494–500.
216. Wasilewska A, Rybi-Szuminska AA, Zoch-Zwierz W. Serum osteoprotegerin (OPG) and receptor activator of nuclear factor κ B

- (RANKL) in healthy children and adolescents. *J Pediatr Endocrinol Metab.* 2009;22:1099–1104.
217. Dorota D, Bogdan KG, Mieczyslaw G, et al. The concentrations of markers of bone turnover in normal pregnancy and preeclampsia. *Hypertens Pregn.* 2012;31:166–176.
 218. Seibold-Weiger K, Wollmann HA, Ranke MB, Speer CP. Plasma concentrations of the carboxyterminal propeptide of type I procollagen (PICP) in preterm neonates from birth to term. *Pediatr Res.* 2000;48:104–108.
 219. Crofton PM, Wade JC, Taylor MRH, Holland CV. Serum concentrations of carboxyl-terminal propeptide of type I procollagen, amino-terminal propeptide of type III procollagen, cross-linked carboxy-terminal telopeptide of type I collagen, and their interrelationships in school children. *Clin Chem.* 1997;43:1577–1581.
 220. Mora S, Pitukcheewanont P, Kaufman FR, et al. Biochemical markers of bone turnover and the volume and the density of bone in children at different stages of sexual development. *J Bone Miner Res.* 1999;14:1664–1671.
 221. Gracia-Marco L, Vicente-Rodriguez G, Valtuena J, et al. Bone mass and bone metabolism markers during adolescence. The HELENA study. *Horm Res Paediatr.* 2010;74:339–350.
 222. Huang Y, Eapen E, Steele S, Grey V. Establishment of reference intervals for bone markers in children and adolescents. *Clin Biochem.* 2011;44:771–778.
 223. Kirmani S, Christen D, van Lenthe GH, et al. Bone structure at the distal radius during adolescent growth. *J Bone Miner Res.* 2009;24:1033–1042.
 224. Rauchenzauner M, Schmid A, Heinz-Erain P, et al. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *J Clin Endocrinol Metab.* 2007;92:443–449.
 225. Drake MT, Fenske JS, Block1, F.A., et al. Validation of a novel, rapid, high [recision sclerostin assay not confounded by sclerostin fragments. *Bone.* 2018;111:36–43.
 226. McNulty M, Singh RJ, Li X, et al. Determination of serum and plasma sclerostin concentrations by enzyme-linked immunoassays. *J Clin Endocrinol Metab.* 2011;96:E1159–E1162.
 227. Amrein K, Amrein S, Drexler C, et al. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. *J Clin Endocrinol Metab.* 2012;97:148–154.
 228. Costa AG, Cremers S, Rubin MR, et al. Circulating sclerostin in disorders of parathyroid gland function. *J Clin Endocrinol Metab.* 2011;96:3804–3810.
 229. Gaudio A, Pennisi P, Bratengeier C, et al. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J Clin Endocrinol Metab.* 2010;95:2248–2253.
 230. Modder UI, Hoey KA, Amin S, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in men and women. *J Bone Miner Res.* 2011;26:373–379.
 231. Kirmani S, Amin S, McCready LK, et al. Sclerostin levels during growth in children. *Osteoporosis Int.* 2012;23:1123–1130.
 232. Robey PG, Boskey AL. The composition of bone. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:32–38.
 233. Gericke A, Qin C, Spevak L, et al. Importance of phosphorylation for osteopontin regulation of biomineralization. *Calc Tiss Int.* 2005;77:45–54.
 234. Chavassieux P, Seaman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures. How determinants of the biomechanical properties of bone are compromised by disease. *Endocr Rev.* 2007;28:151–164.
 235. Rubin C, Rubin J. Biomechanics and mechanobiology of bone. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:36–42.
 236. Hughes-Fulford M. Signal transduction and mechanical stress. *Sci STKE.* 2004. re1.
 237. Specker L, Schonau E. Quantitative bone analysis in children: Current methods and recommendations. *J Pediatr.* 2005;146:726–731.
 238. McCormack SE, Cousminer DL, Chesni A, et al. Association between linear growth and bone accrual in a diverse cohort of children and adolescents. *JAMA Pediatr.* 2017;171(9). e171769.
 239. Gordon CM, Zemel BS, Wren TAL, et al. The determinants of peak bone mass. *J Pediatr.* 2016;138:261–269.
 240. Gilsanz V, Chalfant J, Kalkwarf H, et al. Age at onset of puberty predicts bone mass in adulthood. *J Pediatr.* 2011;158:100–105.
 241. Karasik D, Rivadeneira F, Johnson ML. The genetics of bone mass and susceptibility to bone diseases. *Nat Rev Rheumatol.* 2016;12:323–334.
 242. Ralston SH, Uitterlinden AG. Genetics of osteoporosis. *Endocr Rev.* 2010;31:629–662.
 243. Calabrese GM, Mesner LD, Stains JP, et al. Integrating GWAS and co-expression network data identifies causal bone mineral density genes SPTBN1 and MARK3 and an osteoblast functional module. *Cell Syst.* 2017;41:46–59.
 244. Kalkwarf HJ, Zemel BS, Gilsanz V, et al. The bone mineral density in childhood study: Bone mineral content and density according to age, sex, and race. *J Clin Endocrinol Metab.* 2007;92:2087–2099.
 245. Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: Results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab.* 2011;96:3160–3169. Revised 2012.
 246. Pitukcheewanont P, Punyasavatsut N, Feuilee M. Physical activity and bone health in children and adolescents. *Pediatr Endocrinol Rev.* 2010;7:275–282.
 247. Rikkonen T, Tuppurainen M, Kroger H, et al. Distance of walking in childhood and femoral bone density in perimenopausal women. *Eur J Appl Physiol.* 2006;97:509–515.
 248. Rosenfeld R. Molecular mechanisms of IGF-I deficiency. *Horm Res.* 2006;65(Suppl 1):15–20.
 249. Cruickshank J, Grossman DI, Peng RK, et al. Spatial distribution of growth hormone receptor, insulin-like growth factor-1 receptor and apoptotic chondrocytes during growth plate development. *J Endocrinol.* 2005;184:543–553.
 250. Wang Y, Nishida S, Sakata T, et al. Insulin-like growth factor-I is essential for embryonic bone development. *Endocrinology.* 2006;147:4753–4761.
 251. Cohen P. Overview of the IGF-I system. *Horm Res.* 2006;65(Suppl 1):3–8.
 252. Savage MO, Attie KM, David A, et al. Endocrine assessment, molecular characterization and treatment of growth hormone insensitivity. *Nat Clin Pract Endocrin Metab.* 2006;2:395–407.
 253. Caminos JE, Gualillo O, Lago F, et al. The endogenous growth hormone secretagogue (ghrelin) is synthesized and secreted by chondrocytes. *Endocrinology.* 2005;146:1285–1292.
 254. Ohlsson C, Bengtsson B-A, Isaksson OGP, et al. Growth hormone and bone. *Endocr Rev.* 1998;19:55–79.
 255. Barnard JC, Williams AJ, Rabier B, et al. Thyroid hormones regulate fibroblast growth factor receptor signaling during chondrogenesis. *Endocrinology.* 2005;146:5568–5580.
 256. Zaidi M, Yuen T, Sun L, Rosen CJ. Regulation of skeletal homeostasis. *Endocr Rev.* 2018;39:701–718.
 257. van der Eerden BCJ, Karperien M, Wit J. Systemic and local regulation of the growth plate. *Endocr Rev.* 2003;24:782–801.
 258. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev.* 1999;20:358–417.
 259. Bilezikian JP. Editorial: What's good for the goose's skeleton is good for the gander's skeleton. *J Clin Endocrinol Metab.* 2006;91:1223–1225.
 260. Vanderschueren D, Venken K, Ophoff J, et al. Clinical Review: sex steroids and the periosteum—Reconsidering the roles of androgens and estrogens in periosteal expansion. *J Clin Endocrinol Metab.* 2006;91:378–382.
 261. Ohlsson C, Borjesson AE, Vandenput L. Sex steroids and bone health in men. *BoneKey Rep.* 2012;1:2.
 262. Rauch F, Bailey DA, Baxter-Jones A, et al. The 'muscle-bone unit' during the pubertal growth spurt. *Bone.* 2004;34:771–775.
 263. Hardy RS, Zhou H, Seibel MJ, Cooper MS. Glucocorticoids and bone: Consequences of endogenous and exogenous excess and replacement therapy. *Endocr Rev.* 2018;39:519–568.
 264. Komori T. Glucocorticoid signaling and bone biology. *Horm Metab Res.* 2016;48:755–763.

265. Robson H, Siebler T, Shalet SM, Williams GR. Interactions between GH, IGF-I, glucocorticoids, and thyroid hormones during skeletal growth. *Pediatr Res*. 2003;52:137–147.
266. Lui JC, Baron J. Effects of glucocorticoids on the growth plate. *Endocr Dev*. 2011;20:187–193.
267. Potter LR, Abbey-Hosch A, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev*. 2006;27:47–72.
268. Kanai Y, Yasoda A, Mori KP, et al. Circulating osteocrin stimulates bone growth by limiting C-type natriuretic peptide clearance. *J Clin Invest*. 2017;127:4136–4147.
269. Prickett TMCR, Lynne AM, Barrell GK, et al. Amino-terminal proCNP: A putative marker of cartilage activity in post natal growth. *Pediatr Res*. 2005;58:334–340.
270. Suarez CG, Singer BH, Gebremariam A, et al. The relationship between adiposity and bone density in U.S. children and adolescents. *PLoS ONE*. 2017;12(7). e018157.
271. Jin J, Wang Y, Jiang H, et al. The impact of obesity through fat depots and adipokines on bone homeostasis. *AME Med J*. 2018;3:1.
272. Karsenty G, Oury F. The central regulation of bone mass. The first link between bone remodeling and energy metabolism. *J Clin Endocrinol Metab*. 2010;95:4795–4801.
273. Karsenty G, Oury F. Biology without walls: The novel endocrinology of bone. *Annu Rev Physiol*. 2012;74:87–105.
274. Luo X-H, Guo L-J, Xie H, et al. Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway. *J Bone Miner Res*. 2006;21:1648–1656.
275. Reid IR. Fat and bone. *Arch Biochem Biophys*. 2010;503:20–27.
276. Bachrach LK, Gordon CM. Bone densitometry in children and adolescents. *Pediatrics*. 2016;138. e20162398.
277. Wasserman H, O'Donnell JM, Gordon CM. Use of dual energy X-ray absorptiometry in pediatric patients. *Bone*. 2017;104:84–90.
278. Glorieux FH, Travers R, Taylor A, et al. Normative data for iliac bone histomorphometry in growing children. *Bone*. 2000;26:103–109.
279. Rauch F. Watching bones at work: what we can see from bone biopsies. *Pediatr Nephrol*. 2000;21:457–462.
280. Jameson JR, Albert CI, Busse B, et al. 3D micron-scale imaging of the cortical bone canal network in human osteogenesis imperfecta. In: Weaver JB, Molthen RC, eds. *Medical Imaging 2013: Biomedical Applications in Molecular, Structural, and Functional Imaging*. Proc of SPIE; 2013:86721L8672.
281. Ma J-x, He W-w, Zhao J, et al. Bone microarchitecture and biomechanics of the necrotic femoral head. *Nat Sci Rep*. 2017;7:13345.
282. Khosla S, Melton LJ, Achenbach SJ, et al. Hormonal and biochemical determinants of trabecular microstructure at the ultradistal radius in women and men. *J Clin Endocrinol Metab*. 2006;91:885–891.
283. Bhagat YA, Rajapakse CS, Magland JF, et al. Performance of μ MRI virtual bone biopsy for structural and mechanical analysis at the distal tibia at 7T field strength. *J Magn Reson Imaging*. 2011;33:372–381.
284. Pezzutti IL, Kakehasi AM, Figueiras T, et al. Imaging methods for bone mass evaluation during childhood and adolescence: an update. *J Pediatr Endocrinol Metab*. 2017;30:485–497.
285. Crabtree NJ, Arabi A, Bachrach LK, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2013 ISCD pediatric official positions. *J Clin Densitomet*. 2014;17:225–242.
286. Ward KA, Ashby RL, Roberts SA, et al. UK reference data for the Hologic QDR Discovery dual-energy x ray absorptiometry scanner in healthy children and young adults aged 6-17 years. *Arch Dis Child*. 2007;92:53–59.
287. Gilsanz V, Skaggs DL, Kovanlikaya A, et al. Differential effects of race on the axial and appendicular skeletons of children. *J Clin Endocrinol Metab*. 1998;83:1420–1427.
288. Miller PD, Leonard MB. Clinical use of bone mass measurements in children and adults for the assessment and management of osteoporosis. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:150–161.
289. Utriainen P, Jaaskelainen J, Saarinen A, et al. Body composition and bone mineral density in children with premature adrenarche and the association of LRP5 gene polymorphisms with bone mineral density. *J Clin Endocrinol Metab*. 2009;94:4144Z–4151.
290. Zemel BS, Leonard MB, Kelly A, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab*. 2010;95:1265–1273.
291. Vierucci F, Saggese G, Cimaz R. Osteoporosis in childhood. *Curr Opin Rheumatol*. 2017;29(535–546):2017.
292. Gallo S, Vanstone CA, Weiler HA. Normative data for bone mass in healthy term infants from birth to 1 year of age. *J Osteoporos*. 2012;2012:672403.
293. Xu H, Zhao Z, Wang H, et al. Bone mineral density of the spine in 11,898 Chinese infants and young children: A cross-sectional study. *PLoS ONE*. 2013;8(12). e82098.
294. Koo WWK, Walters J, Bush AJ, et al. Dual-energy x-ray absorptiometry studies of bone mineral status in newborn infants. *J Bone Miner Res*. 1996;11. 1997–1002.
295. McKay HA, Bailey DA, Mirwald RL, et al. Peak bone mineral accrual and age at menarche in adolescent girls: A 6-year longitudinal study. *J Pediatr*. 1998;133:682–687.
296. Wren TALH, Liu X, Pitukcheewanont P, Gilsanz V. Bone densitometry in pediatric patients: Discrepancies in the diagnosis of osteoporosis by DXA and CT. *J Pediatr*. 2005;146:776–779.
297. Di Iorgi N, Maruca K, Patti G, Mora S. Update on bone density measurements and their interpretation in children and adolescents. *Best Pract Res Clin Endocrinol Metab*. 2018;32:477–498.
298. Ashmeade T, Pereda L, Chen Carver JD. Longitudinal measurements of bone status in preterm infants. *J Pediatr Endocrinol Metab*. 2007;20:415–424.
299. Jaworski M, Graff K. Peripheral quantitative computed tomography of the distal and proximal forearm in children and adolescents: bone densities, cross-sectional sizes and soft tissue reference data. *J Musculoskelet Neuronal Interact*. 2018;18:237–247.
300. Zhou B, Wang J, Yu YE, et al. High-resolution peripheral quantitative computed tomography (HR-pQCT) can assess microstructural and biomechanical properties of both human distal radius and tibia: Ex vivo computational and experimental validations. *Bone*. 2016;86:58–67.
301. Rauch F, Schonau E. Peripheral quantitative computed tomography of the distal radius in young subjects - new reference data and interpretation of results. *J Musculoskelet Neuronal Interact*. 2005;5:119–126.
302. Chevalley T, Bonjour JP, van Rietbergen B, et al. Fractures during childhood and adolescence in healthy boys: relation with bone mass, microstructure, and strength. *J Clin Endocrinol Metab*. 2011;96:3134–3142.
303. Zadik Z, Price D, Diamond G. Pediatric reference curves for multi-site quantitative ultrasound and its modulators. *Osteoporos Int*. 2003;14:857–862.
304. Baroncelli GI, Bertelloni S, Ceccarelli C, Saggese G. Measurement of volumetric bone mineral density accurately determines degree of lumbar undermineralization in children with growth hormone deficiency. *J Clin Endocrinol Metab*. 1998;83:3150–3154.
305. Klentrou P, Ludwa IA, Falk B. Factors associated with bone turnover and speed of sound in early and-pubertal females. *Appl Physiol Nutr Metab*. 2011;36:707–714.
306. Majumdar S. Magnetic resonance imaging of bone. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:163–166.
307. Wehrli FW. Structural and functional assessment of trabecular and cortical bone by micro magnetic resonance imaging. *J Magn Reson Imaging*. 2007;25:390–409.
308. Liu XS, Zhang XH, Rajapakse CS, et al. Accuracy of high-resolution in vivo micro magnetic resonance imaging for measurements of microstructural and mechanical properties of human distal tibial bone. *J Bone Miner Res*. 2010;25:2039–2050.

10

Monogenic Diabetes Mellitus: Neonatal Diabetes and Maturity-Onset Diabetes of the Young

Siri Atma W. Greeley*, Mary K. McCauley*, Louis H. Philipson, Mark A. Sperling

*Authors contributed equally to this chapter

CHAPTER OUTLINE

INTRODUCTION	279
DEFINITION	279
INCIDENCE	280
CLINICAL PRESENTATION	280
TRANSIENT NEONATAL DIABETES MELLITUS	282
6q24-Related Transient Neonatal Diabetes Mellitus (TNDM1)	282
K _{ATP} Channel-Related Transient Neonatal Diabetes Mellitus (TNDM2)	283
PERMANENT NEONATAL DIABETES MELLITUS	285
K _{ATP} Mutations: <i>KCNJ11</i> and <i>ABCC8</i>	285
Insulin Gene Mutations	286
Other Genetic Forms of Permanent Neonatal Diabetes Mellitus	286
Syndromic Neonatal Diabetes Mellitus	287
DIAGNOSIS AND TREATMENT OF NEONATAL DIABETES MELLITUS	288
MONOGENIC CAUSES OF DIABETES OF ADOLESCENCE/YOUNG ADULTHOOD: MATURITY-ONSET DIABETES OF THE YOUNG	289
Glucokinase Maturity-Onset Diabetes of the Young (MODY2)	290
<i>HNF1A/HNF4A</i> : Transcription Factor Maturity-Onset Diabetes of the Young (MODY3/MODY1)	290
<i>HNF1B</i> : Maturity-Onset Diabetes of the Young With Renal Cysts (MODY5—RCAD)	291
Other Forms of Monogenic Diabetes	292
AVAILABLE RESOURCES	293
TRANSITION TO ORAL THERAPY	293
FUTURE DIRECTIONS	293
References	294

INTRODUCTION

Diabetes is a heterogeneous disorder with many different possible causes, both genetic and acquired. Risk for the most common causes, type 1 and type 2 diabetes, depends on many different gene loci with intermediate or low effects and are thus considered polygenic. However, approximately 1% to 5% of all diabetes is caused by abnormalities at a single gene or locus and as a group, these entities are termed *monogenic diabetes*.^{1–6} Over 30 genes have now been described as causing the various forms of monogenic diabetes, where they typically fall into one or more of the three main overlapping phenotypic categories: neonatal diabetes, maturity-onset diabetes of the young

(MODY), and syndromic diabetes. In this chapter, we describe several of the more common causes of neonatal diabetes in some detail, and also provide brief descriptions of some of the main forms of MODY and syndromic diabetes, with a focus on diagnosis and treatment.

DEFINITION

Neonatal diabetes mellitus (NDM) was the initial term used to describe the presentation of diabetes within the first few days or weeks of life and was defined further in early reports as severe hyperglycemia occurring in the first month of life, lasting at least 2 weeks, and requiring insulin therapy to control blood glucose.⁷ These strict criteria have been progressively loosened as it became evident that the exact age at which the diabetes was diagnosed was more variable, even when an underlying cause, distinct from autoimmune type 1 diabetes mellitus (T1DM), was suspected, such as a genetic cause for pancreatic malformation, faulty insulin synthesis or secretion. Accumulated evidence has suggested an age under 6 months as being a particularly likely indication of NDM, because the majority of cases with an underlying monogenic cause will be diagnosed under 6 months, whereas autoimmune T1DM is highly unlikely during this window. Increasingly, however, it has become recognized that, for reasons that are poorly understood, several of the genetic forms can initially present with diabetes as late as 9 months to 1 year or even later.^{8–10} Indeed, pedigree analyses conclusively demonstrate that the same defect that causes permanent or transient NDM can be present in parents or other first-degree relatives with a phenotype consistent with MODY, but may have been misdiagnosed as T1DM or type 2 diabetes mellitus (T2DM) as subsequently detailed.^{11,12} Herein lies the importance of understanding the genetic basis of NDM, for although these entities are rare, they have taught us much about the genetic pathways involved in the formation of the exocrine and endocrine pancreas.^{13,14} For example, it has been shown that the specific combination of three transcription factors, *Ngn3*, *Pdx1*, and *Mafa*, known to be implicated in the determination of cell lineage during pancreas formation, can reprogram adult mouse exocrine pancreatic cells into cells that closely resemble pancreatic beta cells.¹⁵ Such information is essential for the ultimate ability to generate beta cells and whole islets as potential therapies for any form of diabetes, including T1DM.¹⁶ Equally important is the demonstration that activating mutations in the pore-forming potassium inward rectifying channel, family 6 subtype2-*KCNJ11* gene (*Kir6.2*) and its regulatory subunit sulfonylurea receptor 1-*ABCC8* gene (*SUR1*) of the K_{ATP}-regulated potassium channel, which keep the channel open and hence limit or preclude insulin secretion resulting in NDM (Fig. 10.1), can be overcome by high-dose sulfonylurea therapy, which restores endogenous insulin secretion in response to feeding.¹⁷ Because of the restoration of endogenous insulin secretion that can be triggered by the incretin effect in response to feeding, this oral treatment provides better metabolic control

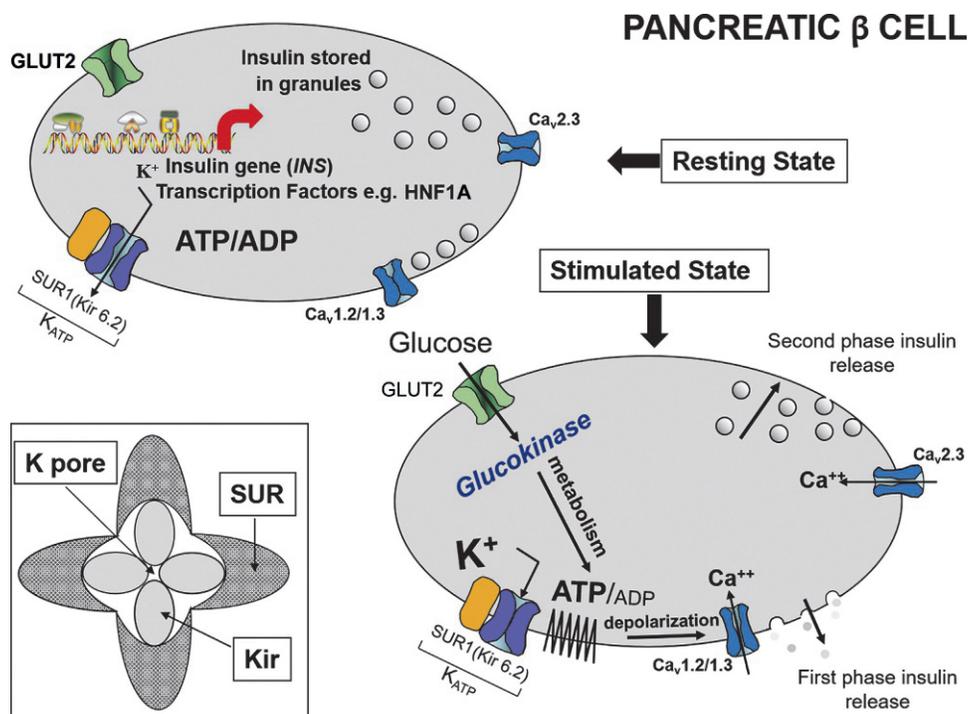


Fig. 10.1 Schematic representation of the role of K_{ATP} channels in nutrient regulation of insulin secretion. In the resting (nonfed) state, depicted in the upper left panel, insulin synthesis and storage are regulated by transcription from the insulin gene (*INS*) and by transcription factors, such as hepatocyte nuclear factor 1 α (*HNF1A*); mutations in *INS* or *HNF1A*, as well as many other beta cell genes can cause maturity-onset diabetes of the young (MODY) or transient or permanent neonatal diabetes mellitus (NDM). The K_{ATP} channel is composed of four subunits of the inward rectifying potassium channel 6.2 (Kir 6.2) encoded by the *KCNJ11* gene on chromosome 11 and four regulatory subunits of sulfonylurea receptor 1 (SUR1), encoded by the *ABCC8* gene, also located on chromosome 11 (inset lower left). In the fasting nonfed state, the K_{ATP} channel remains open. However, in the stimulated (fed) state (panel lower right) glucose concentration increases and enters the beta cell in a concentration-dependent, but insulin-independent manner via the GLUT2 glucose transporter encoded by the gene *SLCA2A*. Glucokinase (GCK) phosphorylates glucose to G6P and its metabolism generates ATP. The resultant change in ATP:ADP causes closure of the K_{ATP} channel, accumulation of intracellular potassium, membrane depolarization, leading to opening of voltage-gated calcium channels and secretion of stored insulin, as depicted in the lower right panel. Metabolism of amino acids, such as glutamate, also generates ATP, which stimulates insulin secretion as described for glucose. The amino acid leucine acts as an allosteric stimulus to glutamate dehydrogenase (GDH), which enables metabolism and generation of ATP. Activating mutations of the K_{ATP} channel maintain it in an open state to varying degrees, in spite of ATP generation, therefore preventing insulin secretion, which leads to diabetes mellitus, including NDM. Inactivating mutations in K_{ATP} genes prevent normal channel opening, maintaining varying degrees of channel closure, and hence constant insulin secretion that causes hyperinsulinism (see Chapters 7 and 23). ADP, Adenosine diphosphate; ATP, adenosine triphosphate; Ca^{++} , calcium; GLUT2, glucose transporter 2; K_{ATP} , ATP-regulated potassium channel; *HNF1A*, hepatocyte nuclear factor 1 α alpha; *INS*, insulin gene; K^+ , potassium; Kir6.2, potassium inward rectifying channel family6 subtype2; SUR1, sulfonylurea receptor1.

than multiple daily injections of insulin or insulin pumps and with a better quality of life.¹⁸ Successful transfer to sulfonylureas is best predicted by in vitro response of specific mutation and diabetes duration.¹⁹ These findings emphasize the benefits of research for understanding pathophysiology and choosing appropriate treatment. Indeed, for those whose NDM is caused by mutations in the K_{ATP} channel that respond to sulfonylureas, these treatments border on the miraculous.

INCIDENCE

Early estimates placed the incidence of NDM at approximately one in 500,000,⁷ but as increased awareness has led to these entities being recognized more frequently, the reported incidence has risen considerably. In populations with high rates of consanguinity, some studies report an incidence of NDM as high as one in 21,000 births.²⁰ A large representative database for pediatric diabetes reported that the incidence of NDM represented approximately one case in 89,000 live births in Germany, and a similar incidence occurred in Italy.^{21,22} In three other European countries, the incidence was reported to be one in 260,000 live births only for those with permanent NDM (PNDM) (suggesting a higher incidence if transient forms of NDM [TNDM] were also

included).²³ In the SEARCH for Diabetes in Youth Study involving 15,829 subjects aged under 20 years diagnosed with diabetes during the years 2001 to 2008, 39 were diagnosed before the age of 6 months. Among these 39 subjects with onset less than 6 months of age, 35 had permanent neonatal diabetes and an additional three had TNDM, leaving one subject whose status remained unknown.⁶ Hence, the total prevalence among children diagnosed with diabetes was approximately 0.246% or approximately 1 in 400. The majority were classified by their primary care providers as having T1DM and treated with insulin; only seven underwent mutational analysis for three of the most common genes (*KCNJ11*, *ABCC8*, and the insulin gene [*INS*]) and five of these seven had mutations in one of these three genes.⁶ The estimated population prevalence of PNDM in those aged less than 20 years in that study was one in 246,000 people.

CLINICAL PRESENTATION

Infants affected with diabetes may have diabetes in isolation, or the underlying genetic defect may also cause a variety of other clinical features (Fig. 10.2). Infants with NDM are more likely to be born small for gestational age, and in many cases there

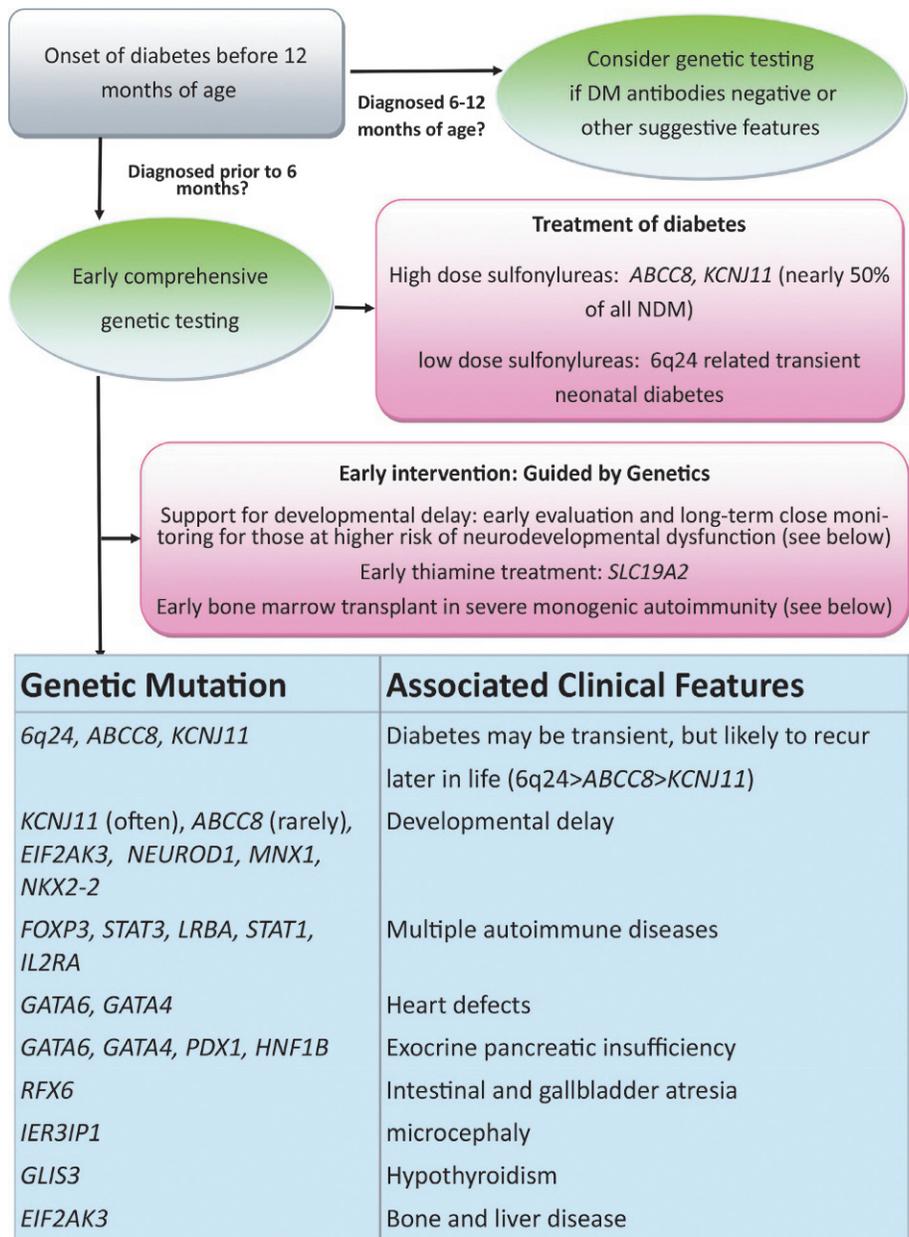


Fig. 10.2 Schematic representation of genetic diagnosis and management of neonatal diabetes mellitus. (Modified from Hattersley, A.T., Patel, K.A. (2017). Precision diabetes: learning from monogenic diabetes. *Diabetologia*, 60(5), 769–777.

may also have been concern for intrauterine growth retardation (IUGR) during their pregnancy, reflecting the in utero deficiency of insulin and emphasizing the role of insulin as a determinant of fetal growth.²⁴ Their small size and low birth weight markedly contrasts with the large birth weight and size of infants with inactivating mutations in the same K_{ATP} genes that instead of diabetes lead to hyperinsulinemic hypoglycemia (see Chapters 7 and 23). A disproportionate number of those with NDM are born prematurely at less than 37 weeks' gestation, which in some cases may be associated with induction of the delivery because of their IUGR status.²⁵ Hyperglycemia leads to osmotic diuresis and avid feeding from breast or bottle despite which the infants often fail to thrive. Delay in diagnosis from not considering the possibility of diabetes mellitus in a newborn may lead to severe dehydration and life-threatening diabetic ketoacidosis (DKA); in fact, a recent retrospective study of a large series of NDM cases in the United States showed that 66% of patients presented in DKA.²⁶ Rare syndromic forms may have severe congenital malformations, such as intestinal

and biliary atresia (*RFX6*), congenital heart defects (*GATA6* or *GATA4*), or brain malformations (*PTF1A*, *NEUROD1*, *IER3IP1*, *MNX1*, *NKX2.2*), or alternatively may have other features, such as skeletal dysplasia (*EIF2AK3*) that are difficult to recognize during the neonatal period. In addition to mutations in *FOXP3* responsible for the IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), several additional genes (*STAT1*, *STAT3*, *LRBA*, *IL2RA*) have now been described to have mutations causing infancy-onset diabetes, along with other autoimmune disorders that result from dysfunction of immune regulation.^{27–30}

Patients with overexpression of genes at chromosome 6q24 present with TNDM and will often have more subtle features, such as macroglossia and umbilical hernia, reminiscent of Beckwith-Wiedemann syndrome. In addition, they rarely may have dysmorphic facies, as well as renal tract anomalies, such as hydronephrosis and vesicoureteral reflux, a variety of cardiac anomalies, hypothyroidism, or hand-finger anomalies.²⁵ A coarse facial appearance together with epilepsy and

later manifestations of developmental delay constitute the developmental delay, epilepsy, neonatal diabetes (DEND) syndrome associated with the most severe mutations of the *KCNJ11* gene¹⁷; however, in most cases of *KCNJ11* or *ABCC8* mutations that maintain the K_{ATP} channel in a variable open state and hence limit insulin secretion, there will not be any abnormal findings on physical examination. Even in those with the severe mutations causing significant neurodevelopmental dysfunction, the abnormalities may often not be recognized until patients are older. Clinical manifestations involving organs other than the pancreas may be part of the syndromes of a variety of genetic causes of NDM. These features in patients with NDM were previously incorporated in a retrospective categorization system to describe permanent, transient, and syndromic forms of NDM. However, now that the monogenic basis of NDM has been well established, genetic testing should be done immediately upon diagnosis of diabetes in the neonatal/infancy period, at which time it will be unclear whether the baby has a permanent or transient form of NDM (see Fig. 10.2). Furthermore, the extrapancreatic manifestations of genes causing syndromic NDM can be phenotypically quite variable and many of these features will often not be readily apparent during the neonatal period.³¹ Expert consensus has therefore shifted toward obtaining comprehensive genetic testing utilizing next-generation sequencing (NGS) panels (or a tiered approach) rather than iterative testing of fewer genes selected based on clinical features.³²

TRANSIENT NEONATAL DIABETES MELLITUS

TNDM is so named because hyperglycemia resolves spontaneously within the first few months of life and no longer requires treatment, although it may reappear later in life. About 70% of these cases will have TNDM1 (6q24-related TNDM), whereas the majority of the remaining cases of TNDM will be caused by mildly activating mutations in the K_{ATP} genes *ABCC8* (*SUR1*) and *KCNJ11* (*Kir6.2*), and only a small minority will be caused by recessive insulin gene mutations, mutations in the transcription factor *HNF1B*, or mutations in *SLCA2A*, the gene encoding the GLUT2 transporter.

6q24-Related Transient Neonatal Diabetes Mellitus (TNDM1)

TNDM1 (or 6q24-related TNDM) is caused by the overexpression of genes at the 6q24 chromosomal locus, including pleomorphic adenoma gene-like 1 (*PLAGL1*), which is a proapoptotic zinc finger protein, and (hydatidiform mole-associated and imprinted transcript (*HYMAI*), encoding an untranslated messenger ribonucleic acid (mRNA). TNDM1 arises as a result of uniparental paternal disomy of the entire—or only a segment that includes 6q24—chromosome 6 (UPD6), a paternally inherited duplication of chromosome 6q24, or relaxation of imprinting of the maternally methylated genes on chromosome 6 (Fig. 10.3). It is important to emphasize that these chromosomal changes cause altered expression of genes, rather than representing mutations. For example, *PLAGL1* (*ZAC*) has antiproliferative properties and is thought to function as a tumor growth suppressor expressed only on the paternal allele³³; overexpression in fetal life is believed to lead to underdevelopment of the pancreas. Although the precise mechanisms by which overexpression of *PLAGL1* lead to TNDM1 are not known, overexpression of *ZAC* in a clonal pancreatic beta cell line impairs glucose-stimulated insulin translation and secretion.³⁴ A transgenic mouse model expressing the human TNDM1 locus (6q24) is characterized by impaired glucose homeostasis with hyperglycemia in the neonatal period and impaired glucose tolerance with reduced insulin responses

to intravenous (IV) glucose as adults.³⁵ The pancreata of these animals display reduced expression of endocrine differentiation factors, notably *PDX1*, *NGN3*, and *PAX6*. There is also a reduction in the number of insulin staining cells and reduced insulin content or insulin secretion despite normal or elevated beta cell mass at all postnatal periods. Thus this model recapitulates TNDM1 and suggests that altered expression of *ZAC/HYMAI* cause impaired development of the endocrine pancreas, as well as impaired beta cell function.^{35,36} This mouse model also demonstrates resolution of abnormal insulin secretion with restoration of normal glucose tolerance during the “juvenile” phase of mouse development between 1.5 and 2 months of life, during which there is an approximate doubling of beta cell number that compensates for the reduced insulin synthesis and secretion of each cell. Also as in humans, the compensatory increase in beta cell mass is not sustained, resulting in a mild diabetes mellitus characterized by normal fasting glucose but hyperglycemia after glucose challenge. Overall, despite the recapitulation of the key features of the human disease, the mouse model displays milder features.^{35,36} One possible reason for this milder phenotype in mice is that pancreatic expression of the mouse ortholog *Zac1* declines drastically during gestation and early postnatal growth in mice, whereas expression of the *ZAC* gene in human pancreas declines between the second trimester and adult life.³⁷ More important, *ZAC* was specifically expressed only in the islets of the human fetus, whereas *Zac1* was predominantly expressed in mesenchyme of the mouse embryo, which may explain the milder features in the mouse model of TNDM1.³⁷

In patients with TNDM1 resulting from hypomethylation of the maternal differentially methylated region (DMR) of chromosome 6q24, there may be hypomethylation of other maternally imprinted loci (HIL) throughout the genome.³⁸ In cases displaying a more generalized HIL, the majority have a mutation in the transcription factor zinc finger protein 57 (*ZFP57*).³⁹ HIL also occurs in Beckwith-Wiedemann syndrome and this likely explains the macroglossia, umbilical hernia, and several congenital abnormalities described in TNDM1.⁴⁰ In a large multinational cohort involving 163 patients with TNDM1,²⁵ the authors describe IUGR with a mean birth weight of 2001 ± 417 g (mean \pm standard deviation) and adjusted Z score for birth weight of -2.5 . The mean age of presentation was 8 ± 12 days with a median of 4 days and a mode of 1 day. Mean gestation was 37.8 ± 2.4 weeks and prematurity was significantly more common than in the general population. Remission occurred at a mean age of 4.5 ± 5.8 months with a median at 3 months. Age at presentation was positively correlated to gestational age, but age at remission was negatively correlated with adjusted birth weight.²⁵ Thus the higher the birth weight, the earlier the remission and vice versa. This would be consistent with the effects of insulin on intrauterine growth, so that the larger infants would have the milder defect and therefore tend to enter remission sooner. Congenital anomalies were significantly more frequent in patients with UPD6 or HIL. Hypomethylation defects were overrepresented in patients born after assisted conception. Thus babies with TNDM1 generally present with diabetes mellitus within the first days of life, are small, and may have been born prematurely. The presence of one or more congenital anomalies suggests UPD or multiple HIL, and among the latter, almost one in seven had been conceived with assisted reproductive techniques. Macroglossia is present in about 50%, umbilical hernia in about 25%, and facial dysmorphism in about 20%. Cardiac and renal anomalies ($\sim 9\%$), hand abnormalities ($\sim 8\%$), and hypothyroidism ($\sim 4\%$) also may be present. Remission, when it occurs, is usually around 3 months and about half of these patients will revert to varying degrees of hyperglycemia in the teen years or later.²⁵ An unusual manifestation following

Mechanisms responsible for differential methylation patterns in TNDM1

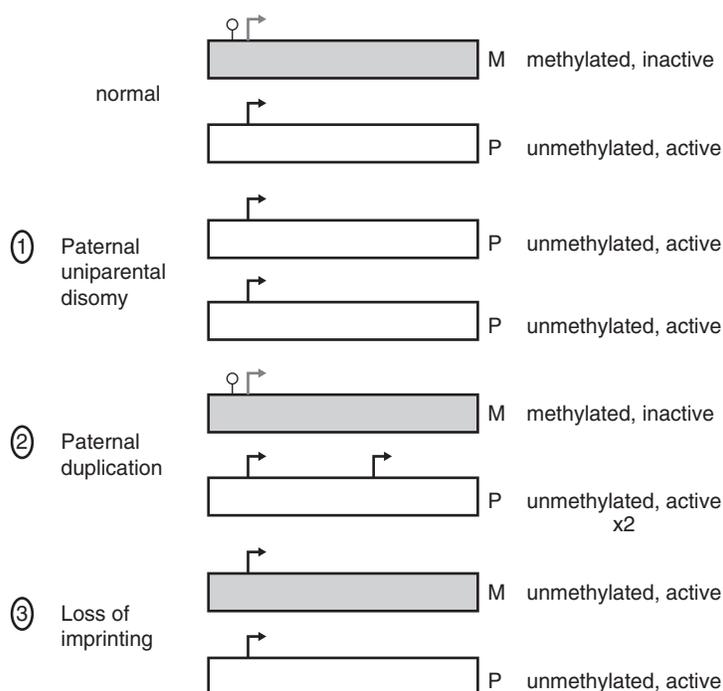


Fig. 10.3 Transient neonatal diabetes mellitus type 1 is caused by overexpression of imprinted genes on chromosome 6q24 (*PLAGL1* and *HYMAI*). Normally the maternal allele remains methylated and inactive, whereas the paternal allele is unmethylated and active. The differential expression of these genes can occur by one of three mechanisms, as illustrated: (1) paternal uniparental disomy, where both alleles are of paternal origin; (2) paternal duplication, so that two active paternal alleles are expressed; (3) loss of imprinting (or “relaxation of imprinting”), whereby the maternal 6q24 allele is also expressed. See text for details.

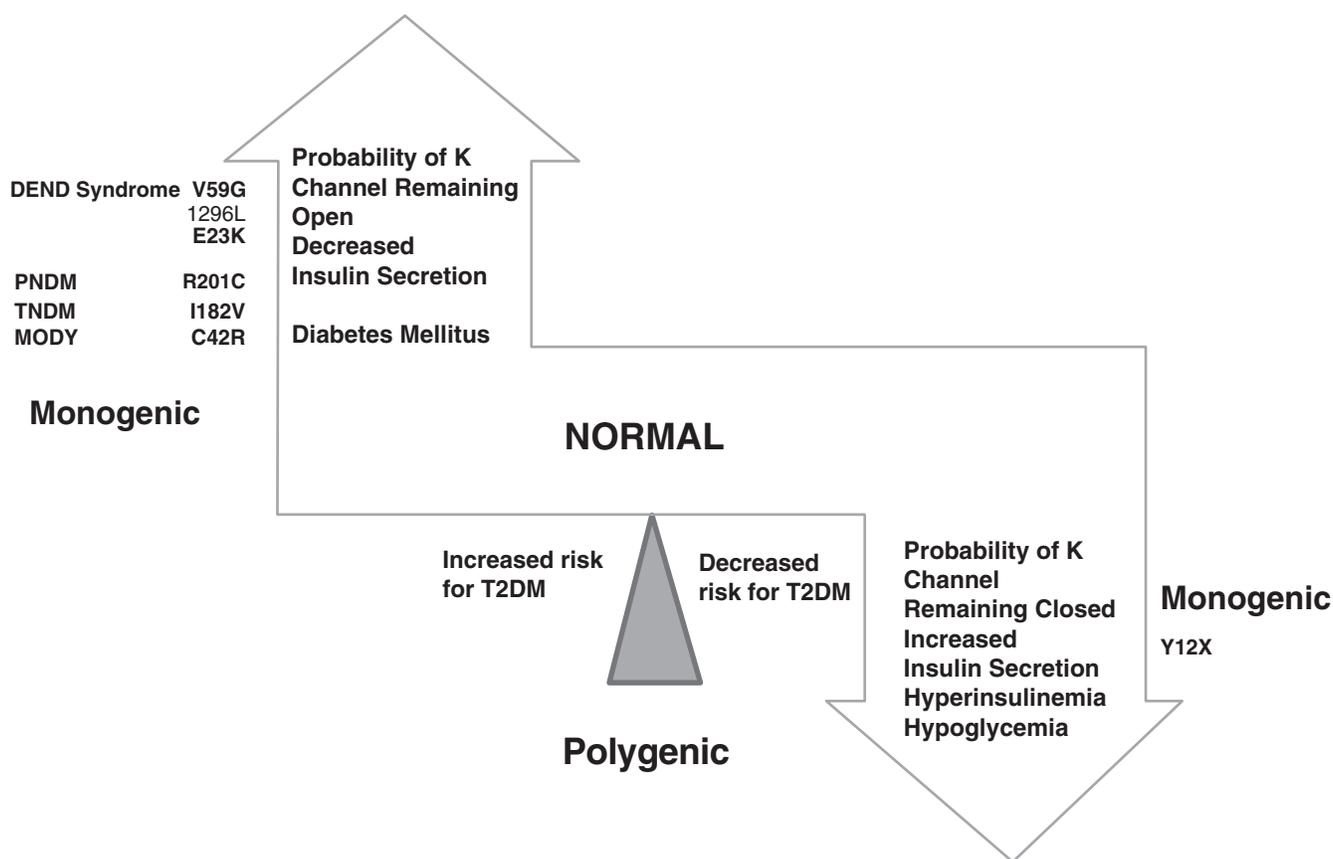
remission of diabetes in patients with 6q24 methylation defects is hypoglycemia with hyperinsulinemia, most often when the cause is UPD6.⁴¹ Modern molecular techniques permit diagnosis to be established which then influences treatment; however, many commercial sequencing panels for monogenic diabetes do not include testing that will reveal overexpression of genes at 6q24 as a cause of diabetes.

Patients with TNDM1 are sensitive to insulin and respond with excellent catch-up growth within several weeks of treatment. Progressive reduction of the insulin dose required to control blood glucose while avoiding hypoglycemia heralds the onset of remission. Although these patients do well with insulin treatment, some reports suggest a variable response to oral sulfonylurea treatment; though it is difficult to discern the extent to which the clinical improvement was caused by medical treatment versus spontaneous resolution of the condition in these observational studies.^{42,43} Of note, rare cases with 6q24-related TNDM will develop significant hypoglycemia within weeks or months after the remission of hyperglycemia. Most had evidence for hyperinsulinism as the cause of hypoglycemia and responded well to diazoxide treatment that was still required after several years in some cases.⁴¹ Even after resolution of diabetes during infancy, it is important that these patients understand that diabetes is highly likely to recur around the time of adolescence. Although a few papers report good response to other drugs besides insulin, the best approach to monitoring during remission, as well as to treatment after recurrence, remain uncertain. Because many questions remain about this condition, it is important that such cases be referred to research centers tracking long-term outcome, even if the diabetes has gone into remission.⁴³

K_{ATP} Channel-Related Transient Neonatal Diabetes Mellitus (TNDM2)

TNDM2 is one classification given to diabetes that remits during infancy and may recur later in life but is caused by mutations in genes regulating insulin secretion rather than expression of imprinted genes. The majority of these entities are caused by activating mutations in the K_{ATP} channel genes *ABCC8* and *KCNJ11*, which, respectively, code for the SUR_1 and Kir6.2 subunits of the K_{ATP} channel (see Fig. 10.1).^{32,44} Recessive loss-of-function mutations in the *INS* itself may occasionally be responsible for TNDM2⁴⁵; the autosomal dominant insulin gene mutations are associated with PNDM.^{8,32,44} There are rare reports of *HNF1B*⁴⁶ and *SLC2A2* mutations⁴⁷ also associated with TNDM.

The normal state of the K_{ATP} channel is to remain open, setting the resting membrane potential of the beta cell. Insulin secretion occurs when the channel closes in response to an increase in adenosine triphosphate (ATP) generated from the metabolism of glucose or amino acids, thereby changing the ATP:adenosine diphosphate (ADP) ratio. Closure of the channel with intracellular retention of K^+ causes depolarization of the plasma membrane, opening of voltage-gated calcium channels, influx of calcium, and secretion of insulin. Activating mutations in *ABCC8* or *KCNJ11* alter the ability of the channel to respond to the change in the ATP:ADP, so that the channel remains open to some extent, efflux of K^+ from the beta cell continues, permitting the cell membrane to remain hyperpolarized and therefore resulting in various degrees of impaired insulin secretion. These same mechanisms are also responsible for the most common form of PNDM, as subsequently discussed.^{32,44}



Relation Between KATP Channel Activity and Insulin Secretion

Fig. 10.4 This is a schematic representation of the relationship between K_{ATP} channel activity and insulin secretion. Activating mutations of the *KCNJ11* or *ABCC8* genes maintain the channel in an open state and hence limit insulin secretion. With progressively increasing probability of the potassium channel remaining open, the severity of the resulting hyperglycemia increases from a mild increased risk for type 2 diabetes mellitus (T2DM), to monogenic diabetes of youth (MODY), transient neonatal diabetes mellitus (TNDM), permanent neonatal diabetes mellitus (PNDM), and in the most severe state the DEND syndrome (developmental delay, epilepsy, neonatal diabetes) as illustrated on the left. In contrast, mutations that increase the probability that the channel remains closed also increase the likelihood of persistent insulin secretion and lead to hyperinsulinemia and hypoglycemia as illustrated on the panel on the right; in milder forms they may decrease the risk for T2DM by maintaining a higher insulin secretion. Common genetic defects are illustrated in each as examples. (Modified from Ashcroft, F.M. (2005). ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest*, 115, 2047–2058. Figure 6.)

It remains unclear how or why remission occurs, but it has been shown in vitro that mutations causing TNDM have a less pronounced effect on channel function compared with mutations that cause PNDM.⁴⁸ The ability of ATP to close the channel in vitro correlates with the severity of NDM, including the severe permanent form associated with the DEND syndrome, which demonstrates the greatest resistance to closure by ATP in vitro.^{48,49} Both resistance to closure caused by activating mutations, or resistance to opening of the channel because of inactivating mutations, segregate with certain mutations as illustrated in Fig. 10.4. As shown in the figure, near the fulcrum of this spectrum, those with minor defects may be prone to either develop milder type 2 diabetes or may be resistant to the development of diabetes by virtue of enhanced insulin secretion (see Fig. 10.4).

In comparison with TNDM1, patients with TNDM2 generally have greater birth weight, present or are diagnosed with diabetes mellitus later, remit later, and recur earlier (Table 10.1). Family members of patients with these forms of TNDM2 may have diabetes that was diagnosed in adulthood and was considered to be T1DM, T2DM, or MODY and yet harbor the same heterozygous mutations as the proband with NDM.^{10–12} This reflects the variable penetrance of these genes or upstream factors that may modify the expression of the gene

in different individuals. Confirming the presence of an *ABCC8* or *KCNJ11* mutation in a case of NDM is important for management because most of these K_{ATP} channel mutations respond to sulfonylurea treatment both at the time of initial diagnosis or later at the time of relapse.^{32,44} During their remission phase, these patients do not require therapy.

Recessive loss of function mutation in the *INS* gene has been reported in rare patients with TNDM.⁴⁵ These patients appear to enter remission at a median age of 12 weeks and insulin was required before remission and after later relapse that was reported in some cases.⁴⁵

Patients with mutations in the transcription factor hepatocyte nuclear factor 1 beta (*HNF1B*) are known to have diabetes associated with renal cysts with onset at a median of 20 years. However, two reported patients had neonatal diabetes: one was diagnosed at age 15 days and required insulin, initially intermittently and then permanently; a second patient diagnosed at the age of 17 days had remission 2 weeks after diagnosis but relapsed at the age of 8 years.⁴⁶ There have also been a few reported cases of mutations in *SLC2A2*, which encodes the glucose transporter type 2 (GLUT2).⁴⁷ Recessive inactivating mutations in this gene cause Fanconi-Bickel syndrome, characterized by glucosuria, galactosuria, aminoaciduria,

TABLE 10.1 Comparison of Clinical Characteristics of Patients With K_{ATP} Channel Mutations to Patients With 6q24 TNDM (data given in median [range])

Characteristic (median)	ABCC8 / KCNJ11 (n = 25)	6q24* (n = 23)	P-Value
Age at diagnosis (weeks)	4 (0–16)	0 (0–4)	<.001
Age at remission (weeks)	35 (2–208)	13 (5–60)	<.001
Age at relapse (years)	4.7 (3–15)	16 (4–25)	.073
Birth weight (g)	2570 (1360–3570)	1950 (1600–2670)	<.001
Percentile birth weight	12 (<1st–89th)	<1st (<1st–21st)	<.001

*Data previously reported (Temple IK, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP: Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. *Diabetes* 49: 1359–1366, 2000). Differences between groups were calculated using Mann-Whitney U and χ^2 tests. Centile birth weights were calculated according to U.K. growth charts (Cole TJ, Freeman JV, Preece MA: British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 17: 407–429, 1998) because the majority of patients were of U.K. white origin.

Modified from Flanagan SE, Patch AM, Mackay DJ, et al. Mutations in ATP-Sensitive K^+ Channel Genes Cause Transient Neonatal Diabetes and Permanent Diabetes in Childhood or Adulthood. *Diabetes* Jul 2007, 56 (7) 1930–1937; Table 2. <https://doi.org/10.2337/db07-0043>. Retrieved from: <https://diabetes.diabetesjournals.org/content/56/7/1930.long>.

proteinuria, and phosphaturia, as well as rickets, poor growth, and short stature with associated glucose and galactose intolerance and enlarged livers. Transient neonatal diabetes has been reported in these patients to occur in association with the classic Fanconi-Bickel syndrome.⁴⁷

PERMANENT NEONATAL DIABETES MELLITUS

The majority of genes that cause NDM result in PNDM without any significant treatment remission. Approximately 80% to 85% of infants with diabetes diagnosed before 6 months of age will have an underlying monogenic cause that can be identified, and the remainder may carry as-yet uncharacterized defects. Although diabetes resulting from monogenic causes can also be diagnosed between 6 and 12 months of age or later, and genetic testing can be considered, the majority of diabetes cases in this age range will have autoimmune diabetes (T1D) that can be suggested by positive anti-beta cell autoantibody testing or type 1 diabetes genetic risk scores (routinely done in most NDM research centers). Except in certain populations with higher frequency of consanguineous births, the vast majority of PNDM is caused by mutations in three genes: the two K_{ATP} channel genes *KCNJ11* coding for the pore-forming protein Kir6.2 (50%) and *ABCC8* coding for SUR1 (together account for 40%–45% of cases; see Figs. 10.1 and 10.2), and mutations in the gene encoding insulin itself (*INS*). Dominant mutations in *GATA6* or *GATA4* causing pancreatic hypoplasia are rarer than other dominant causes, as are X-linked mutations in *FOXP3* causing the IPEX syndrome, or milder IPEX-like syndromes, whereas the remaining gene causes of PNDM are very rare recessive disorders that often include a variety of extrapancreatic syndromic features (see Fig. 10.2).

K_{ATP} Mutations: *KCNJ11* and *ABCC8*

Until the discovery of the genes responsible for the K_{ATP} channel, patients with PNDM were considered to have insulin-dependent diabetes mellitus; now it is known that the majority of patients with K_{ATP} mutations causing PNDM respond to oral therapy with sulfonylurea.^{50,51} Early genetic testing is imperative because those who are responsive to sulfonylurea treatment and have earlier initiation of treatment have been reported to have improved response to therapy and may help improve quality of life.^{50,51} Typically treated with high-dose sulfonylureas, a cohort with this treatment has shown mild and infrequent hypoglycemia.⁵² Patients with PNDM caused by mutations in the K_{ATP} genes or the insulin gene usually present at 2 to 3 months of life, and sometimes later, and may be in severe DKA by the time of the diagnosis. Activating mutations in the Kir6.2 subunit of the K_{ATP} channel were first reported in 2004,⁴⁹ being found in 10 out of 29 subjects with PNDM.

Whereas the insulin secretory response to IV glucagon and glucose was minimal, there was a better response to oral glucose. Patients did respond with insulin secretion to IV tolbutamide, clearly hinting at possible therapy by the administration of sulfonylurea and confirmed by a landmark study published 2 years later.¹⁷ Expression of the mutated Kir6.2 subunit together with a normal SUR1 subunit in *Xenopus laevis* oocytes revealed that the ability to enable channel closure by ATP was greatly impaired. This provided a means to correlate the degree of *in vitro* abnormality with the clinical severity of the diabetes.¹⁷ The affected patients predominantly had *de novo* mutations, with only 20% of the mutations inherited from a parent. It was also noted that 4 of 10 of the patients had severe developmental delay, muscle weakness, and epilepsy, as well as dysmorphic facial features, which was termed the *DEND syndrome*. The degree of muscle weakness was partially ameliorated by treatment with sulfonylurea, raising the possibility that the developmental delay and epilepsy may also be ameliorated, or perhaps prevented, by early recognition and treatment with sulfonylurea.⁴⁴ Subsequently, it was demonstrated in mice that transgenic expression of an activating mutation in Kir6.2 in mouse pancreatic beta cells recapitulates neonatal diabetes⁵³ and that the muscle dysfunction caused by a human K_{ATP} channel mutation is neuronal and not muscular in origin.⁵⁴ K_{ATP} channels exist in other tissues and are known to modulate electric activity and neurotransmitter release at brain synapses in various regions of the brain.⁴⁸ Moreover, K_{ATP} channels in the ventromedial hypothalamic nucleus may be involved in the counterregulatory response to hypoglycemia⁵⁵; in the arcuate nucleus neurons, K_{ATP} channels may be involved in appetite regulation.^{55,56}

A spectrum of clinical disturbances occurs with different mutations ranging from the *DEND syndrome*, to relapsing diabetes,^{48,54,57} permanent diabetes appearing initially in childhood or later in adults.^{57–59} Mutations in adjacent locations may cause either neonatal diabetes or hyperinsulinism because they increase or decrease the open state of the channel.^{57–61} Likewise, mutations in the *ABCC8* gene encoding SUR1 cause transient or permanent NDM, or permanent diabetes diagnosed beyond the newborn period in children, or in adults, and mutations at a similar site in the gene can result in either hyperinsulinism or neonatal diabetes.^{11,62–65}

Initial reports noted certain mutations (such as *KCNJ11* V59M) are characterized by significant global developmental delay (often termed *intermediate DEND syndrome*, or *iDEND*) in about 20% of cases, and anecdotal reports suggested improvement of neurological symptoms of weakness, dyscoordination, and visuomotor impairment after treatment with sulfonylureas (replacing insulin) was initiated.^{66–68} Several groups have since undertaken careful characterizations of large groups of patients using standardized measures that reveal a wide range of dysfunction, including behavioral difficulties

and attention deficit hyperactivity disorder symptoms or diagnosis, lower educational attainment, and difficulties with executive functioning.^{69–73} In one study of 14 patients with mutations previously thought to cause diabetes in isolation, who did not have global developmental delay, IQ was found to be close to normal (91.1 ± 11.3) but significantly lower than 20 sibling controls (111.0 ± 8.3).⁷² Raising the importance of early proper diagnosis and treatment of these conditions, another study revealed better performance on a standardized measure of visuomotor functioning by the three patients who had been started on sulfonylurea treatment under a year, among eight patients with of V59M mutations in *KCNJ11*.⁶⁸

The safety and tolerability of high-dose sulfonylurea treatment, as well as its durability over more than 10 years of treatment, has also been reported in a few recent studies. A survey of 30 patients over a total of 166 patient years revealed no episodes of severe hypoglycemia, whereas mild to moderate hypoglycemia was unrelated to sulfonylurea dose.⁵² A recent follow-up study on 81 patients from a European cohort started on sulfonylureas before 2006 showed no episodes of severe hypoglycemia over 809 patient-years, with sustained excellent glycemic control on stable doses with infrequent mild side effects.⁷⁴

Insulin Gene Mutations

Insulin gene mutations as a cause of PNDM were first reported in 2007 and are now known to be the second most common mutations responsible for these entities.^{75–77} Inheritance was autosomal dominant in the familial cases, but the majority had de novo mutations. The mutations occurred in a critical region of the preproinsulin molecule, predicting misfolding and hence loss of normal trafficking of the proinsulin in the insulin secretory pathway. This misfolding was also proposed to induce the unfolded protein response, with degradation in the endoplasmic reticulum (ER), leading to severe ER stress and apoptosis of the beta cells, processes known to occur in mouse models of dominant insulin gene mutations.⁷⁵ Clinically, the age at diagnosis averaged 13 weeks compared with 5 weeks for *KCNJ11* and 7 weeks for *ABCC8* mutations. With a normal range of gestational ages of 36 to 41 weeks, mean birth weight was also normal at 2846 g. Thus these abnormalities appear to disturb intrauterine growth less than in 6q24 TNDM or those with K_{ATP} channel mutations. These dominant or de novo mutations are not usually associated with TNDM or remission,⁷⁵ whereas recessive mutations of the insulin gene can result in a remitting type of NDM as described earlier.⁴⁵ The initial suggestion of ER stress as a mechanism has been largely confirmed and the spectrum of disorders in the insulin gene extends to a MODY phenotype or onset in adulthood.^{9,76–80} Treatment of these patients is currently limited to insulin, whereby they can be managed very similarly to a patient with autoimmune T1DM. A few case series have demonstrated the effectiveness of insulin pumps and continuous glucose monitors in achieving good glycemic control as early as the neonatal period following diabetes diagnosis.^{81,82} One case study of two sisters suggested the theoretical benefit of early optimization of glycemic control to minimize the need for endogenous beta cells to respond to hyperglycemic excursions by producing mutated insulin that is toxic to beta cells. Limiting the toxic effects of protein production appeared to promote improved cell survival that might allow for at least low level production of insulin via the normal allele.⁸³

Other Genetic Forms of Permanent Neonatal Diabetes Mellitus

Two genes that cause MODY, the enzymatic metabolic gatekeeper glucokinase (*GCK*), and the islet formation transcription factor *PDX1* (previously called *IPF-1*), cause monogenic forms of

diabetes, respectively, known as *MODY2* and *MODY4*, when in the heterozygous state and can more rarely cause PNDM when in the homozygous or compound heterozygous state.

Glucokinase

Glucokinase (*GCK*) has been called the *glucose sensor* of the beta cell. It phosphorylates glucose to glucose-6-phosphate, permitting entry into the glycolytic pathway for metabolism and generation of ATP, which triggers insulin secretion (see Fig. 10.1). *GCK* is also known as *hexokinase IV* or *hexokinase D* and is most active in the physiological range of glucose at 4 to 10 mmol/L (72–180 mg/dL) with a K_m of ~ 8 mmol/L (144 mg/dL). The glucose-stimulated insulin release (GSIR) threshold occurs when glucose phosphorylating capacity reaches 30% of maximum, which generally occurs under normal circumstances at a glucose concentration of ~ 90 mg/dL and is maximum at glucose concentrations of 300 mg/dL or higher but not reaching more than $\sim 80\%$ of full (100%) glucose phosphorylating capacity.

Activating mutations of *GCK* exert these effects at lower glucose concentrations and hence cause hyperinsulinemic hypoglycemia (see Chapters 7 and 23). Inactivating heterozygous mutations in *GCK* cause a mild diabetes because the glucose phosphorylating capacity is shifted to the right, so that the 30% phosphorylating capacity needed for the GSIR occurs when glucose concentrations are around 120 mg/dL rather than 90 mg/dL and reach only a peak of 50% to 60% of the 100% phosphorylating capacity. This is enough to clear all glucose after a meal, but with periods of hyperglycemia before this occurs. *GCK* inactivating mutations are one of the most common forms of MODY (see later), often presenting asymptotically as an incidental finding discovered during blood tests done for other reasons, and there will often be a positive family history of diabetes.

Homozygous inactivating mutations in *GCK* were reported in two patients in 2001,⁸⁴ and the same authors reported an additional three cases in 2003.⁸⁵ The affected subjects were homozygous for the mutation or were compound heterozygous for two different mutations, being a splice site in one and a missense mutation in the other. Each of these five initial subjects was characterized by IUGR, permanent neonatal diabetes from day 1 of life, and hyperglycemia in parents, which was an important diagnostic clue. Apart from some other novel single case reports,^{86,87} an additional four novel cases of PNDM caused by homozygous mutations in *GCK* were reported in 2011.⁸⁸ Overall, these mutations are rare in NDM; in one large cohort of 54 cases of NDM, *GCK* mutations were found in only one case of European ancestry,⁸⁹ and the same was true for a study comparing the etiologies of PNDM in an Arab versus European population.⁹⁰

Pancreatic Duodenal Homeobox 1

Pancreatic duodenal homeobox 1 (*PDX1*), previously known as *insulin promoter factor 1* (*IPF1*), is a critical transcription factor that defines the pancreatic progenitor cell as it differentiates from the endodermal epithelium. Exocrine-duct lineage is then specified by the pancreas transcription factor 1 (PTF1), which defines the exocrine and duct cells. However, in the presence of NGN3, the progenitor cell differentiates into a series of endocrine pancreatic cells under the influence of other transcription factors, including PAX4. Thus *PDX1* is critical for the formation of both the exocrine and endocrine pancreas, and hence homozygous mutations will lead to an absence of pancreas formation with manifestations of neonatal diabetes and pancreatic exocrine insufficiency. The parents may be heterozygous carriers and therefore known to have a form of diabetes known as *MODY4*. The first case of pancreatic agenesis from a monogenic cause in 1997 revealed the etiology as a homozygous loss-of-function frameshift mutation in *PDX1*,⁹¹ whereas the second

case was reported in 2003,⁹² and the third case was reported in 2009⁹³; each of these patients were characterized by pancreatic agenesis with manifestations of both exocrine and endocrine abnormalities. In a more recent study, 103 patients in whom K_{ATP} and *INS* mutations had been excluded and who were not known to have abnormal development or clinical exocrine insufficiency of the pancreas, were tested and found to include four cases with homozygous or compound heterozygous *PDX1* mutations; thus it is possible to have PNDM caused by a mutation in *PDX1* without necessarily having complete pancreatic agenesis.⁹⁴ In this regard, it is important to note that neonatal diabetes may be associated with pancreatic, as well as cerebellar, agenesis as a result of mutations in the *PTF1A* gene (pancreas transcription factor 1A)⁹⁵ and that the *NEUROG3* gene (known as *Ngn3* in mice) can be associated with neonatal diabetes and congenital malabsorptive diarrhea consistent with the role of this gene in pancreas development as briefly described earlier.⁹⁶ Other genes that have been implicated in pancreatic agenesis or hypoplasia include *PTF1A*, *NEUROD1*, *EIF2AK3*, *HNF1B*, *RFX6*, *GATA4*, and *GATA6*, which may also be associated with other features, such as congenital heart defects or cerebellar agenesis.⁹⁷ As a diagnostic clue, if pancreatic imaging shows the absence or hypoplasia of a pancreas, the diagnostic search can be restricted to a series of genes as outlined.⁹⁸

Syndromic Neonatal Diabetes Mellitus

The syndromes associated with NDM together constitute a relatively small fraction of NDM that can vary considerably depending on the frequency of consanguineous unions in the population being studied.^{20,31} Although each form is rare, they are of interest in identifying the spectrum of genetic disorders that can result in abnormal pancreas formation, malfunction, or destruction. It is worth noting that the term syndromic is somewhat subjective but in this context helps to categorize very rare, usually recessive forms of PNDM. Even the most common forms of PNDM caused by K_{ATP} mutations could be considered syndromic based on their frequent variable neurodevelopmental dysfunction, especially with the most severe mutations causing DEND syndrome. In the past, syndromic features could be used to point to specific genes to be sequenced, but consensus opinion now suggests that all NDM patients should undergo comprehensive genetic testing for all known causes either all at once as part of an NGS panel or by using a tiered approach.³²

EIF2AK3: Wollcott-Rallison Syndrome

This rare condition is actually the most common genetic cause of PNDM in consanguineous families,^{90,99} and it may present as isolated nonautoimmune NDM diagnosed at 3 weeks of age or later. Typically, skeletal dysplasia and growth retardation are recognized in the first year or two of life; other manifestations include episodes of liver failure, renal dysfunction, and evidence of pancreatic exocrine insufficiency, neutropenia with recurrent infections, hypothyroidism, and mental retardation.¹⁰⁰ The basis for this condition are mutations in the *EIF2AK3* (eukaryotic translation initiation factor 2 alpha-kinase 3) gene (also known as *PKR-like endoplasmic reticulum kinase* or *PERK*), which participates in the unfolded protein response in the ER. Environmental factors and modification by other genes may influence the spectrum of clinical severity. Skeletal dysplasia with bone fractures and episodes of liver failure plus NDM are pathognomonic of this entity; a review in 2010 indicated that fewer than 60 cases had been reported worldwide¹⁰⁰; two novel mutations were subsequently reported.¹⁰¹ The prognosis is poor for affected patients. Treatment of the diabetes mellitus by pump therapy is recommended; parents should have genetic counseling for risks of recurrence, as this is an autosomal recessive condition.

FOXP3: IPEX Syndrome

The term *IPEX* is an acronym for immune dysregulation, polyendocrinopathy, enteropathy, X-linked and is caused by dysfunction of T regulatory cells caused by mutations in *FOXP3*, which normally is a transcriptional regulator for CD4 regulatory T cells, so that mutations result in the multiorgan autoimmunity. The clinical characteristics are early onset, insulin-dependent diabetes mellitus, or enteritis; eczema and elevated serum immunoglobulin (Ig)E also occur early. Later manifestations include primary hypothyroidism, nephritis, hepatitis, enteritis, and alopecia. Bone marrow stem cell transplant offers potential for cure, but it depends on the availability of a suitable donor.^{102,103} Scurfy mice serve as a model for this condition in humans.¹⁰⁴

Further study of patients with NDM has revealed cases carrying *FOXP3* mutations but without all features of the IPEX syndrome, as well as cases with multiple autoimmune conditions in addition to diabetes attributable to mutations in other genes important for immune regulatory function: *STAT1*, *STAT3*, *LRBA*, and *IL2RA*.^{27,28,105,106}

GLIS3: Neonatal Diabetes Mellitus With Congenital Hypothyroidism

GLIS3 is a member of the GLI-similar zinc finger protein family, which can function both as a repressor and activator of transcription and is specifically involved in the development of pancreatic beta cells, the thyroid, eye, liver, and kidney. An autosomal recessive syndrome, characterized by NDM, IUGR, congenital hypothyroidism, hepatic fibrosis with cholestasis, polycystic kidneys, and congenital glaucoma, was first described in 2003,¹⁰⁷ and the responsible mutation in the *GLIS3* gene was identified in 2006.¹⁰⁸ As noted, this gene is expressed in the early developmental stages of the thyroid, eye, liver, kidney, and the pancreas, particularly in beta cells. Thus mutations in *GLIS3* may have a role in other developmental anomalies and possibly in T1DM or T2DM. Novel mutations associated with the classical findings of NDM and hypothyroidism, plus bilateral sensorineural deafness, osteopenia, and pancreatic exocrine insufficiency, enlarge the clinical spectrum as reported.¹⁰⁹ In animal studies, it appears that *GLIS3* expression is required for pancreatic beta cell function and maintenance of beta cell mass in the adult, so that impaired function could lead to diabetes mellitus.^{110,111} Genome-wide association studies identify *GLIS3* as a locus that affects risk for T1DM,¹¹² and variants of *GLIS3* have been implicated in the predisposition to T2DM.¹¹³

PTF1A: Neonatal Diabetes Mellitus With Pancreatic and Cerebellar Agenesis

The gene *PTF1A* encodes pancreatic transcription factor 1 alpha known to be involved in pancreatic development. Mutations in this gene were identified in two families with NDM associated with pancreatic agenesis, as well as cerebellar agenesis, implicating this factor in normal cerebellar development, as well as pancreatic development, findings confirmed in knockout models of this gene in mice.⁹⁵

RFX6: Mitchell-Riley Syndrome With Biliary and Intestinal Atresia

In mice, it was shown that absence of the gene encoding transcription factor *Rfx6* prevents the formation of any pancreatic endocrine cells, except for pancreatic polypeptide producing cells; mutations in the human ortholog *RFX6* gene caused an autosomal recessive form of neonatal diabetes mellitus.¹¹⁴ In humans, the syndrome of NDM, intestinal atresia, and

hepatobiliary abnormalities had been known as the *Mitchell-Riley syndrome*.¹¹⁵ Clinically, patients are characterized by severe IUGR, fluctuations in glucose concentrations, stabilizing as persistent hyperglycemia and requiring insulin for treatment, cholestatic jaundice and anatomic findings of intestinal atresia, agenesis of the gall bladder, and abnormal formation of the pancreas. Quality of life may also be affected by mental retardation. Life expectancy for those who survive this critical neonatal period requiring intensive surgical and medical interventions is uncertain.¹¹⁶

MXN1 and *NKX2.2*

A fascinating report identified mutations in two other transcription factors previously known to be important for pancreatic beta cell development: *NKX2.2* homozygous mutations were found in two families with diabetes and severe developmental delay, hypotonia, cortical blindness, impaired visual tracking, and hearing impairment, whereas a more variable phenotype was found in two other families with homozygous *MXN1* mutations where both patients had diabetes, but only one had additional features, such as severe neurological complications, hypoplastic lungs, and sacral agenesis.¹¹⁷

NEUROG3: Neonatal Diabetes Mellitus With Congenital Malabsorptive Diarrhea

Neurogenin 3 (*NEUROG3*) is a critical transcription factor for the differentiation of islet endocrine cell types from the pancreatic endoderm. Mice lacking this factor are devoid of intestinal pancreatic endocrine cells and develop diabetes mellitus as newborns. In humans, homozygous mutations in the *NEUROG3* gene cause malabsorptive diarrhea and early-onset diabetes mellitus.^{96,118}

GATA6 and *GATA4*

Both *GATA4* and *GATA6* are critical factors with differential contributions for normal pancreatic organogenesis; double *Gata4* and *Gata6* knockout mice do not develop a pancreas and hence have diabetes.^{119–121} In humans, heterozygous inactivating mutations in *GATA6* were found to be the most common cause of pancreatic agenesis occurring in 15 of 27 subjects with pancreatic agenesis.¹²² These patients required both insulin plus pancreatic enzyme replacement.¹²² In a follow-up study,¹²³ the same investigators sought *GATA6* mutations in 171 subjects with NDM of unknown etiology, having already identified other mutations, including 15 with *GATA6* mutations, in 624 subjects out of a cohort totaling 795. In the new cohort of 171 remaining subjects, an additional nine new cases of *GATA6* mutations were identified for a total of 24 affected out of the original cohort of 795 subjects (3%). In these nine new cases, two had NDM but did not require pancreatic enzyme replacement, and one had TNDM. In addition, four parents were found to have *GATA6* mutations, but the diabetes was diagnosed between the ages of 12 and 46 years. Several of the nine new subjects had subclinical pancreatic enzyme deficiency. Except for one of the parents, subjects with *GATA6* mutations had extra pancreatic features, congenital heart malformations being found in 83%.¹²³ Mutations in *GATA4* were more recently shown to cause infancy-onset diabetes complicated by variable levels of pancreatic hypoplasia and exocrine insufficiency, pancreatic hypoplasia or complete agenesis, congenital heart defects, and neurodevelopmental delay.¹²⁴ Thus *GATA6* or *GATA4* mutations can cause PNDM or occasionally TNDM or adult-onset diabetes, with variable exocrine pancreatic insufficiency from complete agenesis, subclinical, or no deficiency at all.¹²³ Because the majority of cases manifest

congenital heart defects, it is reasonable to consider genetic testing in any individual with a congenital heart defect and diabetes, regardless of the age of onset of diabetes. In evaluating a patient with NDM, pancreatic imaging can be useful in that a finding of pancreatic agenesis or hypoplasia will suggest the need for clinical evaluation of exocrine pancreatic insufficiency or other features that are associated with a more restricted list of genes that include *GATA6*,¹²³ *GATA4*, *PDX1*, and *PTF1A*, as well as *EIF2AK3*, *HNF1B*, *GLIS3*, *NEUROG3*, and *RFX6*.⁹⁸

DIAGNOSIS AND TREATMENT OF NEONATAL DIABETES MELLITUS

NDM is extremely difficult to diagnose clinically, because the cardinal signs of diabetes resulting from osmotic diuresis—polyuria and polydipsia—are masked by the normal high-volume liquid diet of infancy, and clinicians may be reassured rather than worried when babies are feeding frequently and have lots of wet diapers. A diabetes diagnosis can be considered in neonates and infants who display IUGR in utero or at birth, or who fail to thrive despite apparent adequate intake with normal or copious urine output. A positive family history of diabetes mellitus (especially neonatal or infancy-onset) in one or both parents, or in prior siblings, points to a likely genetic cause; however, most cases will be caused by de novo mutations. The diagnosis is established by confirmation of sustained significant hyperglycemia and/or glycosuria, both readily available in hospitals or medical clinics. Recognition of diabetes mellitus within the first week of life often represents TNDM1, because of the differential expression of imprinted genes on chromosome 6q24, particularly if congenital defects, such as macroglossia and umbilical hernia, are present. Somewhat later recognition should also include consideration of defects in the K_{ATP} channel, involving *KCNJ11* and *ABCC8* genes, as well as the *INS* gene; diabetes-associated autoantibodies need not necessarily be determined, unless the baby is close to or older than 6 months of age at the time of diabetes diagnosis, or if other signs of immune dysfunction are present.

Genetic testing is mandatory in any infant diagnosed with diabetes under 6 months of age and should be considered in patients diagnosed up to a year of age or with other features suggestive of an underlying monogenic cause. Although a majority of cases will be related to 6q24 or mutations in *KCNJ11*, *ABCC8*, and *INS*, consensus guidelines recommend urgent comprehensive testing of all known genes either with NGS panels or through a tiered approach.³² A cause can be found in approximately 80% or more of cases diagnosed under 6 months of age, as well as a less certain fraction of cases diagnosed after 6 months of age. A molecular diagnosis is critical not only for guiding the possibility of completely different medical management, but also for guiding monitoring and evaluation of other possible associated features, informing long-term prognosis and outcome, as well as family genetic counseling. Current methodology permits molecular diagnosis to be completed relatively quickly with initial results often available within 1 to 2 weeks. Although commercial testing is available in many countries, consultation with regional centers of expertise can help guide testing decisions and interpretation, as well as allowing for the accrual of accumulated knowledge about best treatment and outcome for these rare disorders. If needed, many of these centers also enable genetic testing without charge as a result of funding from various national agencies (see Available Resources section later).

Insulin therapy is the mainstay of initial treatment in all cases, with the goal of rapidly correcting metabolic derangement and establishing normoglycemia. Because patients will

TABLE 10.2 Inpatient Transition to Oral Sulfonylurea

Day	Glucose Monitoring	Insulin Adjustments	Glyburide Dosing
Prep	Monitor capillary blood glucose (BG) before meals, 2 hours postmeals, bedtime, and 2 a.m. Monitor ketones any time BG is > 300 mg/dL (> 16 mmol/L) Have a plan for hypoglycemia	Maintain usual insulin regimen either via pump or customary basal-bolus injections Reduce basal insulin by 50% (If pump is being used, can be done just before day 1 breakfast; if long-acting insulin is being used, may be reduced on previous evening)	The experience of previous cases with the same mutation will help inform expected response Tablets (easily halved) available: 1.25, 2.5, or 5 mg For infants, tablets can be crushed and suspended in water/formula at the bedside or by any pharmacy
1	If BG before dinner dose is: > 126 mg/dL (7 mmol/L) → < 126 mg/dL (7 mmol/L) →	Administer rapid-acting bolus insulin as needed based on capillary BG: Give usual bolus insulin dose Give 50% of usual insulin dose	0.1 mg/kg before breakfast and dinner (total of 0.2 mg/kg/day), depending on BG: Give 0.1 mg/kg dose at dinner Dinner dose may be skipped
2-7	If BG before SU dose is: > 126 mg/dL (7 mmol/L) → < 126 mg/dL (7 mmol/L) →	Continue decreasing insulin as tolerated: Continue bolus dosing from previous day Decrease insulin bolus by 50%	Each day dose will increase by 0.2 mg/kg/day (0.1 mg/kg/dose) depending on BGs: Increase dose by 0.1 mg/kg/dose May continue dose from previous day
Last	On final day and after discharge continue checking BG at least 4–6 times per day to monitor response. Relative hypoglycemia may necessitate lowering of glyburide dose in following weeks-months	In most SU-responsive cases, insulin can be discontinued within 5–7 days, although mild hyperglycemia may still occur. Treat with rapid-acting bolus insulin as needed, as well as low-dose basal insulin in some cases. It may be possible to discontinue insulin in the following weeks-months	By the end of 5–7 day admission, the patient will either have clearly responded on a lower dose or will be on at least 1 mg/kg/day. The dose may continue to be increased after discharge if needed, with some patients requiring up to 2–2.5 mg/kg/day (which may be lowered eventually in the following weeks-months)
Notes	If expected response is uncertain, C-peptide levels pre- and 90–120 minutes postbreakfast (no insulin given) may be done on day 1 and a later day after the glyburide dose is at least 1 mg/kg/day (usually day 5) If levels pre-SU are nearly undetectable but show a significant increment on glyburide, consider increasing dose as high as 2–2.5 mg/kg/day	BG ranges and insulin adjustments are only a guideline; the physician should be guided by clinical judgment. If there is any indication that glyburide is helping to control BG levels overall, it is often better to decrease the insulin aggressively so as to avoid hypoglycemia If little/no insulin is given with meals, corrections for high BGs (per usual sensitivity/correction scale) may be given postmealtime or at any time	Patients with neurodevelopmental disability or those who are older at the time of transition may experience less responsiveness and require higher doses of glyburide. In such cases, the possible benefit of continuing a high dose for the long term should be carefully considered even if the patient still requires insulin

SU, Sulfonylurea.

Adapted from Pearson, E. R., Flechtner, I., Njolstad, P. R., et al. (2006). Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med*, 355, 467–77. Contact monogenicdiabetes@uchicago.edu for more details.

often have DKA and/or be managed in intensive care units, an IV insulin drip is a viable and effective initial choice, but because this route requires IV access, it is not ideal for the long term. Subsequently, or in the absence of DKA, subcutaneous insulin can be used with regimens similar to older patients, although it should be understood that neonates and infants are often extremely sensitive to very small doses of insulin. A multiple daily injection insulin regimen similar to any type 1 diabetes patient will be effective, but in most cases will require dilution of the typical U100 rapid-acting insulin, which is typically done as a 1:10 dilution (U10, or 10 units/mL). When the appropriate diluent provided by the manufacturer is used, the diluted insulin should be as stable as typical insulin. A very small dose (often the minimal feasible dose of 0.5 unit) of long-acting basal insulin is favored over giving a larger dose that may be tolerated while the infant is feeding frequently but may ultimately be more than appropriate for basal requirements and cause significant hypoglycemia as the baby sleeps better and starts to space out the timing of feedings. Another excellent option if available is an insulin pump, with which it is usually not necessary to use diluted insulin because of the extremely low doses of bolus and basal insulin that can be programmed.¹²⁵

Once the patient is stable and genetic testing has been sent, some experts recommend that a brief glyburide trial could be considered while the family receives diabetes education and preparations are being made for discharge.⁴³ In

the absence of consanguinity or features suggestive of a rare syndromic cause, the chances of carrying a sulfonylurea responsive mutation approaches 50%.³¹ A standard protocol (Table 10.2) should be followed whereby the dose of glyburide is increased daily if hyperglycemia persists, and meanwhile correcting with appropriate modest doses of insulin to maintain reasonable glycemic control. It is important to discuss the relative risks and benefits with the family and be mindful of the high likelihood of failure. If no significant improvement of hyperglycemia is seen after a few days of increasing to a dose approaching 1 mg/kg/day, then the patient should resume insulin monotherapy pending genetic testing results.

MONOGENIC CAUSES OF DIABETES OF ADOLESCENCE/YOUNG ADULTHOOD: MATURITY-ONSET DIABETES OF THE YOUNG

MODY was described as a clinical phenotype distinct from other forms of diabetes by astute clinical scientists who recognized the high prevalence of diabetes occurring at a relatively young age within certain families that suggested an autosomal dominant pattern of inheritance. MODY is now understood to have somewhat different clinical presentations depending on the underlying gene cause and has now been reported to be caused by mutations in up to 12 to 14 different genes,¹²⁶

although some of these gene causes remain under dispute as to their actual pathogenicity (Table 10.3). The most common forms of MODY result from highly penetrant autosomal dominant rare mutations in genes important for beta cell function. Affected patients often have a mild disease presentation marked by modest elevation of glucose often without ketosis, that may be revealed by incidental laboratory testing in asymptomatic patients or may become clinically apparent during intercurrent illness, pregnancy, or puberty that unmasks the limited insulin secretion.^{44,127} Traditional clinical criteria used to define the phenotype of MODY are not absolute indicators of an underlying monogenic diagnosis but help to identify patients who may benefit from genetic testing:

- Dominant inheritance with at least two (and preferably three) consecutive affected generations (but de novo mutations can occur)
- Diabetes onset typically before age 25 to 30 years (some patients will not be diagnosed until older ages; consider whether other family members were diagnosed at younger ages)
- Evidence of significant but impaired residual insulin secretion reflected in C-peptide levels whether or not the patient is being treated with insulin
- Autoantibody tests associated with T1DM will in most cases be negative (very rare exceptions have been reported)
- A distinct phenotype of stable, mild, nonprogressive hyperglycemia in asymptomatic individuals suggests GCK-MODY

Overall, monogenic forms of diabetes may represent up to 2% to 5% of diabetes in those diagnosed under 30 to 35 years of age and may represent as much as 0.4% of all diabetes. GCK mutations appear to have a high population prevalence, with a higher fraction of GCK mutations found in those with suspected MODY who are younger because the stable mild hyperglycemia may be incidentally detected any time after birth. Microvascular complications are rare in GCK-MODY but will occur in those with *HNF1A/HNF4A* mutations (“transcription factor MODY”) because the disease is progressive over time. Diabetes in *HNF1A/4A*-MODY is most often diagnosed in adolescence and young adulthood and is rarely diagnosed before 10 years of age. *HNF1B*-MODY (MODY5) is characterized by renal cysts or other genitourinary anomalies with diabetes.^{44,127} Other rare but important causes of diabetes with a MODY phenotype include milder defects in the K_{ATP} genes *KCNJ11* and *ABCC8* (who will usually respond to oral sulfonylurea treatment), as well as defects in the *INS* itself (see Table 10.3).^{44,127}

Glucokinase Maturity-Onset Diabetes of the Young (MODY2)

GCK, a hexokinase family enzyme, acts in the pancreatic beta cells as the “glucose sensor,” catalyzing the first reaction in the glycolytic pathway by phosphorylating glucose to glucose-6-phosphate. Inactivating heterozygous mutations causing decreased GCK activity leads to insulin release occurring at slightly higher glucose levels, and individuals with these mutations consequently exhibit mild fasting asymptomatic hyperglycemia.^{128,129} There is an important contribution from the liver, which also requires normal levels of GCK, and the enzyme is also present in the appetite centers of the brain. Homozygous or compound heterozygous inactivating mutations, although rare, may result in permanent neonatal diabetes, requiring insulin therapy, whereas homozygous activating mutations can rarely result in neonatal hyperinsulinemic hypoglycemia (see Chapter 7).¹³⁰

The most characteristic feature of GCK-MODY that distinguishes it from other forms of monogenic diabetes is mild stable fasting hyperglycemia, reflected in mildly elevated hemoglobin (Hb)A1c levels that are rarely above 7.5%. In addition to fasting hyperglycemia, a relatively low 2-hour glucose value is seen following oral glucose tolerance test. This may be caused by the shifted glucose sensing defect causing insulin secretion at a higher set point, but peak insulin secretion is not blunted, and first-phase insulin secretion generally is preserved.^{128,129} In a cross-sectional study, patients with GCK-MODY were shown to have a low prevalence of clinically significant microvascular complications (1%) compared with control group (2%) and those with young-onset type 2 diabetes (36%).¹³¹

Some have reported the population prevalence of GCK-MODY as 1.1 in 1000 or 0.11%, and in pregnant women, 9 in 1000, or 0.93%.¹³² GCK-MODY commonly presents in pregnancy because this may be the first time a woman’s blood glucose level was ever checked. The prevalence in pregnancy highlights the importance of correctly diagnosing GCK-MODY because management options will differ from other types of gestational diabetes mellitus. Using the combination of body mass index less than 25 and fasting glucose of 100 or more, the Atlantic Diabetes in Pregnancy cohort used these tools for 68% sensitivity and 99% specificity for distinguishing GCK-MODY from GDM, with 2.7 needing to undergo genetic testing to diagnose one case of GCK-MODY.¹³² Treatment recommendations in GCK-MODY pregnancies are also affected by the fetal genetic status. An unaffected fetus born to a mother with GCK-MODY will have increased insulin secretion caused by mild maternal hyperglycemia, presenting a risk for increased birth weight and macrosomia, whereas an affected fetus will likely have normal birth because they share an altered set point for hyperglycemia resulting from the mutation. A fetus with paternally inherited GCK-MODY born to an unaffected mother would have a lower birth weight.¹³³ As invasive sampling is not currently recommended, routine ultrasounds beginning at 26 weeks allow for monitoring of accelerated fetal growth. If accelerated growth is noted, insulin therapy in the mother may be considered, but may not be effective in preventing macrosomia in a fetus presumed to be negative for mutations.¹³³

HNF1A/HNF4A: Transcription Factor Maturity-Onset Diabetes of the Young (MODY3/MODY1)

The most common MODY phenotype is caused by mutations encoding either of two important pancreatic transcription factors, *HNF1A* (MODY3) or *HNF4A* (MODY1). The patients are usually diagnosed with diabetes in adolescence or young adulthood (most often, but not always, >10 years of age and <30 years of age), and will often have an autosomal dominant pattern of diabetes in several generations of the family. Well-preserved beta cell function would be expected earlier in the disease course, with presenting HbA1c usually under 10%, and often under 7.5%. Although significant ketosis or DKA is unlikely, it has been reported in rare cases. *HNF4A* mutations are a much rarer cause of transcription factor MODY than *HNF1A*, but more often *HNF4A* patients and/or family members will have a history of higher birth weights and/or hyperinsulinemic hypoglycemia during the neonatal period (the mechanisms for which are poorly understood). A classic feature of *HNF1A* mutations that is often shared by those with *HNF4A* mutations is that they often do well with low-dose insulin treatment but are especially sensitive to treatment with low doses of sulfonylureas. In fact, some patients or family members will

TABLE 10.3 Genes Associated With MODY

Gene	OMIM # / Designation	Importance of Genetic Diagnosis	Comments
Genes for which genetic diagnosis has significant treatment implications; causal variants have high penetrance			
<i>GCK</i>	125851 / MODY2	Caused by highly penetrant heterozygous loss-of-function variants; most patients will not require ANY treatment, except possibly during pregnancy	Relatively high population prevalence of causal variants (approximately 1:1000) Inheritance of biallelic variants can cause neonatal diabetes
<i>HNF1A</i>	600496 / MODY3	Caused by highly penetrant heterozygous loss-of-function variants; usually responsive to low-dose oral sulfonylureas	Most common cause of treatment-requiring MODY. Glucosuria is common. Risk for benign hepatic adenomas (rarely can become large and/or complicated)
<i>HNF4A</i>	125850 / MODY1	Caused by highly penetrant heterozygous loss-of-function variants; usually responsive to low-dose oral sulfonylureas	Patient or family members may have history of large birth weights and/or hyperinsulinemic hypoglycemia
<i>HNF1B</i>	137920 / MODY5	Caused by heterozygous loss-of-function variants; renal manifestations appear to be more penetrant than diabetes; best treatment for diabetes not well established; genetic diagnosis will inform monitoring and management of other features	Other features may include genitourinary malformations (particularly renal cysts), altered liver function tests, hypomagnesemia, hyperuricemia, exocrine pancreatic insufficiency, and/or developmental delay (as part of chromosome 17q deletion syndrome)
<i>ABCC8</i>	600509 / MODY12	Caused by rare highly penetrant heterozygous missense mutations; usually respond to medium-high dose oral sulfonylureas	Same or similar mutations may also cause TNDM and similar but more severe mutations cause PNDM (most often heterozygous missense mutations)
<i>KCNJ11</i>	616329 / MODY13	Caused by rare highly penetrant heterozygous missense mutations; usually respond to medium-high dose oral sulfonylureas	Same or similar heterozygous missense mutations may also cause TNDM and similar but more severe mutations are the most common cause of PNDM
Genes with good evidence for causality but genetic diagnosis less likely to result in treatment difference; causal variants may have lower penetrance			
<i>INS</i>	613370 / MODY10	Very rare heterozygous variants cause MODY-like phenotype; best treatment not established	Pathogenic heterozygous variants more often cause neonatal diabetes, but certain rare variants are more often associated with a MODY-like phenotype
<i>RFX6</i>	N/A	Very rare pathogenic heterozygous variants associated with MODY-like diabetes with incomplete penetrance. Best treatment not established	Inheritance of biallelic variants can cause neonatal diabetes with other features
<i>NEUROD1</i>	606394 / MODY6	Very rare pathogenic heterozygous variants associated with MODY-like diabetes with incomplete penetrance. Best treatment not established	Inheritance of biallelic variants can cause neonatal diabetes with other features
<i>CEL</i>	609812 / MODY8	Very rare pathogenic heterozygous mutations in the VNTR of <i>CEL</i> associated with MODY-like diabetes. Best treatment not established	Very likely to include exocrine pancreatic insufficiency (can be assessed by fecal elastase)
<i>APPL1</i>	616511 / MODY14	Very rare pathogenic heterozygous variants associated with MODY-like diabetes with incomplete penetrance. Best treatment not established	Only two families reported, with diabetes onset in third to fourth decades of life
<i>PDX1</i>	606392 / MODY4	Very rare pathogenic heterozygous variants (e.g., 188delC, Pro63fs, ClinVar variation ID 8857) associated with MODY-like diabetes with incomplete penetrance. Best treatment not established.	Nonpathogenic variants, or those of uncertain significance (VUS) are more common than pathogenic variants. Inheritance of rare pathogenic biallelic variants can cause NDM with pancreatic hypoplasia
Genes for which the evidence for causality remains uncertain			
<i>KLF11</i>	610508 / MODY7	The few published reports describe MODY-like patients who carry variants that are present in genomic databases at frequencies higher than expected for variants causing monogenic diabetes	
<i>PAX4</i>	612225 / MODY9		
<i>BLK</i>	613375 / MODY11		

MODY, Maturity-onset diabetes of the young; *PNDM*, permanent neonatal diabetes mellitus; *TNDM*, transient neonatal diabetes mellitus.

Note: If a variant is extremely rare or even novel, it does not necessarily mean it is causal. Allele frequency depends greatly on particular genes, as well as specific populations, but in general, any variant present in population databases at a frequency greater than or equal to 0.0001 (0.01%) is highly unlikely to be causal for a monogenic form of diabetes. In case of any uncertainty, consider consultation with experts familiar with monogenic diabetes genes and interrogation of genomic information (see Available Resources section).

note a history of hypoglycemia with the use of sulfonylurea drugs, even from the initial dose. The long-term sustainability of sulfonylurea treatment has not been conclusively demonstrated, but several studies report the superiority of sulfonylurea therapy compared with insulin, particularly early in treatment.¹³⁴ A few small studies, and some case reports, have suggested the benefit of additional medications, such as GLP-1 receptor agonists if sulfonylurea monotherapy is not sufficient.¹³⁵ Glinide medications have a shorter half-life and may allow for a lower risk of hypoglycemia in some circumstances.¹³⁶ There are excellent recent reviews that provide guidance on management of *HNF1A*- and *HNF4A*-MODY during pregnancy.^{133,137}

***HNF1B*: Maturity-Onset Diabetes of the Young With Renal Cysts (MODY5—RCAD)**

Heterozygous mutations or large deletions of the pancreatic beta cell transcription factor *HNF1B* are a relatively rare cause of MODY that are characterized by renal cysts and/or developmental defects of the genitourinary system and diabetes.

HNF1B mutations seem to have a somewhat lower penetrance for diabetes than other forms of MODY, so some family members may exhibit renal abnormalities without a diabetes diagnosis.

These patients do not exhibit a similar sensitivity to sulfonylurea therapy as *HNF1A/HNF4A*-MODY, so the best

treatment approach, while not established, may well be insulin. In one large recent study of 201 *HNF1B* patients, 49% were treated with insulin at diabetes diagnosis, and 79% were treated with insulin at follow-up.¹³⁸ Over half (57%) exhibited some responsiveness to sulfonylurea or repaglinide within the first few years of diabetes diagnosis; however, switching from insulin to sulfonylurea or repaglinide was only successful in 30% of patients. One case report described successful treatment of a 17-year-old patient with oral medications (sitagliptin and glimepiride) for 6 years, but thereafter insulin was required.¹³⁹

Other Forms of Monogenic Diabetes

Mitochondrial Diabetes

Mitochondrial genetic defects that cause diabetes are commonly but not invariably associated with neuromuscular disorders, including deafness, migraine, seizures, focal segmental glomerulosclerosis, and mental retardation. For example, the MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) syndrome may initially present in childhood with short stature, go on to deafness in teen years, and develop diabetes and encephalopathy in midlife. Diabetes mellitus may be the only (or early) manifestation of a mitochondrial disorder encoded by a gene defect within the mitochondria (all of which are maternally inherited) or a nuclear deoxyribonucleic acid (DNA)-encoded gene necessary to the oxidative phosphorylation sequence within mitochondria, which would be predicted to have more devastating effects because all mitochondria should be affected. The variability of the syndrome is caused by heteroplasmy, as the number of mitochondria with the mutation (usually encoding the mitochondrial transfer [t]RNA-leu) varies from a few percent and up, and there is a variation from tissue to tissue.

This defective energy pathway leads to progressive impairment of insulin secretion, and thus an initially mild hyperglycemia may progressively worsen. This is the case with the most common form of diabetes caused by a mitochondrial gene mutation at nucleotide pair (np) 3243 of the mitochondrial genome in the tRNA-leu, often associated with (low-frequency) deafness. Remarkably, this same genetic defect causing maternally inherited diabetes with deafness (MIDD) may then later on be associated with the MELAS syndrome. Initially, patients with np 3243 mutations can be controlled by diet alone but later may require insulin. Metformin has been used but because it may be a mild mitochondrial toxin, it may not be the most appropriate therapy.

Diabetes mellitus presenting in infancy and severe from the outset and requiring insulin may be associated with large mitochondrial DNA deletions, as seen in the Kearns-Sayre syndrome and Pearson syndrome.^{140,141}

WFS1: Wolfram Syndrome

Wolfram syndrome 1 (the classic form) is characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (previously termed *DIDMOAD*).^{142–144} There is a selective loss of functional beta cells, which is responsible for the diabetes mellitus. Genetic linkage studies in consanguineous families with autosomal-recessive inheritance led to the positional cloning of a gene on the short arm of chromosome 4 (termed *WFS1*, and now named *wolframin*).

WFS1 encodes an ER protein, which has functions including regulation of ER stress signaling and calcium homeostasis.¹⁴⁵ Loss-of-gene function is believed to result in progressive decline of endocrine and neurological function by inducing chronic ER stress-mediated apoptosis of pancreatic beta cells and neuronal cells.¹⁴⁶

WFS1 is expressed in many tissues (most abundantly in beta cells, compared with the exocrine pancreas), including the

brain. Mutations in wolframin have been identified in many families with Wolfram syndrome. Affected individuals are often compound heterozygotes, but homozygous cases will be more common in populations with higher rates of consanguineous unions. The *WFS1* gene may have a role in beta cell and neural tissue survival, and there does not appear to be a correlation between the observed mutation and severity of disease. Defects in wolframin have been implicated in the idiopathic common nonimmune form (antiislet antibody negative) of clinically suspected type 1 diabetes mellitus.¹⁴⁴ A second locus of Wolfram syndrome (*WFS2*) was mapped to the *CISD2* gene on chromosome 4 based on several consanguineous Jordanian families.¹⁴⁴ In these patients, diabetes insipidus was not a feature—but upper gastrointestinal bleeding and ulceration were prominent.

Although a mitochondrial form of Wolfram syndrome has been proposed, a defect in mitochondrial DNA could not be confirmed in one large cohort.¹⁴⁵ It has been suggested that diabetes mellitus (before age 15 years) and progressive optic atrophy are highly predictive of Wolfram syndrome. The sequence of appearance of the stigmata may be variable but most often nonautoimmune clinical “type 1 diabetes” will occur first, within the first decade of life, whereas optic atrophy will usually occur early in the second decade; central diabetes insipidus and sensorineural deafness (low frequency deafness is seen) in two-thirds to three-fourths of the patients within the second decade; renal tract anomalies in approximately half within the third decade; and neurological complications, such as cerebellar ataxia and myoclonus, in half to two-thirds by the fourth decade. Other features include primary gonadal atrophy in the majority of males and a progressive neurodegenerative course with neurorespiratory death at a median age of 30 years. Depression has been reported as a frequent feature of relatives of patients with Wolfram syndrome.¹⁴⁴ Genetic testing can be considered among the 5% to 10% of young people with clinical type 1 diabetes who will be antiislet antibody negative, but such testing is more urgently indicated in any such patient who also exhibits other features, such as hearing or vision loss.

SLC19A: Thiamine-Responsive Megaloblastic Anemia and Diabetes (Rogers Syndrome)

This syndrome is characterized by megaloblastic anemia, diabetes mellitus, and sensorineural deafness—all of which may respond to vitamin B₁ (thiamine). Diabetes mellitus is mild to moderate, insulin secretion may improve with thiamine therapy, and there are no associated autoimmune markers. It may be caused by a defect in the thiamine transporter,^{147,148} which has now been identified as being caused by mutations in the *SLC19A* gene. This gene encodes the membrane-bound thiamine transporter THTR1.

Uptake of thiamine by the combined pathways of an active high-affinity carrier and a passive low-affinity carrier leads to accumulation of intracellular thiamine, which is then converted to its active form, thiamine pyrophosphate. This cofactor enables proper function of transketolase, which is important in the pentose phosphate shunt, the key to ribose synthesis and hence nucleic acid production and of pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and branched chain acid dehydrogenase—all of which are key to oxidative decarboxylation. Mutations in the high-affinity transporter THTR1 lead to cell death in those cells that have a high rate of nucleic acid turnover (such as bone marrow cells) and activity (such as pancreatic beta cells), thereby explaining the association of thiamine-responsive anemia with diabetes in those affected by this mutation.

Other Forms of Monogenic Diabetes. With the advent of NGS platforms, other genes have also been rarely implicated in monogenic diabetes and the list is growing. Consultations with experts in the field are strongly recommended. Another

reason for seeking consultation is that many genetic reports contain listings of variants of uncertain significance (VUS). Although these may be disease-causing, more often they are not, and have not yet been reported. Experts in genetic analysis of monogenic diabetes can provide important information, and changes should never be made in treatment based on a reported VUS sequence alteration.

It is important to note that we have focused in this chapter on genes that result in beta cell defects. Other genes that control insulin action, such as the insulin receptor itself (*INSR*), and genes associated with lipodystrophy may also lead to diabetes. As the availability of NGS testing increases, additional genes associated with monogenic diabetes are likely to be found.

AVAILABLE RESOURCES

The following resources offer advice on diagnostic management of patients with known or suspected monogenic diabetes and may also be able to provide genetic testing free of charge through funding from national governmental agencies:

www.diabetesgenes.org (Exeter, United Kingdom)

<https://monogenicdiabetes.uchicago.edu> (Chicago, United States)

www.mody.no (Norway)

Resource for finding laboratories offering clinical testing:

<https://www.ncbi.nlm.nih.gov/gtr/> (NCBI Genetic Testing Registry)

TRANSITION TO ORAL THERAPY

1. K_{ATP} (*KCNJ11/ABCC8*) mutations. Once the diagnosis of a mutation in the K_{ATP} channel likely to respond to sulfonylurea is established, transition to oral treatment from insulin treatment may be best accomplished on an inpatient basis, where blood sugars can be monitored frequently, the dose of insulin reduced quickly, and the dose of sulfonylurea increased rapidly. Depending on the age of the patient, whole or half-tablets, or a suspension of glyburide prepared in a pharmacy may be needed to facilitate the swallowing of the medication. The starting dose is typically 0.2 mg/kg/day in two divided doses, but

this starting dose may be decreased by half in newborns who may be especially insulin-sensitive and responsive to treatment. Table 10.2 outlines the sequential steps to be taken in transitioning the patient and is based on recommendations from NDM centers in both the United Kingdom and the United States.

2. *HNF1A* and *HNF4A*. *HNF1A/4A* mutations often respond dramatically to low-dose sulfonylureas, especially in younger patients. A starting dose equivalent to 0.625 mg of glyburide is often sufficient to allow for rapid withdrawal of insulin (so as to avoid hypoglycemia). Gradual increase of sulfonylurea dose may be necessary over time, based on blood glucose monitoring and possibly C-peptide levels. Additional agents, such as GLP-1 receptor agonists, may be helpful if glycemic control remains nonoptimal at sulfonylurea doses at the higher range recommended for type 2 diabetes (*HNF1A/4A* should not require very high doses as needed by K_{ATP} mutation patients).

FUTURE DIRECTIONS

The discovery of the genes responsible for various forms of NDM and MODY has been spectacular in its impact on the diagnosis and treatment of affected patients and its relevance to understanding the global burden of diabetes mellitus. The same genetic defects that cause NDM and/or MODY have been found in parents or unrelated individuals considered to have T2DM or classic-appearing T1DM that is nonautoimmune in origin. Thus these discoveries have informed potential mechanisms by which insulin secretion is impaired at the level of pancreas formation and development, insulin synthesis, and insulin secretion. In addition, this offers the possibility to overcome the defect through the pharmacogenetics of the K_{ATP} channel in which activating mutations can be overcome by sulfonylurea treatment. However, the cause of diabetes occurring under 6 months of age remains unknown in about 15% of cases, and many others diagnosed after 6 months of age may not have typical autoimmune type 1 diabetes. Whole genome sequencing¹⁴⁹ along with improved understanding of noncoding regulatory regions may reveal the basis for these undefined syndromes in the near future.

BOX 10.1 Neonatal Diabetes Mellitus

1. Genetic testing will reveal a monogenic cause for diabetes occurring under 6 months of age in about 85% of cases
2. A monogenic cause will be found in a lower fraction of those diagnosed 6–12 months of age or even later, whereas autoimmune type 1 diabetes becomes increasingly more likely. Genetic testing should be considered if islet cell antibodies are negative, there is a strong family history of diabetes, and/or other syndromic features (e.g., congenital heart defects, developmental delay or multiple autoimmune problems) are present
3. Comprehensive and rapid genetic testing is essential to guide specific treatment, anticipate possible remission and familial recurrence risk, and assess associated syndromes
4. Many infants with NDM will enter a remission phase for a variable period of time (transient diabetes mellitus); of these, about 2/3 will be caused by overexpression of genes on 6q24 (see Fig. 10.3), about 1/3 caused by mutations in the K_{ATP} genes *ABCC8* or *KCNJ11*, and very rarely resulting from other gene causes
5. Permanent diabetes mellitus is most commonly caused by heterozygous mutations in *KCNJ11*, *INS*, and *ABCC8* (see Fig. 10.2) that are most often de novo but can be inherited in an autosomal dominant fashion
6. Rarer causes often include syndromic features, such as heterozygous *GATA6/GATA4* mutations causing pancreatic hypoplasia with congenital heart defects, x-linked *foxp3* mutations causing NDM with other autoimmune dysfunction, and a long list of very rare recessive gene causes (see Fig. 10.2)
7. The majority of patients with mutations in *KCNJ11/ABCC8* respond to high-dose oral sulfonylurea medications with improved endogenous insulin secretion, improved HbA1c and quality of life; neurodevelopmental improvement may also occur, especially when treatment starts early
8. Up to 66% of new cases of neonatal diabetes will present in diabetic ketoacidosis (less likely with 6q24-related diabetes)
9. Any baby with diabetes is at greater risk of developmental delay and should be referred for early assessment and provision of any needed therapies (speech, physical, occupational, developmental), as well as ongoing monitoring during childhood for behavioral or learning difficulties, as well as seizures. Certain gene causes (including the most common: *KCNJ11* AND *ABCC8*) affect brain development and function and require close monitoring and support

BOX 10.2 MODY

1. MODY constitutes about 3%–5% of childhood diabetes (about 0.4% of all diabetes) and should be suspected in those with any of these features (none of which are absolute):
 - A. Autosomal dominant family history of diabetes with individuals affected across 2 to 3 generations
 - B. Onset before age 25 to 35 years (usually) but also usually after 8 to 10 years of age
 - C. Absence of markers of anti-beta cell autoimmunity
 - D. Absence of indicators of insulin resistance, such as acanthosis nigricans, characteristic dyslipidemia and/or significant obesity (note: individuals with these features could still have MODY)
 - E. “Mild diabetes” with initial HbA1c <10%, absence of significant ketosis, persistently low insulin requirements with surprisingly good glycemic control even 3 to 5 years after diagnosis
2. GCK-MODY (MODY2) has a distinct phenotype in addition to the earlier points
 - A. Mild, persistent, nonprogressive, fasting hyperglycemia (often 110–140 mg/dL)
 - B. HbA1c rarely in excess of 7.5%
 - C. Extremely low risk of any hyperglycemia-related complications
 - D. GCK-MODY does not require any treatment (not even diet modification)
 - E. The one exception is that treatment may be considered under certain circumstances during pregnancy (to try to prevent macrosomia in unaffected fetus)
 - F. Fasting hyperglycemia also present in children as this is a life-long condition
 - G. Caused by dominant mutations in glucokinase (GCK), with population frequency around 0.1%
3. *HNF1A* / *HNF4A* MODY (MODY3/MODY1)
 - A. Frequently respond to low doses of sulfonylurea (SU) drugs (less so with *HNF4A*)
 - B. Greater SU responsiveness (even hypoglycemia) earlier in the course of the disease
 - C. Beta cell dysfunction is characterized by insufficient insulin secretion and is progressive
 - D. Eventually additional hypoglycemic medications may be required (SGLT2 inhibitors may be contraindicated)
 - E. All diabetes-related complications may occur, dependent on metabolic control
 - F. Lipid profile better than in type 2 diabetes, but still at higher cardiovascular risk than unaffected relatives
4. Rarer forms of MODY are listed in Table 10.3 and include other causes for which treatment could change, such as *KCNJ11* and *ABCC8* that often respond to higher doses of SU drugs

MODY, Maturity-onset diabetes of the young; SGLT2, sodium glucose transport protein 2.

REFERENCES

1. Ehtisham S, Hattersley A, Dunger D, Barrett T. First UK survey of paediatric type 2 diabetes and MODY. *Arch Dis Child.* 2004; 89(6):526.
2. Fendler W, Borowiec M, Baranowska-Jazwiecka A, Szadkowska A, Skala-Zamorowska E, Deja G, et al. Prevalence of monogenic diabetes amongst Polish children after a nationwide genetic screening campaign. *Diabetologia.* 2012;55(10):2631–2635.
3. Irgens H, Molnes J, Johansson B, Ringdal M, Skrivarhaug T, Undlien D, et al. Prevalence of monogenic diabetes in the population-based Norwegian Childhood Diabetes Registry. *Diabetologia.* 2013;56(7):1512–1519.
4. Pihoker C, Gilliam LK, Ellard S, Dabelea D, Davis C, Dolan LM, et al. Prevalence, characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in *HNF1A*, *HNF4A*, and glucokinase: results from the SEARCH for diabetes in youth. *J Clin Endocrinol Metab.* 2013;98(10):4055–4062.
5. Shepherd M, Shields B, Hammersley S, Hudson M, McDonald TJ, Coldough K, et al. Systematic population screening, using biomarkers and genetic testing, identifies 2.5% of the U.K. pediatric diabetes population with monogenic diabetes. *Diabetes Care.* 2016;39(11):1879–1888.
6. Shankar R, Pihoker C, Dolan LM, Standiford D, Badaru A, Dabelea D, et al. Permanent neonatal diabetes mellitus: prevalence and genetic diagnosis in the SEARCH for Diabetes in Youth Study. *Pediatr Diabetes.* 2013;14(3):174–180.
7. von Mühlendahl K, Herkenhoff H. Long-term course of neonatal diabetes. *N Engl J Med.* 1995;333(11):704–708.
8. Stoy J, Greeley SW, Paz VP, Ye H, Pastore AN, Skowron KB, et al. Diagnosis and treatment of neonatal diabetes: a United States experience. *Pediatr Diabetes.* 2008;9(5):450–459.
9. Polak M, Dechaume A, Cavé H, Nimri R, Crosnier H, Sulmont V, et al. Heterozygous missense mutations in the insulin gene are linked to permanent diabetes appearing in the neonatal period or in early infancy: a report from the French ND (Neonatal Diabetes) Study Group. *Diabetes.* 2008;57(4):1115–1119.
10. Rubio-Cabezas O, Flanagan SE, Damhuis A, Hattersley AT, Ellard S. KATP channel mutations in infants with permanent diabetes diagnosed after 6 months of life. *Pediatr Diabetes.* 2012; 13(4):322–325.
11. Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, et al. Activating mutations in the *ABCC8* gene in neonatal diabetes mellitus. *N Engl J Med.* 2006;355(5):456–466.
12. Bowman P, Flanagan S, Edghill E, Damhuis A, Shepherd M, Paisey R, et al. Heterozygous *ABCC8* mutations are a cause of MODY. *Diabetologia.* 2012;55(1):123–127.
13. Rieck S, Bankaitis ED, Wright C. Lineage determinants in early endocrine development. *Semin Cell Dev Biol.* 2012;23(6):673–684.
14. Wilson ME, Scheel D, German MS. Gene expression cascades in pancreatic development. *Mechanism Dev.* 2003;120(1):65–80.
15. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to β -cells. *Nature.* 2008;455(7213):627.
16. Zhou Q, Melton DA. Pancreas regeneration. *Nature.* 2018; 557:351–358.
17. Pearson ER, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B, et al. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med.* 2006;355(5):467–477.
18. Sperling MA. ATP-sensitive potassium channels — neonatal diabetes mellitus and beyond. *N Engl J Med.* 2006;355(5):507–510.
19. Babiker T, Vedovato N, Patel K, Thomas N, Finn R, Männikkö R, et al. Successful transfer to sulfonylureas in *KCNJ11* neonatal diabetes is determined by the mutation and duration of diabetes. *Diabetologia.* 2016;59(6):1162–1166.
20. Habeb AM, Al-Magamsi MS, Eid IM, Ali MI, Hattersley AT, Hussain K, et al. Incidence, genetics, and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. *Pediatr Diabetes.* 2012;13(6):499–505.
21. Grulich-Henn J, Wagner V, Thon A, Schober E, Marg W, Kapellen T, et al. Entities and frequency of neonatal diabetes: data from the diabetes documentation and quality management system (DPV). *Diabetic Med.* 2010;27(6):709–712.
22. Iafusco D, Massa O, Pasquino B, Colombo C, Iughetti L, et al. Minimal incidence of neonatal/infancy onset diabetes in Italy is 1:90,000 live births. *Acta Diabetologica.* 2011;49(5):405–408.
23. Slingerland A, Shields B, Flanagan S, Bruining G, Noordam K, Gach A, et al. Referral rates for diagnostic testing support an incidence of permanent neonatal diabetes in three European

- countries of at least 1 in 260,000 live births. *Diabetologia*. 2009;52(8):1683–1685.
24. Sperling MA. Neonatal diabetes mellitus: from understudy to center stage. *Curr Opin Pediatr*. 2005;17(4):512.
 25. Docherty L, Kabwama S, Lehmann A, Hawke E, Harrison L, Flanagan S, et al. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in an international cohort of patients. *Diabetologia*. 2013;56(4):758–762.
 26. Letourneau LR, Carmody D, Wroblewski K, Denson AM, Sanyour M, Naylor RN, et al. Diabetes presentation in infancy: high risk of diabetic ketoacidosis. *Diabetes Care*. 2017;40:e147–e148.
 27. Uzel G, Sampaio EP, Lawrence MG, Hsu AP, Hackett M, Dorsey MJ, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol*. 2013;131(6):1611–1623.
 28. Flanagan SE, Haapaniemi E, Russell MA, Caswell R, Allen L, De Franco E, et al. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat Genet*. 2014;46(8):812–814.
 29. Johnson MB, De Franco E, Allen H, Senani A, Elbarbary N, Siklar Z, et al. Recessively inherited LRBA mutations cause autoimmunity presenting as neonatal diabetes. *Diabetes*. 2017;66(8):2316–2322.
 30. Roth TL, Puig-Saus C, Yu R, Shifrut E, Carnevale J, Li JP, et al. Reprogramming human T cell function and specificity with non-viral genome targeting. *Nature*. 2018;559:405–409.
 31. De Franco E, Flanagan SE, Houghton JA, Allen H, Mackay DJ, Temple KI, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet*. 2015;386(9997):957–963.
 32. Hattersley AT, Greeley SA, Polak M, Rubio-Cabezas O, Njølstad PR, Mlynarski W, et al. ISPAD Clinical Practice Consensus Guidelines 2018: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2018;19:47–63.
 33. Abdollahi A. PLAGL1 (pleomorphic adenoma gene-like 1). *Atlas Genet Cytogenet Oncol Haematol*. 2008;12(2):697–700.
 34. Du X, Ounissi-Benkhalha H, Loder MK, Rutter GA, Polychronakos C. Overexpression of ZAC impairs glucose-stimulated insulin translation and secretion in clonal pancreatic beta-cells. *Diabetes Metab Res Rev*. 2012;28(8):645–653.
 35. Ma D, Shield JP, Dean W, Leclerc I, et al. Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. *J Clin Invest*. 2004;114(3):339–348.
 36. Hattersley AT. Unlocking the secrets of the pancreatic beta cell: man and mouse provide the key. *J Clin Invest*. 2004;114(3):314–316.
 37. Du X, Rousseau M, Ounissi-Benkhalha H, Marchand L, Jetha A, Paraskevas S, et al. Differential expression pattern of ZAC in developing mouse and human pancreas. *J Mol Histol*. 2011;42(2):129–136.
 38. Mackay D, Temple KI. Transient neonatal diabetes mellitus type 1. *Am J Med Genet C Semin Med Genet*. 2010;154C(3):335–342.
 39. Mackay DJ, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, et al. Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. *Nat Genet*. 2008;40(8):949–951.
 40. Blik J, Verde G, Callaway J, Maas SM, de Crescenzo A, Sparago A, et al. Hypomethylation at multiple maternally methylated imprinted regions including PLAGL1 and GNAS loci in Beckwith-Wiedemann syndrome. *Eur J Hum Genet*. 2009;17(5):611–619.
 41. Flanagan S, Mackay D, Greeley SAW, McDonald TJ, Mericq V, Hassing J, et al. Hypoglycaemia following diabetes remission in patients with 6q24 methylation defects: expanding the clinical phenotype. *Diabetologia*. 2013;56(1):218–221.
 42. Garcin L, Kariyawasam D, Busiah K, Fauret-Amsellem A-L, Bourgeois F, Vaivre-Douret L, et al. Successful off-label sulfonylurea treatment of neonatal diabetes mellitus due to chromosome 6 abnormalities. *Pediatr Diabetes*. 2018;19(3):197–199.
 43. Carmody D, Bell CD, Hwang JL, Dickens JT, Sima DI, Felipe DL, et al. Sulfonylurea treatment before genetic testing in neonatal diabetes: pros and cons. *J Clin Endocrinol Metab*. 2014;99:E2709–E2714.
 44. Murphy R, Ellard S, Hattersley AT. Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. *Nat Clin Pract Endocrinol Metab*. 2008;4(4):200–213.
 45. Garin I, Edghill E, Akerman I, Rubio-Cabezas O, Rica I, Locke J, et al. Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc Natl Acad Sci*. 2010;107(7):3105–3110.
 46. Yorifuji T, Kurokawa K, Mamada M, Imai T, Kawai M, Nishi Y, et al. Neonatal diabetes mellitus and neonatal polycystic, dysplastic kidneys: phenotypically discordant recurrence of a mutation in the hepatocyte nuclear factor-1beta gene due to germline mosaicism. *J Clin Endocrinol Metab*. 2004;89(6):2905–2908.
 47. Sansbury F, Flanagan S, Houghton J, Shen SF, Al-Senani A, Habebe A, et al. SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion. *Diabetologia*. 2012;55(9):2381–2385.
 48. Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest*. 2005;115(8):2047–2058.
 49. Gloy AL, Pearson ER, Antcliff JF, Proks P, Bruining JG, Slingerland AS, et al. Activating mutations in the gene encoding the atp-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med*. 2004;350(18):1838–1849.
 50. Thurber BW, Carmody D, Tadie EC, Pastore AN, Dickens JT, Wroblewski KE, et al. Age at the time of sulfonylurea initiation influences treatment outcomes in KCNJ11-related neonatal diabetes. *Diabetologia*. 2015;58(7):1430–1435.
 51. Greeley SW, John PM, Winn AN, Ornelas J, Lipton RB, Philipson LH, et al. The cost-effectiveness of personalized genetic medicine. *Diabetes Care*. 2011;34(3):622–627.
 52. Lanning MS, Carmody D, Szczerbiński Ł, Letourneau LR, Naylor RN, Greeley SW. Hypoglycemia in sulfonylurea-treated KCNJ11-neonatal diabetes: Mild-moderate symptomatic episodes occur infrequently but none involving unconsciousness or seizures. *Pediatr Diabetes*. 2018;19(3):393–397.
 53. Girard CA, Wunderlich TF, Shimomura K, Collins S, Kaizik S, Proks P, et al. Expression of an activating mutation in the gene encoding the KATP channel subunit Kir6.2 in mouse pancreatic beta cells recapitulates neonatal diabetes. *J Clin Invest*. 2009;119(1):80–90.
 54. Clark RH, McTaggart JS, Webster R, Mannikko R, Iberl M, Sim X, et al. Muscle dysfunction caused by a KATP channel mutation in neonatal diabetes is neuronal in origin. *Science*. 2010;329(5990):458–461.
 55. Fan X, Ding Y, Cheng H, Gram DX, Sherwin RS, McCrimmon RJ. Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K⁺ channel opener? *Diabetes*. 2008;57(12):3327–3334.
 56. Park Y, Choi Y, Park S, Kim J, Kim S, Song D, et al. ATP-sensitive potassium channel-deficient mice show hyperphagia but are resistant to obesity. *Diabetes Metab J*. 2011;35(3):219–225.
 57. Koster JC, Cadario F, Peruzzi C, Colombo C, Nichols CG, Barbetti F. The G53D mutation in Kir6.2 (KCNJ11) is associated with neonatal diabetes and motor dysfunction in adulthood that is improved with sulfonylurea therapy. *J Clin Endocrinol Metab*. 2008;93(3):1054–1061.
 58. Clark R, Proks P. Advances in experimental medicine and biology. *Adv Exp Med Biol*. 2010;654:165–192.
 59. Gloy AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, et al. Relapsing diabetes can result from moderately activating mutations in KCNJ11. *Hum Mol Genet*. 2005;14(7):925–934.
 60. Flanagan SE, Patch A-M, Mackay D, Edghill EL, Gloy AL, Robinson D, et al. Mutations in ATP-sensitive K⁺ channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. *Diabetes*. 2007;56(7):1930–1937.
 61. Shimomura K, Flanagan SE, Zadek B, Lethby M, Zubcevic L, Girard CA, et al. Adjacent mutations in the gating loop of Kir6.2 produce neonatal diabetes and hyperinsulinism. *EMBO Mol Med*. 2009;1(3):166–177.
 62. de Wet H, Proks P, Lafond M, Aittoniemi J, Sansom MS, Flanagan SE, et al. A mutation (R826W) in nucleotide-binding domain 1 of ABCC8 reduces ATPase activity and causes transient neonatal diabetes. *EMBO Rep*. 2008;9(7):648–654.
 63. Proks P, Arnold AL, Bruining J, Girard C, Flanagan SE, et al. A heterozygous activating mutation in the sulfonylurea receptor SUR1

- (ABCC8) causes neonatal diabetes. *Hum Mol Genet.* 2006;15(11):1793–1800.
64. Patch A, Flanagan S, Boustred C, Hattersley A, Ellard S. Mutations in the ABCC8 gene encoding the SUR1 subunit of the KATP channel cause transient neonatal diabetes, permanent neonatal diabetes or permanent diabetes diagnosed outside the neonatal period. *Diabetes Obes Metab.* 2007;9(s2):28–39.
 65. Männikkö R, Flanagan SE, Sim X, Segal D, Hussain K, Ellard S, et al. Mutations of the same conserved glutamate residue in NBD2 of the sulfonylurea receptor 1 subunit of the KATP channel can result in either hyperinsulinism or neonatal diabetes. *Diabetes.* 2011;60(6):1813–1822.
 66. Slingerland AS, Hurkx W, Noordam K, Flanagan S, Jukema J. Sulfonylurea therapy improves cognition in a patient with the V59M KCNJ11 mutation. *Diabetic Med.* 2008;25(3):277–281.
 67. Slingerland AS, Nuboer R, Hadders-Algra M, Hattersley A, Bruining G. Improved motor development and good long-term glycaemic control with sulfonylurea treatment in a patient with the syndrome of intermediate developmental delay, early-onset generalised epilepsy and neonatal diabetes associated with the V59M mutation in the. *Diabetologia.* 2006;49(11):2559–2563.
 68. Shah RP, Spruyt K, Kragie BC, Greeley SW, Msall ME. Visuomotor performance in KCNJ11-related neonatal diabetes is impaired in children with DEND-associated mutations and may be improved by early treatment with sulfonylureas. *Diabetes Care.* 2012;35(10):2086–2088.
 69. Busiah K, Drunat S, Vaivre-Douret L, Bonnefond A, Simon A, Flechtner I, et al. Neuropsychological dysfunction and developmental defects associated with genetic changes in infants with neonatal diabetes mellitus: a prospective cohort study. *Lancet Diabetes Endocrinol.* 2013;1(3):199–207.
 70. Beltrand J, Elie C, Busiah K, Fournier E, Boddart N, Bahi-Buisson N, et al. Sulfonylurea therapy benefits neurological and psychomotor functions in patients with neonatal diabetes owing to potassium channel mutations. *Diabetes Care.* 2015;38(11):2033–2041.
 71. Landmeier KA, Lanning M, Carmody D, Greeley SW, Msall ME. ADHD, learning difficulties and sleep disturbances associated with KCNJ11-related neonatal diabetes. *Pediatr Diabetes.* 2017;18(7):518–523.
 72. Carmody D, Pastore A, Landmeier K, Letourneau L, Martin R, Hwang J, et al. Patients with KCNJ11-related diabetes frequently have neuropsychological impairments compared with sibling controls. *Diabetic Med.* 2016;33(10):1380–1386.
 73. Rowman P, Broadbridge E, Knight B, Pettit L, Flanagan S, Reville M, et al. Psychiatric morbidity in children with KCNJ11 neonatal diabetes. *Diabetic Med.* 2016;33(10):1387–1391.
 74. Bowman P, Sulen Å, Barbetti F, Beltrand J, Svalastoga P, Codner E, et al. Effectiveness and safety of long-term treatment with sulfonylureas in patients with neonatal diabetes due to KCNJ11 mutations: an international cohort study. *Lancet Diabetes Endocrinol.* 2018;6(8):637–646.
 75. Stoy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, et al. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci U S A.* 2007;104(38):15040–15044.
 76. Glaser B. Insulin mutations in diabetes: the clinical spectrum. *Diabetes.* 2008;57(4):799–800.
 77. Edghill EL, Flanagan SE, Patch A-M, Boustred C, Parrish A, Shields B, et al. Insulin mutation screening in 1,044 patients with diabetes. *Diabetes.* 2008;57(4):1034–1042.
 78. Meur G, Simon A, Harun N, Virally M, Dechaume A, Bonnefond A, et al. Insulin gene mutations resulting in early-onset diabetes: marked differences in clinical presentation, metabolic status, and pathogenic effect through endoplasmic reticulum retention. *Diabetes.* 2010;59(3):653–661.
 79. Molven A, Ringdal M, Nordbø AM, Raeder H, Støy J, Lipkind GM, et al. Mutations in the insulin gene can cause MODY and autoantibody-negative type 1 diabetes. *Diabetes.* 2008;57(4):1131–1135.
 80. Colombo C, Porzio O, Liu M, Massa O, Vasta M, Salardi S, et al. Seven mutations in the human insulin gene linked to permanent neonatal/infancy-onset diabetes mellitus. *J Clin Invest.* 2008;118(6):2148–2156.
 81. Ortolani F, Piccinno E, Grasso V, Papadia F, Panzeca R, Cortese C, et al. Diabetes associated with dominant insulin gene mutations: outcome of 24-month, sensor-augmented insulin pump treatment. *Acta Diabetol.* 2015;1–3.
 82. Marin MT, Coffey ML, Beck JK, Dasari PS, Allen R, Krishnan S. A novel approach to the management of neonatal diabetes using sensor-augmented insulin pump therapy with threshold suspend technology at diagnosis. *Diabetes Spectrum.* 2016;29(3):176–179.
 83. Letourneau LR, Carmody D, Philipson LH, Greeley SW. Early intensive insulin use may preserve beta-cell function in neonatal diabetes due to mutations in the proinsulin gene. *J Endocr Soc.* 2017;2(1):1–8.
 84. Njolstad P, Sovik O, Cuesta-Munoz A, Bjorkhaug L, Massa O, Barbetti F, et al. Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med.* 2001;344(21):1588–1592.
 85. Njolstad PR, Sagen JV, Bjorkhaug L, Odili S, Shehadeh N, Bakry D, et al. Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. *Diabetes.* 2003;52(11):2854–2860.
 86. Porter JR, Shaw NJ, Barrett TG, Hattersley AT, Ellard S, Gloyn AL. Permanent neonatal diabetes in an Asian infant. *J Pediatr.* 2005;146(1):131–133.
 87. Rubio-Cabezas O, González DF, Aragonés A, Argente J, Campos-Barros A. Permanent neonatal diabetes caused by a homozygous nonsense mutation in the glucokinase gene. *Pediatr Diabetes.* 2008;9(3 Pt 1):245–249.
 88. Bennett K, James C, Mutair A, Al-Shaikh H, Sinani A, Hussain K. Four novel cases of permanent neonatal diabetes mellitus caused by homozygous mutations in the glucokinase gene. *Pediatr Diabetes.* 2011;12(3pt1):192–196.
 89. Russo L, Iafusco D, Brescianini S, Nocerino V, Bizzarri C, Toni S, et al. Permanent diabetes during the first year of life: multiple gene screening in 54 patients. *Diabetologia.* 2011;54(7):1693–1701.
 90. Habeb AM, Flanagan SE, Deeb A, Al-Alwan I, Alawneh H, Balafrej AA, et al. Permanent neonatal diabetes: different aetiology in Arabs compared to Europeans. *Arch Dis Child.* 2012;97(8):721–723.
 91. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet.* 1997;15(1):106–110.
 92. Schwitzgebel VM, Mamin A, Brun T, Ritz-Laser B, Zaiko M, Maret A, et al. Agenesis of human pancreas due to decreased half-life of insulin promoter factor 1. *J Clin Endocrinol Metab.* 2003;88(9):4398–4406.
 93. Thomas IH, Saini NK, Adhikari A, Lee JM, Kasa-Vubu JZ, Vazquez DM, et al. Neonatal diabetes mellitus with pancreatic agenesis in an infant with homozygous IPF-1 Pro63fsX60 mutation. *Pediatr Diabetes.* 2009;10(7):492–496.
 94. De Franco E, Shaw-Smith C, Flanagan S, Edghill E, Wolf J, Otte V, et al. Biallelic PDX1 (insulin promoter factor 1) mutations causing neonatal diabetes without exocrine pancreatic insufficiency. *Diabetic Med.* 2013;30(5):e197–e200.
 95. Sellick GS, Barker KT, Stolte-Dijkstra I, Fleischmann C, Coleman RJ, Garrett C, et al. Mutations in PTF1A cause pancreatic and cerebellar agenesis. *Nat Genet.* 36(12), 1301–1305.
 96. Pinney SE, Oliver-Krasinski J, Ernst L, Hughes N, Patel P, Stoffers DA, et al. Neonatal diabetes and congenital malabsorptive diarrhea attributable to a novel mutation in the human neurogenin-3 gene coding sequence. *J Clin Endocrinol Metab.* 2011;96(7):1960–1965.
 97. Bonnefond A, Sand O, Guerin B, Durand E, Graeve DF, Huyvaert M, et al. GATA6 inactivating mutations are associated with heart defects and, inconsistently, with pancreatic agenesis and diabetes. *Diabetologia.* 2012;55(10):2845–2847.
 98. Haldorsen IS, Ræder H, Vesterhus M, Molven A, Njolstad PR. The role of pancreatic imaging in monogenic diabetes mellitus. *Nat Rev Endocrinol.* 2012;8(3):148.
 99. Rubio-Cabezas O, Patch A-M, Minton JA, Flanagan SE, Edghill EL, Hussain K, et al. Wolcott-Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. *J Clin Endocrinol Metab.* 2009;94(11):4162–4170.
 100. Julier C, Nicolino M. Wolcott-Rallison syndrome. *Orphanet J Rare Dis.* 2010;5(1):29.

101. Reis AF, Kannengiesser C, Jennane F, Manna T, Cheurfa N, Oudin C, et al. Two novel mutations in the EIF2AK3 gene in children with Wolcott-Rallison syndrome. *Pediatr Diabetes*. 2011;12(3 Pt 1):187–191.
102. d'Hennezel E, Dhuban K, Torgerson T, Piccirillo C. The immunogenetics of immune dysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet*. 2012;49(5):291–302.
103. Barzagli F, Passerini L, Bacchetta R. Immune dysregulation, polyendocrinopathy, enteropathy, x-linked syndrome: a paradigm of immunodeficiency with autoimmunity. *Front Immunol*. 2012;3:211.
104. Suscovich TJ, Perdue NR, Campbell DJ. Type-1 immunity drives early lethality in scurfy mice. *Eur J Immunol*. 2012;42(9):2305–2310.
105. Johnson MB, De Franco E, Allen H, Senani A, Elbarbary N, Siklar Z, et al. Recessively inherited LRBA mutations cause autoimmunity presenting as neonatal diabetes. *Diabetes*. 2017;66(8):2316–2322.
106. Garg G, Tyler JR, Yang JH, Cutler AJ, Downes K, Pekalski M, et al. Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. *J Immunol*. 2012;188(9):4644–4653.
107. Taha D, Barbar M, Kanaan H, Balfe J. Neonatal diabetes mellitus, congenital hypothyroidism, hepatic fibrosis, polycystic kidneys, and congenital glaucoma: a new autosomal recessive syndrome? *Am J Med Genet A*. 2003;122A(3):269–273.
108. Senée V, Chelala C, Duchatelet S, Feng D, Blanc H, Cossec JC, et al. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. *Nat Genet*. 2006;38(6):682–687.
109. Dimitri P, Warner J, Minton J, Patch A, Ellard S, Hattersley A, et al. Novel GLIS3 mutations demonstrate an extended multisystem phenotype. *Eur J Endocrinol*. 2011;164(3):437–443.
110. Yang Y, Chang B, Chan L. Sustained expression of the transcription factor GLIS3 is required for normal beta cell function in adults. *EMBO Mol Med*. 2013;5(1):92–104.
111. Watanabe N, Hiramatsu K, Miyamoto R, Yasuda K, Suzuki N, Oshima N, et al. A murine model of neonatal diabetes mellitus in Glis3-deficient mice. *FEBS Letters*. 2009;583(12):2108–2113.
112. Consortium T, Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*. 2009;41(6):703–707.
113. Rees SD, Hydrie ZM, O'Hare PJ, Kumar S, Shera SA, Basit A, et al. Effects of 16 genetic variants on fasting glucose and type 2 diabetes in south asians: ADCY5 and GLIS3 variants may predispose to type 2 diabetes. *Plos One*. 2011;6(9):e24710.
114. Smith SB, Qu H-Q, Taleb N, Kishimoto NY, Scheel DW, Lu Y, et al. Rfx6 directs islet formation and insulin production in mice and humans. *Nature*. 2010;463(7282):775.
115. Mitchell J, Punthakee Z, Lo B, Bernard C, Chong K, Newman C, et al. Neonatal diabetes, with hypoplastic pancreas, intestinal atresia and gall bladder hypoplasia: search for the aetiology of a new autosomal recessive syndrome. *Diabetologia*. 2004;47(12):2160–2167.
116. Spiegel R, Dobbie A, Hartman C, de Vries L, Ellard S, Shalev SA. Clinical characterization of a newly described neonatal diabetes syndrome caused by RFX6 mutations. *Am J Med Genet A*. 2011;155(11):2821–2825.
117. Flanagan SE, De Franco E, Allen H, Zerah M, Abdul-Rasoul MM, Edge JA, et al. Analysis of transcription factors key for mouse pancreatic development establishes NKX2-2 and MNX1 mutations as causes of neonatal diabetes in man. *Cell Metab*. 2014;19(1):146–154.
118. Wang J, Cortina G, Wu VS, Tran R, Cho J-H, Tsai M-J, et al. Mutant neurogenin-3 in congenital malabsorptive diarrhea. *N Engl J Med*. 2006;355(3):270–280.
119. Xuan S, Borok MJ, Decker KJ, Battle MA, Duncan SA, Hale MA, et al. Pancreas-specific deletion of mouse Gata4 and Gata6 causes pancreatic agenesis. *J Clin Invest*. 2012;122(10):3516–3528.
120. Carrasco M, Delgado I, Soria B, Martín F, Rojas A. GATA4 and GATA6 control mouse pancreas organogenesis. *J Clin Invest*. 2012;122(10):3504–3515.
121. Rodríguez-Seguí S, Akerman I, Ferrer J. GATA believe it: new essential regulators of pancreas development. *J Clin Invest*. 2012;122(10):3469–3471.
122. Allen H, Flanagan SE, Shaw-Smith C, De Franco E, Akerman I, Caswell R, et al. GATA6 haploinsufficiency causes pancreatic agenesis in humans. *Nat Genet*. 2012;44(1):20.
123. De Franco E, Shaw-Smith C, Flanagan SE, Shepherd MH, Consortium I, Hattersley A, et al. GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes*. 2013;62(3):993–997.
124. Shaw-Smith C, De Franco E, Allen LH, Battle M, Flanagan S, Borowiec M, et al. GATA4 Mutations are a cause of neonatal and childhood-onset diabetes. *Diabetes*. 2014;63(8):2888–2894.
125. Olinder A, Kernell A, Smide B. Treatment with CSII in two infants with neonatal diabetes mellitus. *Pediatr Diabetes*. 2006;7(5):284–288.
126. Vaxillaire M, Froguel P. Monogenic diabetes: implementation of translational genomic research towards precision medicine. *J Diabetes*. 2016;8(6):782–795.
127. Fajans S, Bell G, Polonsky K. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*. 2001;345(13):971–980.
128. Byrne M, Sturis J, Clément K, Vionnet N, Pueyo M, Offel, et al. Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations. *J Clin Invest*. 1994;93(3):1120–1130.
129. Velho G, Froguel P, Clément K, Pueyo ME, Zouali H, Cohen D, et al. Primary pancreatic beta-cell secretory defect caused by mutations in glucokinase gene in kindreds of maturity onset diabetes of the young. *Lancet*. 1992;340(8817):444–448.
130. Amed S, Oram R. Maturity-onset diabetes of the young (MODY): making the right diagnosis to optimize treatment. *Can J Diabetes*. 2016;40(5):449–454.
131. Steele AM, Shields BM, Wensley KJ, Colclough K, Ellard S, Hattersley AT. Prevalence of vascular complications among patients with glucokinase mutations and prolonged, mild hyperglycemia. *JAMA*. 2014;311(3):279–286.
132. Chakera AJ, Spyer G, Vincent N, Ellard S, Hattersley AT, Dunne FP. The 0.1% of the population with glucokinase monogenic diabetes can be recognized by clinical characteristics in pregnancy: the atlantic diabetes in pregnancy cohort. *Diabetes Care*. 2014;37(5):1230–1236.
133. Dickens LT, Naylor RN. Clinical management of women with monogenic diabetes during pregnancy. *Curr Diabetes Rep*. 2018;18(3):12.
134. Raile K, Schober E, Konrad K, Thon A, Grulich-Henn J, Meissner T, et al. Treatment of young patients with HNF1A mutations (HNF1A-MODY). *Diabetic Med*. 2015;32(4):526–530.
135. Østoft SH, Bagger JL, Hansen T, Pedersen O, Faber J, Holst JJ, et al. Glucose-lowering effects and low risk of hypoglycemia in patients with maturity-onset diabetes of the young when treated with a GLP-1 receptor agonist: a double-blind, randomized, crossover trial. *Diabetes Care*. 2014;37(7):1797–1805.
136. Becker M, Galler A, Raile K. Meglitinide analogues in adolescent patients with HNF1A-MODY (MODY 3). *Pediatrics*. 2014;133(3):775–779.
137. Shepherd M, et al. Management of sulfonylurea-treated monogenic diabetes in pregnancy: implications of placental glibenclamide transfer. *Diabetic Med*. 2017;34:1332–1339.
138. Dubois-Laforgue D, et al. Diabetes, associated clinical spectrum, long-term prognosis, and genotype/phenotype correlations in 201 adult patients with hepatocyte nuclear factor 1B (HNF1B) molecular defects. *Diabetes Care*. 2017;40:1436–1443.
139. Carrillo E, Lomas A, Pinés P, Lamas C. Long-lasting response to oral therapy in a young male with monogenic diabetes as a part of HNF1B-related disease. *Endocrinol Diabetes Metab Case Rep*. 2017;1.
140. Ho J, Pacaud D, Rakic M, Khan A. Diabetes in pediatric patients with Kearns-Sayre syndrome: clinical presentation of 2 cases and a review of pathophysiology. *Can J Diabetes*. 2014;225–228.
141. Williams TB, Daniels M, Puthenveetil G, Chang R, et al. Pearson syndrome: unique endocrine manifestations including Neonatal Diabetes and adrenal insufficiency. *Mol Genet Metab*. 2012;106:104–107.
142. Khanim F, Kirk J, Latif F, Barrett TG. WFS1/Wolframin mutations, Wolfram syndrome, and associated diseases. *Hum Mutat*. 2001;17:357–367.

143. Barrett TG, Scott-Brown M, Seller A, et al. The mitochondrial genome in Wolfram syndrome. *J Med Genet.* 2000;37:463–466.
144. López de Heredia M, Clères R, Nunes V. Genotypic classification of patients with Wolfram syndrome: insights into the natural history of the disease and correlation with phenotype. *Genet Med.* 2013;15(7):497–506.
145. Rigoli L, Lombardo F, Di Bella C. Wolfram syndrome and WFS1 gene. *Clin Genet.* 2011;79:103–117.
146. Rohayem J, Ehlers C, Wiedemann B, et al. Diabetes and neurodegeneration in Wolfram Syndrome: a multicenter study of phenotype and genotype. *Diabetes Care.* 2011;34:1503–1510.
147. Labay V, Raz T, Baron D, et al. Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nature.* 1999;22:300–304.
148. Olsen BS, Hahnemann JMD, Schwartz M, Østergaard E. Thiamine-responsive megaloblastic anaemia: a cause of syndromic diabetes in childhood. *Pediatr Diabetes.* 2007;8:239–241.
149. Bonnefond A, Durand E, Sand O, De Graeve F, et al. Molecular diagnosis of neonatal diabetes mellitus using next-generation sequencing of the whole exome. *PLoS One.* 2010;5(10). e13630.

11 Disorders of Childhood Growth

Alexander A.L. Jorge, Adda Grimberg, Mehul T. Dattani, Jeffrey Baron

CHAPTER OUTLINE

INTRODUCTION	299
CHILDHOOD GROWTH	299
The Biological Basis of Linear Growth	299
Regulation of Linear Growth	299
Clinical Evaluation of Growth	310
DISORDERS OF CHILDHOOD GROWTH	314
Short Stature	314
Tall Stature	342
References	347

INTRODUCTION

Human growth is an astonishing process. Its beginnings are intertwined with the enormously intricate mechanisms that transform a single cell into a complex embryo. Once formed, the human fetus and then child continue to grow over the course of approximately 15 years, reaching a body mass more than 10,000,000 times greater than that of the zygote from which the child originated. The early growth rate is enormous. The fetus grows in length at more than 100 cm per year, but, by birth, the linear growth rate has slowed to 50 cm per year and by midchildhood to 5 cm per year. This decline in the growth rate is briefly interrupted by the pubertal growth spurt but then resumes, causing body growth to gradually grind to a halt as the child approaches adulthood. During the period of body enlargement, growth of the different organs, such as the kidney, heart, lung, liver, and bones, occurs synchronously, orchestrated to maintain body proportions.

In the majority of children, the largely mysterious mechanisms governing body growth proceed like clockwork, yielding an adult height most often between 1.5 and 2 m. However, in some children, body growth is either greater than or less than the normal range, often prompting the concerned family to seek medical evaluation.

The healthcare provider is then called upon to answer two questions. First, why is the child's growth outside the normal range and second what, if anything, should be done about it. The first question, "why," is important for several reasons. Many children are short or tall because they have inherited from their parents a large set of normal polymorphisms that place the child in one tail of the normal height distribution or the other. Such polygenic short or tall stature is generally benign. However, some children are short or tall because of a single gene abnormality that may have important health implications, such as a propensity to cardiac malformations or to malignancies. Other children are short because of a subtle systemic acquired disease, such as inflammatory bowel disease (IBD), autoimmune thyroiditis, or a central nervous system (CNS) tumor causing pituitary dysfunction. Thus the healthcare provider who evaluates children's growth faces the

challenge of sorting out the few children with important underlying pathology from the many children with a benign condition.

The second question, "what to do about it," can also be challenging. If the diagnostic evaluation uncovers an underlying, treatable disorder, such as celiac disease, the course is clear. But often, no simple treatable cause is found. Then, the provider and the family must try to assess whether the short or tall stature is sufficiently abnormal and sufficiently distressing to the child, or likely to be distressing in the future, so that the possible benefits of medical therapy outweigh the effort, costs, discomforts, and potential risks of intervening.

Addressing these two central questions requires a deep understanding of normal childhood growth and the many disorders that affect it, the determination to look diligently for underlying causes, as well as the willingness and ability to listen compassionately to the concerns of the child and family. In this chapter, we have tried to present the healthcare provider with a broad overview of the knowledge necessary for this endeavor.

CHILDHOOD GROWTH

The Biological Basis of Linear Growth

In children, many tissues and organs grow simultaneously. However, most children who present to the pediatric endocrinologist for evaluation of growth do so because the child's height is abnormal. The height is primarily determined by the length of the long bones in the lower extremities and the height of the vertebral bodies. These bone lengths in turn are determined by the action of the skeletal growth plates, thin layers of cartilage located near the ends of the long bones and vertebrae.

Within the growth plates, the chondrocytes are arranged in columns parallel to the long axis of the bone (Fig. 11.1A). In the upper portion (closer to the ends of the bone) of the columns, the chondrocytes proliferate (Fig. 11.1B). In the lower portion of the columns, the chondrocytes stop dividing and instead physically enlarge (Fig. 11.1B). These two processes, cell proliferation and cell hypertrophy, lead to chondrogenesis, the production of cartilage. In isolation, this chondrogenesis would cause progressive thickening of the cartilaginous growth plate. However, simultaneously, at the bottom of the growth plate, the newly formed cartilage is remodeled into bone. The net result is that new bone tissue is progressively created at the bottom of the growth plates, the overall bone grows longer, and the child grows taller.

Thus linear growth (height gain) in children results from growth plate chondrogenesis. Therefore short stature is caused by decreased growth plate chondrogenesis and tall stature is caused by increased growth plate chondrogenesis.

Regulation of Linear Growth

Because linear growth results from growth plate chondrogenesis, the regulation of linear growth results from the regulation of growth plate chondrocytes. Factors that stimulate growth

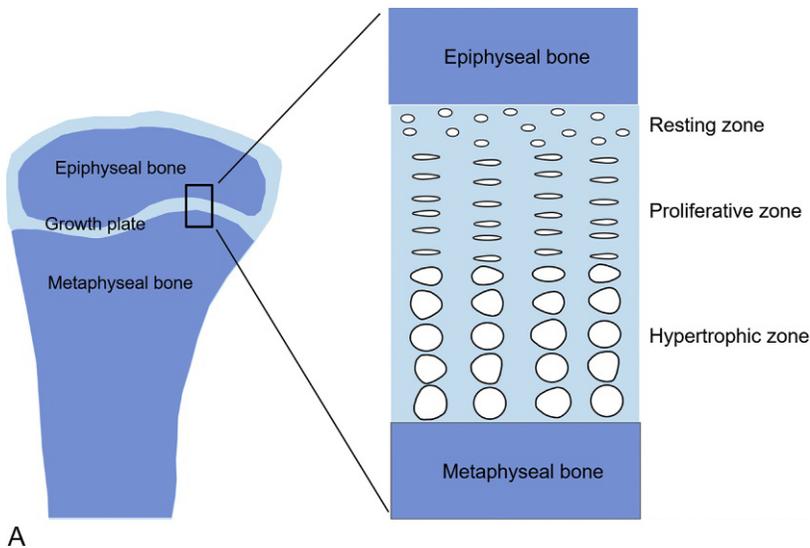


Fig. 11.1A Growth plate structure. In children, linear growth (height gain) results from growth plate chondrogenesis. The growth plate is a thin layer of cartilage found near the ends of long bones and in vertebrae. The growth plate is composed of three zones.

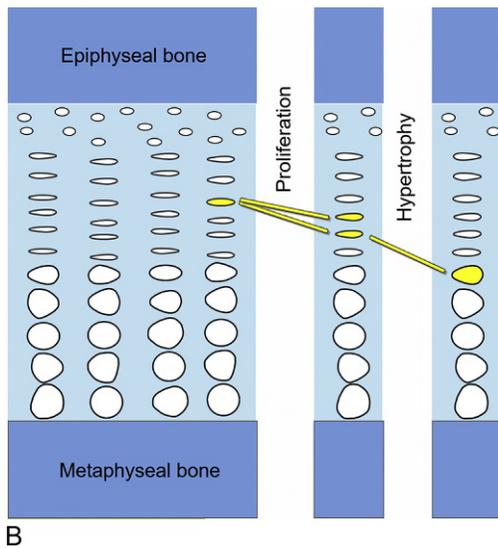


Fig. 11.1B Growth plate function. Within the growth plate, chondrocytes first proliferate and later hypertrophy. The result is the production of more cartilage (chondrogenesis). At the bottom of the growth plate, the newly formed cartilage is remodeled into bone (not shown). The net result is that new bone is created at the bottom of the growth plate, gradually pushing apart the ends of the bone, causing the bones to grow longer and the child to grow taller.

plate chondrocyte proliferation and hypertrophy stimulate linear growth, whereas factors that inhibit growth plate chondrocyte proliferation and hypertrophy inhibit linear growth. This regulation of growth plate chondrocyte function occurs at multiple different levels; endocrine signals, inflammatory cytokines, nutritional intake, paracrine/autocrine signals, extracellular matrix effects, and intracellular systems can all modulate chondrocyte proliferation and hypertrophy and therefore affect the rate of linear growth. Consequently, abnormalities at any of these levels can cause short or tall stature.

Endocrine Regulation of Linear Growth

Growth Hormone.

The Pituitary Gland. The pituitary gland lies in the sella turcica, the hypophyseal fossa of the sphenoid bone, which is

located in the center of the cranial base. The concept of the pituitary as a “master gland” controlling the endocrine activities of the body has become outdated and has been replaced by an appreciation of the importance of the brain, particularly of the hypothalamus, in regulating hormonal production and secretion. Nevertheless, the pituitary gland remains central to our understanding of the regulation of growth, metabolism and homeostasis, response to stress, lactation, and reproduction.

Embryologically, the pituitary gland is formed from two distinct sources, namely Rathke’s pouch, a diverticulum of the primitive oral cavity (stomodeal ectoderm), which gives rise to the adenohypophysis, and the neural ectoderm of the floor of the forebrain, which gives rise to the neurohypophysis, the posterior lobe. The adenohypophysis normally constitutes 80% of the weight of the pituitary and consists of the pars distalis (also known as the *pars anterior* or *anterior lobe*), the pars intermedia (also known as the *intermediate lobe*), and the pars tuberalis (also known as the *pars infundibularis* or *pars proximalis*).

Much of our knowledge of normal hypothalamopituitary development is derived from animal, particularly rodent, models. In the mouse, a thickening of the ectoderm in the midline of the anterior neural ridge, forming the hypophyseal placode, heralds the onset of pituitary development at 7.5 dpc (days postcoitum). The formation of a rudimentary Rathke’s pouch follows at 9 dpc, with formation of a definitive pouch by 12 dpc and subsequently, the anterior pituitary consisting of five different cell types secreting six different hormones (Fig. 11.2).

In humans, the pars distalis is the largest portion of the adenohypophysis and houses the great majority of hormone-producing cells. In contrast with the mouse, the pars intermedia is rudimentary and consists of several cystic cavities lined by a single layer of cuboidal epithelium as it largely disappears during embryogenesis. The pars distalis and intermedia are separated by a cleft, a vestigial structure of Rathke’s pouch from which it develops. This structure may often develop as a cyst (Rathke’s cleft cyst). The pars tuberalis represents an upward extension of the pars distalis onto the pituitary stalk and may contain a limited number of gonadotropin-producing cells. The posterior pituitary (neurohypophysis) consists of the infundibular stem or hypophyseal stalk, the median eminence of the tuber cinereum, and the infundibular process (posterior lobe, neural lobe). The posterior pituitary contains the terminal axonal projections of magnocellular neurons from the paraventricular and supraoptic nuclei of the hypothalamus; these

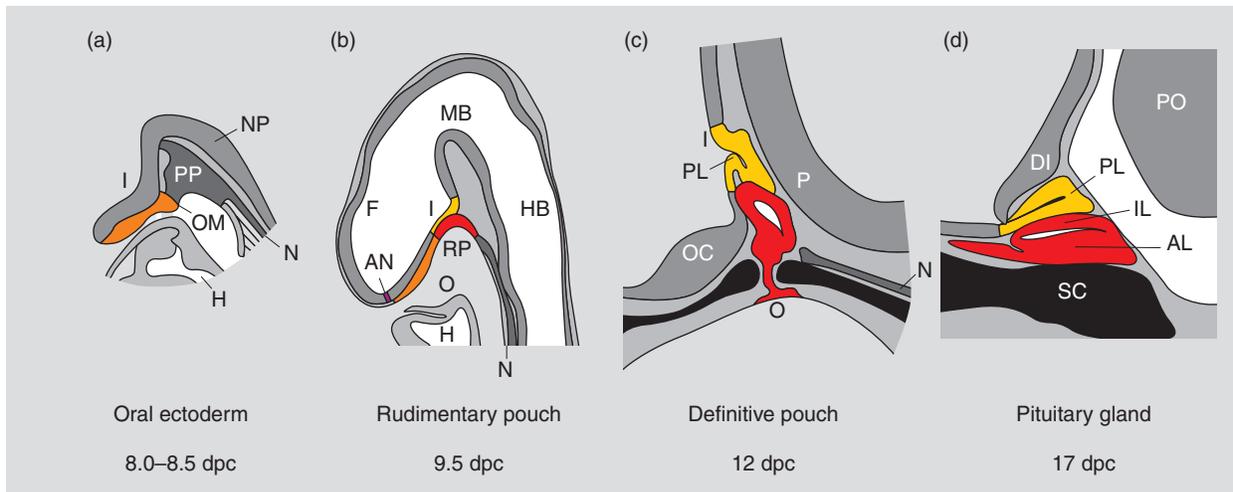


Fig. 11.2 Mouse pituitary development in sagittal section. Stages of development are indicated in dpc. AL, Anterior lobe; AN, anterior neural pore; DI, diencephalon; F, forebrain; H, heart; HB, hindbrain; I, infundibulum; IL, intermediate lobe; MB, midbrain; N, notochord; NP, neural plate; O, oral cavity; OC, optic chiasma; OM, oral membrane; P, pontine flexure; PL, posterior lobe; PO, pons; PP, prechordal plate; RP, Rathke's pouch; SC, sphenoid cartilage. (Modified from Sheng, H. Z., & Westphal, H. (1999). Early steps in pituitary organogenesis. *Trends Genet.* 15:236–240. With permission from Elsevier.)

produce oxytocin, which is required during lactation and parturition, and vasopressin, which is required for osmotic regulation. It has no known function in the regulation of growth and will not be discussed further in this chapter.

Rathke's pouch, the origin of the adenohypophysis, can be identified in the 3-mm embryo during the third week of pregnancy in humans. Rathke's pouch then begins to develop, resulting in a complete pouch disconnected from the oral ectoderm by the end of the sixth gestational week. Growth hormone (GH)-producing cells can be identified by 9 weeks of gestation. It is at about this time that the vascular connections between the anterior lobe of the pituitary and the hypothalamus develop, although it has been demonstrated that hormonal production by the pituitary can occur in the absence of connections with the hypothalamus. Somatotropes are thus frequently demonstrable in the pituitary of an anencephalic newborn. Nevertheless, it appears likely that the initiation of development of the anterior pituitary is dependent on responsiveness of the oral ectoderm to inducing factors from the ventral diencephalon. Infrequently, the craniopharyngeal canal (marking the embryonic migration of Rathke's pouch) remains patent and may contain small nests of adenohypophyseal cells—giving rise to a pharyngeal hypophysis that may be capable of hormone synthesis.

Maintained apposition and interaction between the oral ectoderm and neuroectoderm is critical for normal anterior pituitary development.^{1,2} Experimental manipulation of embryos from several species, as well as Rathke's pouch explant experiments in rodents, have shown that signals from the diencephalon are essential not only for the induction and maintenance of Rathke's pouch, but also for the regionalization within the pouch that allows the emergence of the different endocrine cell types. During gestation, proliferating progenitor cells are enriched around the pouch lumen, and they appear to delaminate as they exit the cell cycle and differentiate. During late mouse gestation and the postnatal period, anterior lobe progenitors reenter the cell cycle and expand the populations of specialized, hormone-producing cells. At birth, all cell types are present, and their localization appears stratified, based on cell type. Current models of cell specification in the anterior lobe suggest that opposing gradients of fibroblast growth factor (FGF) and bone morphogenic protein (BMP) signaling pattern the progenitor cells within Rathke's pouch before they move

on to the anterior lobe where they differentiate. Several studies have revealed that normal pituitary development is dependent upon a complex cascade of transcription factors and signaling molecules that are expressed in a spatiotemporal manner.

Signaling molecules implicated in pituitary development are either intrinsic, emanating from the oral ectoderm, such as sonic hedgehog (Shh), or extrinsic from the neuroectoderm, such as Nkx2.1, FGFs (e.g., FGF 8), and BMPs (e.g., BMP4) (Fig. 11.3).¹ These molecules may activate or repress transcription factors, such as *Hex1*, *Lhx3*, and *Lhx4*. They may also act as morphogens creating the appropriate environment for cell differentiation, thus playing a critical role in cell fate. Such signaling molecules include members of the Shh family, FGFs, transforming growth factors- β (TGFs)/BMPs, *Wingless/Wnts*, and molecules in the Notch pathway to mention a few.

Recently, Davis and colleagues have challenged the current dogma of pituitary cell specification,³ showing evidence that, in mice, the pattern of cell specification that results in the rostral location of gonadotropes, the caudal location for somatotropes and a more intermediate location for corticotropes and thyrotropes, does not appear to be the result of an ordered cell cycle exit, as previously suggested. All anterior lobe cell types appear to begin the differentiation process concurrently (E11.5-E14.5), rather than in a temporally discrete manner.

To date, not many pituitary phenotypes have been reported in association with mutations in these signaling molecules. Importantly, the Wnt signaling pathway has recently been implicated in pituitary tumorigenesis. For example, there is clear evidence that the Wnt/ β catenin pathway is involved in the pathogenesis of craniopharyngioma, a rare tumor in the hypothalamopituitary region.^{4,5}

Multiple pituitary-specific transcription factors are involved in the determination of pituitary cell lineages and cell-specific expression of anterior pituitary hormones (see Fig. 11.3). Several homeodomain transcription factors have been shown to be involved in human anterior pituitary development and differentiation. Defects in several of these have now been associated with various combinations of pituitary hormone deficiencies (Table 11.1). Because additional gene defects have been implicated in abnormal murine hypothalamopituitary development,

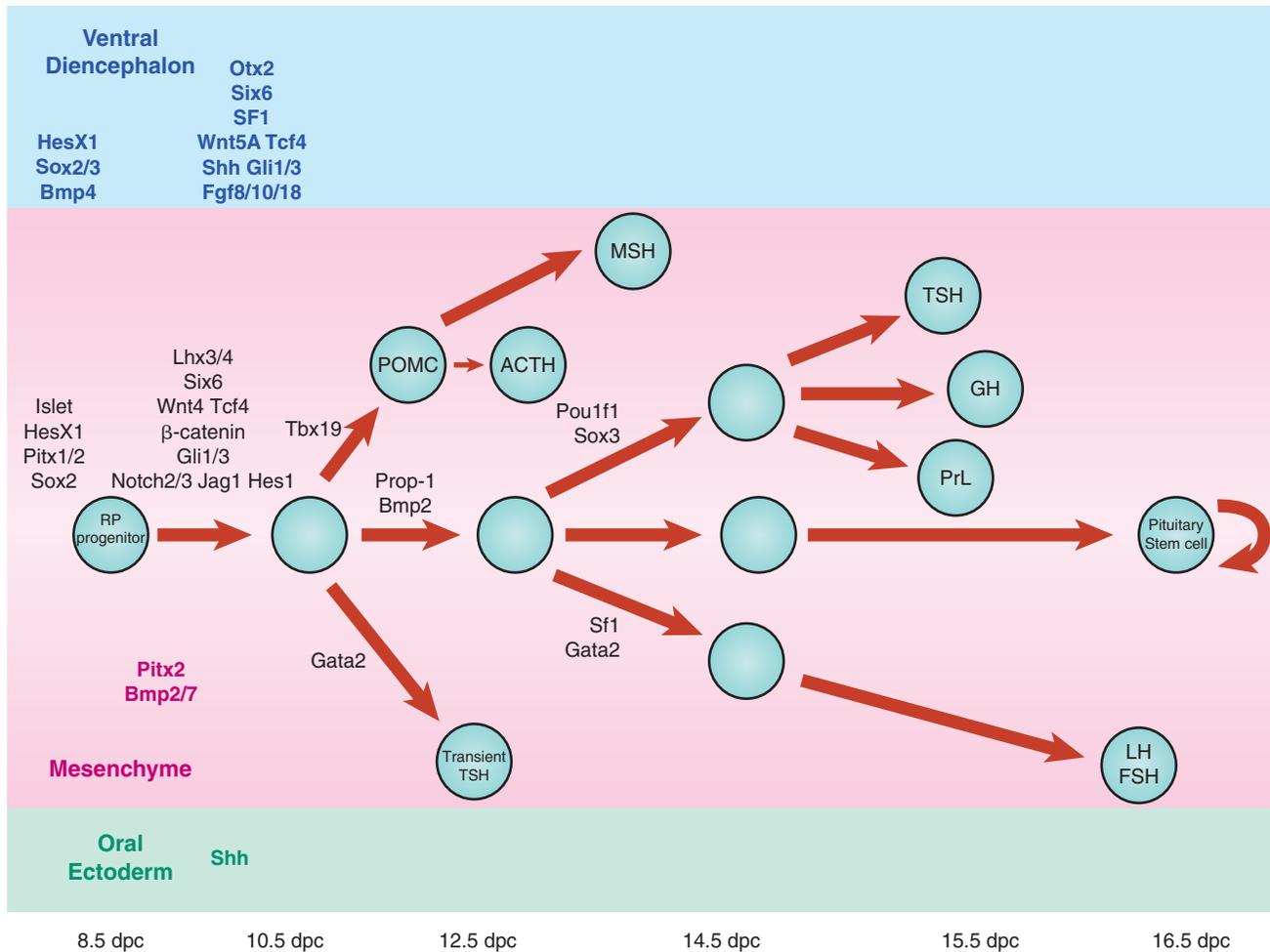


Fig. 11.3 Schematic representation of the developmental cascade of genes implicated in human pituitary development with particular reference to pituitary cell differentiation. (From Kelberman, D., Rizzoti, K., Lovell-Badge, R., et al. (2009). Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev*, 30, 790–829.)

it seems likely that the number of known human genetic defects will expand.^{1,6}

In the human adult, the pituitary has a mean weight of 600 mg, with a range of 400 to 900 mg. Pituitary weight is slightly greater in women than in men, and typically increases during puberty and pregnancy. In the newborn, pituitary weight averages about 100 mg. The pituitary normally resides in the sella turcica immediately above and partially surrounded by the sphenoid bone. The volume of the sella turcica provides a good measure of pituitary size, which may be reduced in the child with pituitary hypoplasia. It is important, however, to recognize that considerable variation in pituitary size occurs normally. The pituitary is covered superiorly by the diaphragma sellae, and the optic chiasm is directly above the diaphragma. The anatomic proximity between the optic chiasm and the pituitary is important because hypoplasia of the optic chiasm may occur together with hypothalamic/pituitary dysfunction, as in the condition of septo-optic dysplasia (SOD), and because pituitary tumors may in turn impact on the optic chiasm, leading to visual impairment. The patient with congenital blindness or nystagmus should be initially evaluated and then subsequently monitored carefully for hypopituitarism, as this can evolve. In addition, suprasellar growth of a pituitary tumor may initially manifest with visual complaints or evidence of progressive impairment of peripheral vision, particularly bitemporal hemianopia.

The existence of a portal circulatory system within the pituitary is critical to normal pituitary function. The blood supply of the pituitary derives from the superior and inferior hypophyseal arteries, branches of the internal carotid. The anterior and posterior branches of the superior hypophyseal artery may terminate within the infundibulum and the proximal portion of the pituitary stalk. Hypothalamic peptides, produced in neurons that terminate in the infundibulum, enter the primary plexus of the hypophyseal portal circulation and are transported by means of the hypophyseal portal veins to the capillaries of the anterior pituitary. This portal system thus provides a means of communication between the neurons of the hypothalamus and the hormone-producing cells of the anterior pituitary. The blood supply of the neurohypophysis is separate, deriving from the inferior hypophyseal artery. Regulation of the posterior lobe of the pituitary does not involve the hypophyseal portal circulation but, rather, is mediated through direct neural connections.

The definitive Rathke's pouch comprises proliferative progenitors that will gradually relocate ventrally, away from the lumen as they differentiate. A proliferative zone containing progenitors is maintained in the embryo in the periluminal area and was recently found to persist in the adult. The exact nature of progenitor cells in the pituitary gland, however, remains unknown. Members of the Sox family of transcription factors are likely involved in the earliest steps of pituitary stem cell

TABLE 11.1 Genetic Defects of Pituitary Development and Their Phenotype³⁹⁵

Gene	Pituitary Deficiencies	MRI Phenotype	Inheritance	Other Phenotypic Features
<i>POU1F1</i>	GH, TSH, prolactin	Small or normally sized anterior pituitary	AR and AD	
<i>PROP1</i>	GH, TSH, LH, FSH, prolactin, evolving ACTH deficiencies	Small, normal or enlarged anterior pituitary – may evolve over time	AR	
<i>HESX1</i>	Isolated GH deficiency through to panhypopituitarism with TSH, LH, FSH, ACTH, and prolactin deficiencies	Optic nerve hypoplasia, absence of the septum pellucidum, ectopic posterior pituitary, anterior pituitary hypoplasia	AR and AD	Developmental delay, visual abnormalities, SOD
<i>LHX3</i>	GH, TSH, LH, FSH, prolactin with late ACTH deficiencies	Small, normal, or enlarged anterior pituitary	AR	Short neck with limited rotation
<i>LHX4</i>	GH, TSH, ACTH, and gonadotrophin deficiencies	Small anterior pituitary, ectopic posterior pituitary, cerebellar abnormalities, corpus callosum hypoplasia	AD (variable penetrance)	
<i>SOX3</i>	GH, TSH, LH, FSH, ACTH deficiencies. Most commonly isolated GH deficiency	Anterior pituitary and infundibular hypoplasia, ectopic posterior pituitary, corpus callosum abnormalities including cysts; persistent craniopharyngeal canal	X-linked recessive	Learning difficulties
<i>SOX2</i>	LH, FSH variable GH deficiency	Anterior pituitary hypoplasia, optic nerve hypoplasia, SOD, hypothalamic hamartoma	AD (usually de novo)	Microphthalmia, anophthalmia, micropenis, sensorineural deafness, gastrointestinal tract defects with esophageal atresia
<i>GLI2</i>	GH, TSH, and ACTH with variable gonadotrophin deficiencies	Anterior pituitary hypoplasia	AD	Holoprosencephaly, cleft lip and palate, anophthalmia, postaxial polydactyly, imperforate anus, laryngeal cleft, renal agenesis
<i>GLI3</i>	GH, TSH, LH, FSH, ACTH	Anterior pituitary hypoplasia	AD	Pallister-Hall syndrome Postaxial polydactyly, hamartoma
<i>OTX2</i>	GH, TSH, LH, FSH, and ACTH deficiencies	Normal or small AP, pituitary stalk agenesis, ectopic posterior pituitary, Chiari I malformation	AD (usually de novo)	Microcephaly, bilateral anophthalmia, developmental delay, cleft palate
<i>FGFR1</i>	GH, TSH, LH, FSH, and ACTH deficiencies	Normal or small anterior pituitary, corpus callosum agenesis	AD	ASD and VSD, brachydactyly, brachycephaly, preauricular skin tags, ocular abnormalities, seizures
<i>FGF8</i>	GH, TSH, ACTH, gonadotrophin, and ADH deficiencies	Absent corpus callosum, optic nerve hypoplasia, holoprosencephaly	AD or AR	
<i>PROKR2</i>	GH, TSH, ACTH and gonadotrophin deficiencies	Hypoplastic corpus callosum, normal or small anterior pituitary	AD	
<i>ARNT2</i>	GH, TSH, ACTH, LH, FSH, ADH deficiencies	Absent PP, thin stalk, thin corpus callosum, delayed myelination	AR	Hip dysplasia, hydronephrosis, vesicoureteric reflux, neuropathic bladder, microcephaly, prominent forehead, deep-set eyes, retrognathia
<i>TCFL7</i>	GH	Absent posterior pituitary, anterior pituitary hypoplasia, optic nerve hypoplasia, partial agenesis of corpus callosum, thin anterior commissure	AD	
<i>IGSF1</i>	GH (transient/partial), TSH, prolactin	Normal in the majority of cases. Frontoparietal hygroma, hypoplasia of the corpus callosum, and small stalk lesion reported	X-linked recessive	Macroorchidism, delay in puberty
<i>RNPC3</i>	GH deficiency		AR	Maternally inherited gingival fibromatosis
<i>KCNQ1</i>	GH, ACTH, TSH, and gonadotrophin deficiencies	Small anterior pituitary with normal stalk and ectopic posterior pituitary Normal/small AP	AR	

ACTH, Adrenocorticotropic hormone; *AD*, autosomal dominant; *ADH*, antidiuretic hormone; *AP*, anterior pituitary; *AR*, autosomal recessive; *ASD*, aortic septal defect; *FSH*, follicle-stimulating hormone; *GH*, growth hormone; *LH*, luteinizing hormone; *MRI*, magnetic resonance imaging; *PP*, posterior pituitary; *SOD*, septooptic dysplasia; *TSH*, thyroid-stimulating hormone; *VSD*, ventricular septal defect.

Adapted and updated from Alatzoglou KS, Gregory LC, Dattani MT. Development of the pituitary gland. *Compr Physiol*. 2020;10(2):389–413. <https://doi.org/10.1002/cphy.c150043>. PMID: 32163208.

proliferation and the earliest transitions to differentiation.^{7–9}

The transcription factor Prophet of Pit1 (*PROP1*) and the *NOTCH* signaling pathway may then regulate the transition to differentiation. It has been proposed that the niche of progenitors may be the marginal zone around the lumen of Rathke's pouch, between the anterior and intermediate lobes of mouse pituitary, because cells in this region are able to give birth to all five pituitary hormone cell lineages (see Fig. 11.3).

Stem cells have been shown to play a role in tumorigenesis in some tissues, and their role in pituitary hyperplasia, pituitary adenomas, and tumors is an important area for future

investigation. The ability to cultivate and grow pituitary stem cells in a predifferentiation state might also be helpful for the long-term treatment of pituitary deficiencies. Indeed, a seminal study resulted in the efficient self-formation of a three-dimensional anterior pituitary in an aggregate culture of mouse embryonic stem (ES) cells.¹⁰ Various endocrine cells were generated, and these cells were able to respond to trophic hormones. When these ES-derived cell aggregates were implanted under the kidney capsule in hypophysectomized mice, corticosterone was produced. These studies may therefore reflect the first step toward stem cell treatment.

Terminally differentiated secreting cells are not distributed randomly in a patchwork-like fashion throughout the pituitary gland. Instead, these cells appear to organize themselves in same-cell-type networks. The connectivity between the cells of this network is important to deliver coordinated secretory pulses of hormones to their target tissues and to facilitate the coordinated physiological response to stimuli.

Growth Hormone Chemistry. Human GH is produced from somatotrope cells within the anterior pituitary as a single-chain, nonglycosylated, 191-amino-acid, 22-kDa protein that comprises a core of four helices in a parallel/antiparallel orientation with two disulfide bonds between cysteines 53–165 and 182–189. The 217-amino-acid GH precursor is cleaved to remove the signal peptide before secretion.

GH is homologous with several other proteins produced by the pituitary or placenta, including prolactin, chorionic somatomammotropin (CS, placental lactogen), and a 22-kDa GH variant hGH-V, secreted only by the placenta. The latter differs from pituitary GH by 13 amino acids. The genes for these proteins have probably descended from a common ancestral gene, even though the genes are now located on different chromosomes.

GH1 is located on the long arm of chromosome 17 (17q22-24) within a cluster of five homologous genes encompassing a distance of about 65 kb (*CSHP* [CS pseudogene], *CSH-1* [CS gene], *GH-2* and *CSH-2*). Expression of *GH1* is regulated by the highly polymorphic proximal promoter and a locus control region (LCR) 15–32 kb upstream of the gene that confers the pituitary-specific and high-level expression of GH. Normally, the vast majority of GH (75%) produced by the pituitary is of the mature 22-kDa form. Alternative splicing results in deletion of amino acids 32 through 46, yielding a 20-kDa form that normally accounts for less than 10% of pituitary GH. The remainder of pituitary GH includes desamidated and N-acetylated forms, as well as various GH oligomers. A 17.5-kDa variant that results from complete skipping of exon 3 and lacks amino acids 32–71 is much less abundant (1%–5%).¹¹

Growth Hormone Secretion. GH can be identified in fetal serum by the end of the first trimester. The pulsatile pattern characteristic of GH secretion largely reflects the interplay of multiple regulators, including two hypothalamic regulatory peptides: GH-releasing hormone (GHRH) and somatostatin (somatotropin release-inhibiting factor [SRIF]). *GHRHR* encodes a G-protein-coupled receptor comprising seven transmembrane domains. The expression of *GHRHR* is upregulated by pituitary-specific transcription factor 1 (*POU1F1*) and is required for the proliferation of somatotrophs.

Regulation of GH production by GHRH is largely transcriptionally mediated and is dependent on stimulation of adenylyl cyclase and increases in intracellular cyclic adenosine monophosphate (cAMP) concentrations. Solid tumors secreting GHRH are a rare cause of GH excess. GHRH is used diagnostically, especially for the identification of adult GH deficiency, when it is frequently used in combination with arginine. However, it is not conventionally used for therapeutic purposes in patients with GH deficiency.

The actions of the 14-amino-acid peptide somatostatin appear to regulate the timing and amplitude of pulsatile GH secretion, rather than GH synthesis. The binding of somatostatin to its specific receptor results in an inhibition of adenylyl cyclase activity and a reduction in intracellular calcium concentrations. The pulsatile secretion of GH observed *in vivo* is believed to result from a simultaneous reduction in hypothalamic somatostatin release and increase in GHRH activity. Conversely, a trough of GH secretion occurs when somatostatin release is increased in the presence of diminished GHRH activity. The net effects of these two hypothalamic hormones is modulation of GH secretion, as well as the timing and amplitude of peaks, resulting in pulsatile GH secretion.

Multiple neurotransmitters and neuropeptides are involved in regulating the release of these hypothalamic factors, including serotonin, histamine, norepinephrine, dopamine, acetylcholine, gamma-aminobutyric acid (GABA), thyroid-releasing hormone, vasoactive intestinal peptide, gastrin, neurotensin, substance P, calcitonin, neuropeptide Y, vasopressin, corticotrophin-releasing hormone, and galanin. These factors are implicated in the alterations of GH secretion observed in a wide variety of physiological states (such as stress, sleep, hemorrhage, fasting, hypoglycemia, and exercise) and form the basis for a number of GH-stimulatory tests used in the evaluation of GH secretory capacity/reserve. GH secretion is also impacted by a variety of nonpeptide hormones, including androgens, estrogens, thyroxine, and glucocorticoids. The precise mechanisms by which these hormones regulate GH secretion are complex, potentially involving actions at the hypothalamic and pituitary levels. Practically speaking, hypothyroidism and glucocorticoid excess may each blunt spontaneous and provocative GH secretion (and therefore should be corrected before GH testing). Sex steroids, at the onset of puberty or administered pharmacologically, appear to be responsible for the rise in GH secretion characteristic of puberty. Synthetic hexapeptides capable of stimulating GH secretion have been developed and termed *GH-releasing peptides* (GHRPs). These peptides, later recognized as analogues of the gastric hormone ghrelin, are capable of directly stimulating GH release and enhancing the GH response to GHRH. These agents have the potential advantage of oral administration, and, in the patient with an intact pituitary, may be capable of greatly enhancing GH secretion. When these agents were administered chronically to elderly patients and to some GH-deficient children, the amplitudes of GH pulses were significantly increased. Ghrelin-mimetic ligands were used to characterize a common receptor termed the *GH secretagogue receptor* (GHS-R) for the GH-releasing substances. The GHS-R is a G-protein-coupled receptor, which is distinct from the GHRH receptor. The receptor is strongly expressed in the hypothalamus, but specific binding sites for GHRPs have also been identified in other regions of the CNS and peripheral endocrine and nonendocrine tissues in both humans and other organisms. Ghrelin is a 28-amino-acid peptide that has been identified as the endogenous ligand for GHS-R.¹² It is expressed predominantly in the stomach, but smaller amounts are also produced within the bowel, pancreas, kidney, the immune system, placenta, pituitary, testis, ovary, and hypothalamus. Ghrelin is a unique gene product that requires octanoylation for normal function. Intravenous, intracerebroventricular, and intraperitoneal administration of ghrelin in animal models stimulates food intake and obesity and raises plasma GH concentrations, and, to a lesser extent, prolactin (PRL) and adrenocorticotrophic hormone (ACTH) concentrations. In addition, it influences endocrine pancreatic function and glucose metabolism, gonadal function, and behavior. It also controls gastric motility and acid secretion and has cardiovascular and antiproliferative effects. Both ghrelin and GHRPs release GH synergistically with GHRH but the efficacy of these compounds as growth-promoting agents is poor. Variants in the ghrelin receptor have been identified as a possible cause of idiopathic short stature (ISS) and GH deficiency.^{13,14} However, note that mouse models with targeted deletion of the receptor (*ghsr*^{-/-}) have a near normal phenotype.¹⁵ A second peptide encoded by the same gene as ghrelin has been identified and termed *obestatin*. This gene appears to regulate weight but not GH secretion.

In addition to the complex regulatory processes described previously, the synthesis and secretion of GH are also regulated by feedback by the insulin-like growth factor (IGF) polypeptides. IGF receptors have been identified in the pituitary. Inhibition of GH secretion by IGF-1 has been demonstrated

in multiple systems. In addition, inhibition of spontaneous GH secretion has been demonstrated in humans treated with subcutaneous injections of recombinant IGF-1.¹⁶ GH also regulates its own secretion by acting directly on hypothalamic GH receptors (GHRs), via a short loop negative feedback.¹⁶

During puberty, sex steroids increase GH pulse amplitude contributing to the very high serum concentrations of IGF-1 characteristic of puberty. GH secretion begins to decline by late adolescence and continues to fall throughout adult life. Indeed, puberty may be considered with some justification a period of “acromegaly,” whereas aging (with its characteristic decrease in GH secretion) has been termed the *somatopause*.

In addition to aging, a wide variety of physiological conditions affect GH secretion. These include stage of sleep, nutritional status, acute fasting, exercise, stress, and sex steroids. Ho and associates³⁹⁴ have reported that neither age nor sex influenced the integrated serum concentrations of GH when the effects of estradiol were removed from analysis. The effects of testosterone on serum IGF-1 concentrations may be at least in part independent of GH because individuals with mutations of the GHR still experience a rise in serum IGF-1 during puberty.

The pulsatile nature of GH secretion is readily demonstrable by frequent serum sampling, especially when coupled with sensitive assays for GH. Such assays demonstrate that, under normal conditions, serum GH concentrations are less than 0.2 ng/mL between bursts of GH secretion. It is consequently impractical to assess GH secretion by a single, random serum sample. Maximal GH secretion occurs during the night, especially at the onset of the first slow-wave sleep (stages III and IV). Rapid-eye-movement (REM) sleep is, on the other hand, associated with low GH secretion.

Normal young men generally experience, on average, 12 GH secretory bursts per 24 hours. Obesity is characterized by decreased GH secretion, reflected by a decreased number of GH secretory bursts. Fasting increases the number and amplitude of GH secretory bursts, presumably reflecting decreased somatostatin secretion. The impact of the pulsatile secretory nature of GH secretion on its biological actions remains uncertain.

Growth Hormone Receptor/Growth Hormone-Binding Protein. The GHR comprises an extracellular, hormone-binding domain, followed by a single membrane-spanning domain, and a cytoplasmic domain. Two genomic *GHR* isoforms that exist only in humans have arisen from ancestral homologous recombination. They differ in the retention or deletion of exon 3. Exon 3 of the GHR has been shown to be deleted in a number of normal individuals. This delta-3 GHR polymorphism has been shown by some, but not all, investigators to affect responsiveness to GH and to be associated with birth size and postnatal growth.¹⁷ There are also short isoforms of the GHR because of alternative splicing, resulting in a truncated receptor with loss of a large portion of the cytoplasmic domain.

The GHR is a member of the class 1 hematopoietic cytokine family and is highly homologous with the prolactin receptor and shares sequence homology with many of the receptors for interleukins (ILs), as well as receptors for erythropoietin (EPO), leptin, granulocyte-macrophage colony-stimulating factor, and interferon.

Examination of the crystal structure of the GH-GHR complex revealed that the complex consists of one molecule of GH bound to two GHR molecules, indicating a GH-induced receptor dimerization—which is necessary for GH action. Interestingly, a genetically engineered fusion complex of GH and the GHR has been shown to have a significantly improved efficacy and a dramatically longer half-life compared with GH alone when tested in rodent models.

The GHR, like its family group member EPO-R, is preformed as a dimer and is transported in a nonligand bound state to the cell surface.¹⁸ GH then binds in a sequential manner to the GHR dimer where the first GHR binds to the stronger site 1 of the GH molecule followed by the second GHR binding to the weaker site 2.¹⁹ Binding of GH results in a conformational change whereby rotation of the GHRs results in repositioning of the intracellular domains and of Box11-1-associated Janus Kinase 2 (JAK2), a major GHR-associated tyrosine kinase. As a result, JAK2 is autophosphorylated and activated, which in turn leads to cross-phosphorylation of distal tyrosine residues of GHR. This enables SH2 (*Src* homology 2) domain molecules to dock to these sites. The GHR itself appears to have no intrinsic kinase activity. It is likely that colocalization of two JAK2 molecules by the dimerized GHR results in transphosphorylation of one JAK2 by the other, leading to JAK2 activation. Signal transducer and activator of transcription (Stat)5a and 5b molecules contain SH2 domains and bind to these phosphorylated tyrosine sites, and then they in turn become phosphorylated. Phosphorylated Stat5 molecules (homo- and hetero-) dimerize and translocate to the nucleus where they bind deoxyribonucleic acid (DNA), as dimers or as tetramers, and activate target genes. Importantly, the dimerization of the GHR by GH is exploited by the use of Pegvisomant, a genetically modified form of GH, which binds to one receptor only and therefore cannot dimerize the receptor. It therefore acts as a GH antagonist and can be used to treat pituitary gigantism and acromegaly.²⁰

Both Stat5a and Stat5b can be activated by GH, and they have both overlapping and distinct functions. Gene inactivation mouse models have shown that Stat5b is of greater importance for stimulation of growth than Stat5a.²¹

Negative Regulation of Growth Hormone Signaling. Activation of JAK-STAT signaling occurs rapidly, within minutes after GH stimulation, but is transient because of the tight control of the termination of signaling. This negative regulation of signaling occurs at several levels: GHR internalization, suppressors of cytokine signaling (SOCS), protein tyrosine phosphatases (PTPs), and protein inhibitors of activated Stats (PIAS).

In humans, the circulating GH-binding protein (GHBP) appears to derive from proteolytic cleavage of the extracellular domain of the GHR. GHBP has an identical affinity for GH as GHR. It binds to GH with high specificity and affinity, but with relatively low capacity. It increases GH half-life in the circulation and may serve a function in the transportation of GH to target tissues and subsequent binding to the receptor. GHBP is, like GHR, present in many tissues, but GHBP in the circulation is mostly derived from the liver. Although GHR and GHBP are regulated by and are very sensitive to GH, and GHR and GHBP often change in parallel, measurement of plasma GHBP has not been shown to reflect GHR and GH responsiveness. Measurement of serum GHBP concentrations may, however, aid in identifying patients with GH insensitivity (GHI) caused by genetic abnormalities of the GHR. Patients with GHI from nonreceptor abnormalities, abnormalities of the intracellular portion of the GHR, or inability of the receptor to dimerize may have normal serum concentrations of GHBP. Recently, a complex of the GH and the GHBP molecule has been shown to be more effective than GH alone—indicating a physiological and possible therapeutic role for GHBP.

Growth Hormone Actions. According to the somatomedin hypothesis, the anabolic actions of GH are mediated through the IGF polypeptides.²² Although this hypothesis is at least in part true, it appears that GH is capable of stimulating a variety of effects that are independent of IGF activity. Indeed, the effects of GH and IGF are on occasion contradictory, as for example, the “diabetogenic” actions of GH and the

glucose-lowering activity of IGFs. Green and colleagues³⁹⁶ attempted to resolve some of these differences in a “dual-effector” model in which GH stimulates precursor cells, such as prechondrocytes, to differentiate. When differentiated cells or neighboring cells then secrete IGFs, these peptides act as mitogens and stimulate clonal expansion. This hypothesis is based on the ability of IGF peptides to work not only as classic endocrine factors that are transported through the blood but as paracrine or autocrine growth factors. GH also stimulates a variety of metabolic effects, some of which appear to occur independently of IGF production, such as lipolysis, amino acid transport in diaphragm and heart, and production of specific hepatic proteins. Thus there are multiple sites of GH action, and it is not entirely clear which of these actions are mediated through the IGF system and which might represent IGF-1–independent effects of GH.

Insulin-Like Growth Factor-1.

Insulin-Like Growth Factor-1 Historical Background. The IGFs (or somatomedins) constitute a family of peptides that are at least in part GH-dependent and that are believed to mediate many of the anabolic and mitogenic actions of GH. Although they were originally identified in 1957 by their ability to stimulate [^{35S}]sulfate incorporation into rat cartilage, it has been established over the ensuing 45 years that they are involved in diverse metabolic activities.

Insulin-Like Growth Factor Structure and Molecular Biology. IGF-1, previously termed *somatomedin-C*, is a basic polypeptide of 70 amino acids, whereas IGF-2 is a slightly acidic polypeptide of 67 amino acids. The two peptides are structurally related, sharing 45 of 73 possible amino acid positions. They have approximately 50% amino acid homology to insulin. Like insulin, both IGFs have A and B chains connected by disulfide bonds and an intervening connecting (C-peptide) region. This structural homology explains the ability of both IGFs to bind to the insulin receptor and for insulin to bind to the type 1 IGF receptor. On the other hand, structural differences explain the failure of insulin to bind to the IGF-binding proteins.

Serum Concentrations of Insulin-Like Growth Factors. In human fetal serum, IGF-1 concentrations are relatively low and are positively correlated with gestational age. A correlation between fetal cord serum IGF-1 concentrations with birth weight has been reported by some groups, although others have reported no correlation. IGF-1 concentrations in human newborn serum are generally 30% to 50% of adult concentrations. There is a slow, gradual rise in serum concentrations during childhood, with attainment of adult concentrations at the onset of sexual maturation.²³ During puberty, IGF-1 concentrations increase to 2 to 3 times the concentrations seen in adults. Thus concentrations during adolescence correlate better with Tanner stage (bone age) than with chronological age. Girls with gonadal dysgenesis show no adolescent increase in serum IGF-1, clearly establishing the association of the pubertal rise in IGF-1 with the production of sex steroids. Estrogen stimulates GH secretion, which increases hepatic IGF-1 production. It is of note, however, that patients with GHI because of *GHR* mutations show a pubertal rise in serum IGF-1, which may be mediated by direct effects of androgen on IGF-1.

After adolescence, or at least after 20 to 30 years of age, serum IGF-1 concentrations demonstrate a gradual and progressive age-associated decline. It has been suggested that this decline may be responsible for the negative nitrogen balance, decrease in body musculature, and osteoporosis characteristic of aging. Although this provocative hypothesis remains unproven at this time, it has generated considerable interest in the potential use of GH and/or IGF-1 therapy in normal aging.

Human newborn concentrations of IGF-2 are generally 50% of adult concentrations. By 1 year of age, however, adult concentrations are attained with little, if any, subsequent decline even out to the seventh or eighth decade of life. This is in contrast to rodents, whose IGF-2 expression declines early following birth. Lack of rodent models has impeded our understanding of the physiological role of IGF-2 persistence in humans.

Insulin-Like Growth Factor Receptors. IGFs bind (although generally with low affinity) to insulin receptors, thus providing an explanation for their insulin-like activity. In addition, IGFs bind to at least two classes of IGF receptors.

The type 1 IGF receptor is closely related to the insulin receptor. Both are heterotetramers composed of two identical membrane-spanning alpha subunits and two identical intracellular beta subunits. The alpha subunits contain the binding sites for IGF-1 and are linked by disulfide bonds. The beta subunits contain a transmembrane domain, an adenosine triphosphate (ATP)-binding site, and a tyrosine kinase domain, which constitutes the presumed signal transduction mechanism for the receptor.

Although the type 1 IGF receptor has been commonly referred to as the *IGF-1 receptor*, the receptor is capable of binding both IGF-1 and IGF-2 with high affinity—and both IGF polypeptides appear capable of activating tyrosine kinase by binding to this receptor. Affinity of the type 1 receptor for insulin is generally 100-fold less, thereby providing one of the mechanisms for the mitogenic effects of insulin. IGF-1 acts primarily through IGF1R but can bind with lower affinity to the highly homologous insulin receptor (IR), and to IGF1R/IR heterodimers. Vice versa, insulin is able to signal through the IGF1R. Signs of such alternative signaling can become apparent in pathological IGF-1 or insulin signaling.

The type 1 IGF receptor mediates IGF actions on multiple cell types, and these actions are diverse and tissue specific. In general, it is believed that all of the effects of IGF receptor activation are mediated by tyrosine kinase activation and phosphorylation of substrates, which activate specific cellular pathways, leading to various biological actions. Among these effects is induction of cell growth through activation of the cell cycle machinery, maintenance of cell survival (prevention of apoptosis) mediated by effects on the Bcl family members, and induction of cellular differentiation, which occurs by as yet incompletely characterized mechanisms.

The substrates phosphorylated by the IGF receptor include members of the insulin receptor substrate family (particularly IRS-1 and IRS-2); knockout of these genes results in poor growth in mice (as well as insulin resistance). In addition, several other signaling molecules respond to IGF receptor activation. Blockade of the type 1 receptor has been proposed as a cancer therapy.

Mutations that decrease IGF signaling lead to an extension of life expectancy in nematodes, flies, and mice.²⁴ It is unclear, however, what relevance the IGF-1/IR has for human longevity. Recent data have suggested that loss-of-function variants in IGF1R are overrepresented among female centenarians, suggesting a role of this pathway in modulation of human life span.²⁵

The type 2 IGF receptor, however, bears no structural homology with either the insulin or the type 1 IGF receptor. The type 2 receptor does not contain an intrinsic tyrosine kinase domain or any other recognizable signal transduction mechanism and has been found to be identical to the cation-independent mannose-6-phosphate (CIM6P) receptor, a protein involved in the intracellular lysosomal targeting of a variety of acid hydrolases and other mannosylated proteins. Unlike the type 1 IGF receptor, which binds both IGF polypeptides with high affinity and insulin with 100-fold

lower affinity, the type 2 receptor only binds IGF-2 with high affinity. IGF-1 binds with substantially lower affinity, and insulin not at all. Most studies have indicated that the classic mitogenic and metabolic actions of IGF-1 and IGF-2 are mediated through the type 1 IGF receptor, with its tyrosine kinase signal transduction mechanism.²⁶

Insulin-Like Growth Factor-Binding Protein Superfamily. Although insulin and the IGFs share significant structural homology, and despite the structural-functional similarity of the insulin and type 1 IGF receptors, the IGFs differ from insulin in that the IGFs circulate in plasma complexed to a family of binding proteins.²⁷ These carrier proteins extend the serum half-life of the IGF peptides, transport the IGFs to target cells, and modulate the interaction of the IGFs with their surface membrane receptors. Six distinct IGF-BPs have been identified. Additional lower affinity IGF binding proteins (named *IGFBP related proteins, IGFBPrPs*) were found by in silico searches for homology to the known IGF-BPs; many of these molecules were previously known in other contexts, serving roles in normal or neoplastic growth.²⁷

Under most conditions, the IGF-BPs appear to inhibit IGF action—presumably by competing with IGF receptors for IGF polypeptides. This concept is supported by the observation that IGF analogues with decreased affinity for IGF-BPs generally appear to have increased biological potency. In addition, IGFBP-3 appears to inhibit cell growth even in the absence of added IGF—suggesting a direct inhibitory role of the binding protein. Under specific conditions, however, several of the IGF-BPs apparently are capable of enhancing IGF action—perhaps by facilitating IGF delivery to target receptors.

Evidence indicates that IGF-BPs are essentially bioactive molecules that, in addition to binding IGFs, have a variety of IGF-1-independent functions. These include growth inhibition in some cell types, growth stimulation in other tissues, direct induction of apoptosis, and modulation of the effects of other non-IGF growth factors. These effects of IGF-BPs are mediated by binding to their own receptors. Because IGFBP-3 is regulated by GH, it is intriguing that in vivo, IGFBP-3 enhances IGF-1 action when given to hypophysectomized rats (rather than inhibiting it). The mechanisms involved in this effect have not been elucidated but may explain the limited effects of IGF-1 therapy on the growth of Laron patients.

The relative amounts of each of the IGF-BPs vary among biological fluids. IGFBP-1 is the major IGF-BP in human amniotic fluid. IGFBP-2 is prominent in cerebrospinal fluid and seminal plasma. IGFBP-3 is the major IGF-BP in normal human serum and demonstrates clear GH dependence. Among the IGF-BPs, IGFBP-3 and IGFBP-5 are unique in that they normally circulate in adult serum as part of a ternary complex consisting of IGFBP-3 or IGFBP-5, an IGF polypeptide, and an acid-labile subunit (ALS).

Analysis of IGF-BPs is further complicated by the presence of IGF-BP proteases, capable of various levels of IGF-BP degradation. Initially reported in the serum of pregnant women, proteases for IGFBP-3, -4, and -5 have already been demonstrated in a variety of biological fluids. Proteolysis of IGF-BPs complicates their assay by both Western ligand blotting and radioimmunoassay methodologies and must be taken into consideration when concentrations of the various IGF-BPs in biological fluids are reported. The physiological significance of limited proteolysis of IGF-BPs remains to be determined, although evidence suggests that protease activity results in decreased affinity of the IGF-BP for IGF peptides.²⁸ Recently, recessive mutations in the metalloproteinase pregnancy-associated plasma protein A2 (*PAPP-A2*) were associated with short stature. The mutations lead to increased binding of IGF-1 within the ternary complex and reduced free IGF-1.²⁸

Targeted Disruption of Components of the Insulin-Like Growth Factor System. The critical role of the IGF system in fetal and postnatal growth was demonstrated in a series of elegant gene knockout studies in mice.²⁹ Unlike GH and GHR knockouts, which are near normal size at birth, Igf1 null mice have a birth weight 60% of normal. Postnatal growth is also abnormal. A similar prenatal and postnatal growth phenotype has been observed in a reported human case of an *Igf1* gene deletion.³⁰ Igf2 null mice also show diminished pre- and postnatal growth. The *Igf2* gene is imprinted in humans, such that only the paternal gene is expressed. Therefore loss-of-function mutations on the paternal allele cause growth impairment whereas mutations on the maternal allele do not.³¹ When the gene for the type 1 IGF receptor is knocked out (*Igf1r* null mice), the mice are severely growth retarded. In humans, dominant and recessive *IGF1R* mutations are associated with intrauterine growth retardation (IUGR), postnatal growth failure, and learning difficulties³² (see section on Disorders of Childhood Growth for details). The relationship between GH and IGF-1 in controlling postnatal growth was analyzed in mouse mutants lacking GHR, IGF-1, or both. This demonstrated that GH and IGF-1 promote postnatal growth by independent and common functions.

Thyroid Hormone. Thyroid hormone is required for normal linear growth. Newborns with hypothyroidism are of normal birth length, suggesting that fetal growth is not greatly affected by fetal thyroid hormone production. In contrast, severe, long-standing postnatal hypothyroidism can impair linear growth and delay skeletal maturation.³³ Mutations in either thyroid hormone receptor-alpha or -beta cause some impairment in linear growth, suggesting that both of these receptors contribute to linear growth regulation.^{34,35} Thyroid hormone has both a direct effect on growth plate chondrocytes, particularly supporting chondrocyte hypertrophy, and an indirect effect, mediated by a stimulatory effect on growth hormone secretion.³⁶

Glucocorticoids. Glucocorticoids, when present in concentrations that exceed physiological levels, impair linear growth. This linear growth impairment arises in part from a direct effect of glucocorticoids on growth plate chondrocyte proliferation.³⁶ Glucocorticoids may also indirectly affect growth plate chondrogenesis by altering GH secretion. However, not all studies show an effect, and any effects may be caused by the glucocorticoid-induced obesity. Circulating concentrations of IGF-1, a marker and mediator of GH action, do not appear to be reduced by glucocorticoid excess, which would be consistent with the idea that the decreased GH concentrations are primarily caused by obesity. The inability of GH treatment to fully compensate for the linear growth impairment caused by glucocorticoid excess³⁷ further suggests that the growth impairment is primarily caused by a direct action of glucocorticoids on growth plate chondrocytes.

Estrogens. In adolescents, both male and female, estrogens stimulate linear growth, contributing to the pubertal growth spurt.³⁶ In boys, the estrogen that stimulates the adolescent growth spurt is produced by aromatization of testicular and adrenal androgens. The growth acceleration is mediated in part by the stimulatory effect of estrogens on GH secretion. In addition, estrogen appears to have direct actions on growth plate chondrocytes.³⁶ Estrogen stimulates growth plate senescence, the developmental program in the growth plate that is responsible for the progressive slowing of linear growth with age. Consequently, estrogen exposure leads to more rapid “aging” of the growth plate, earlier growth cessation, and earlier epiphyseal fusion. This effect of estrogen accounts for the early epiphyseal

fusion in children with precocious puberty and the late epiphyseal fusion in individuals with hypogonadism, aromatase deficiency, and estrogen resistance.

Thus the effect of estrogen on linear growth is a “two-edged sword” in that it has two opposing effects on adult height. Estrogen stimulates the linear growth rate, which would be expected to increase adult height, but also accelerates growth plate senescence, which would be expected in decreased adult height. In general, the latter effect predominates. As a result, untreated precocious puberty can cause initial childhood tall stature but eventual adult short stature, whereas untreated hypogonadism or delayed puberty can cause adolescent short stature but eventual adult tall stature. These concepts also suggest that gonadotropin-releasing hormone (GnRH) analogues and aromatase inhibitors would increase adult height (see section on Treatment of Short Stature, later in this chapter).

Androgens. Androgens also can accelerate linear growth and thus contribute to the pubertal growth spurt. Androgens, secreted by the gonads or adrenal glands can be aromatized to estrogens in various peripheral tissues, including adipose tissue and thereby affect linear growth. Aromatase is also expressed in growth plate cartilage³⁸; thus local conversion into estrogen may occur. In addition to androgen’s role as a precursor for estrogen, androgen per se appears also to stimulate linear growth. For example, dihydrotestosterone, which is not aromatizable, appears to accelerate linear growth in adolescent boys.³⁹ Animal and in vitro studies suggest that these effects are mediated, at least in part, through a direct effect on the growth plate.³⁶

Insulin. Insulin positively regulates human fetal growth. Consequently, fetal growth retardation occurs in individuals with decreased fetal insulin production because of permanent neonatal diabetes mellitus⁴⁰ and those with decreased insulin sensitivity because of severe biallelic insulin receptor defects.⁴¹ Conversely, increased fetal growth occurs in pregnancies complicated by gestational diabetes. This macrosomia, which primarily involves weight rather than length,⁴² is thought to be caused by the high glucose concentrations stimulating high fetal insulin concentrations.

Nutritional Regulation of Linear Growth

Inadequate nutritional intake causes functional GH insensitivity, with decreased circulating IGF-1 concentrations and increased GH concentrations. Proposed mechanisms include decreased insulin concentrations, causing downregulation of GHR expression^{43,44} and increased FGF-21 concentrations, causing a decrease in hepatic GHR and phosphorylated STAT5 (which is involved in GH signal transduction).^{44,45} The increased FGF-21 concentrations in malnutrition may impair GH sensitivity not only in the liver but also in the growth plate.⁴⁵ In addition, undernutrition can decrease thyroid hormone concentrations, as part of the nonthyroidal illness syndrome,⁴⁶ increase cortisol concentrations,⁴³ and decrease sex steroids (mediated by decreased leptin and hence gonadotropins),^{43,44} all of which may contribute to the decrease in linear growth.

Conversely, overnutrition can stimulate linear growth. Obese children tend to be tall with an increased bone age.^{45,47,48} Because of the accelerated skeletal maturation and, in girls, the earlier puberty, there is little effect on adult height.⁴⁹ Proposed mechanisms by which obesity affects linear growth include increased levels of estrogens because of peripheral aromatization and increased free IGF-1, leptin, prolactin, and adrenal androgens.⁴⁵

Cytokine Regulation of Linear Growth

Impaired linear growth often occurs in children with systemic inflammatory disorders, including juvenile idiopathic arthritis (JIA), IBD, and cystic fibrosis. The underlying mechanisms are likely complex and involve multiple mediators, including malnutrition and glucocorticoid excess. In addition, there is evidence that elevated levels of proinflammatory cytokines contribute to the poor growth. Various proinflammatory cytokines, including tumor necrosis factor- α (TNF- α), IL-1 β , and IL-6, act directly on growth plate chondrocytes to suppress bone growth.^{50,51} Proinflammatory cytokines may also act indirectly on the growth plate by decreasing circulating IGF-1, sex steroid, and thyroid hormone concentrations.^{50,52} The effects of these cytokines may be mediated in part by upregulation of an intracellular protein called SOCS, which decreases GHR signaling.

Autocrine/Paracrine Regulation of Linear Growth

Many different autocrine/paracrine factors are secreted by growth plate chondrocytes and act locally on other chondrocytes. These factors serve to orchestrate the complex processes of proliferation and differentiation in the growth plate. Consequently, genetic defects in these local growth factors, their receptors, or other genes involved in growth factor signaling can impair growth plate chondrogenesis and therefore linear growth of the child. Depending on the specific paracrine system involved and the severity of the genetic abnormality, the bones might be both short and malformed, presenting clinically as a chondrodysplasia, or short but not malformed, presenting clinically as isolated short stature.⁵³ Following are some examples of autocrine/paracrine growth factors that regulate growth plate chondrocytes and are clinically important.

C-Type Natriuretic Peptide. C-type natriuretic peptide (CNP) was named based on its structural similarity to atrial and brain natriuretic peptides. However, CNP serves different functions. It acts locally in the growth plate to stimulate chondrocyte proliferation and hypertrophy. Consequently, loss-of-function mutations in NPR2, the receptor for CNP, cause short stature whereas gain-of-function mutations cause tall stature (see section on Disorders of Childhood Growth for details).⁵⁴ CNP analogues are in clinical trials to augment bone growth in achondroplasia (ACH).

Fibroblast Growth Factors. FGFs, acting through FGF receptor-3 (FGFR3), negatively regulate growth plate chondrogenesis. As a result, gain-of-function mutations in FGFR3 impair linear growth. Depending on the severity of the mutation, the clinical presentation can include several skeletal dysplasias with severe short stature. Recent evidence suggests that milder gain-of-function mutations can present as isolated short stature without an evident skeletal dysplasia.⁵⁵ Conversely, loss-of-function mutations cause tall stature.⁵⁶

Insulin-like Growth Factors. IGF-1 and -2 act through the receptor IGF1R to stimulate growth of many tissues. In the growth plate, they act on chondrocytes to stimulate proliferation and hypertrophy. IGF-1, produced primarily in the liver, acts in an endocrine fashion on the growth plate (see section Regulation of Linear Growth-Endocrine Regulation of Linear Growth), but IGF-1 is also thought to be produced locally in the growth plate, by cells in the perichondrium and/or chondrocytes, and act locally on chondrocytes as a paracrine factor.⁵⁷ In rodents, both endocrine and paracrine effects are important, but the relative importance in humans is unclear. IGF-2 acts primarily as a paracrine factor and is expressed at high levels in fetal tissues. The *IGF2* gene is

imprinted and consequently is expressed only by the paternal allele. In humans, mutations in IGF1, IGF2, and IGFR1 can all impair both fetal and postnatal growth (see section on Disorders of Childhood Growth for details)^{31,58} indicating that both IGF-1 and IGF-2 are important for growth before and after birth.

Parathyroid Hormone-Related Protein and Indian Hedgehog. Parathyroid hormone-related protein (PTHrP) is expressed in the chondrocytes near the ends of the long bones in the embryo and in the resting zone of the growth plate postnatally. It diffuses down through the growth plate. PTHrP acts through PTH1R (the same receptor used by PTH) and, in part, through Gs-alpha, to prevent proliferative zone chondrocytes from undergoing hypertrophic differentiation until they reach a sufficient distance from the source of PTHrP. Indian hedgehog (IHH) is produced by chondrocytes that have just begun hypertrophic differentiation. IHH stimulates production of PTHrP, forming a negative feedback loop.⁵⁹ Mutations in genes that encode components of this system impair bone growth. For example, loss-of-function mutations in PTH1R cause Blomstrand lethal chondrodysplasia, and gain-of-function mutations in PTH1R cause Jansen metaphyseal chondrodysplasia. Similarly, loss-of-function mutations in IHH cause brachydactyly type A1⁶⁰ or short stature with nonspecific skeletal abnormalities,⁶¹ and loss-of-function mutations in Gs-alpha cause Albright hereditary osteodystrophy. Mutations in genes affecting subsequent steps in the PTHrP signal transduction pathway cause acrodyostosis.

Cartilage Matrix Regulation of Linear Growth

Growth plate chondrocytes secrete a cartilage matrix that serves a structural function, allowing the growth plate cartilage to bear the considerable mechanical forces required of the skeletal system. The cartilage matrix also provides the milieu through which endocrine and paracrine factors must travel to reach growth plate chondrocytes. This matrix is a complex mixture of molecules, including multiple types of collagen, for example, collagen types II and X, and proteoglycans, such as aggrecan. Mutations in many of the encoding genes, for example, *COL2A1*, *COL10A1*, and *ACAN* (aggrecan), cause chondrodysplasias. Heterozygous mutations in *ACAN* can also present as short stature with advanced bone age (see section on Disorders of Childhood Growth for details). Mutations in the collagen 1 genes, *COL1A1* and *COL1A2*, cause osteogenesis imperfecta. Affected patients have bone fragility, reflecting the important role of collagen type I in bone matrix, but also can have short stature.

Intracellular Regulation of Linear Growth

Proliferation and hypertrophic differentiation of growth plate chondrocytes is also under complex control by intracellular factors. For example, the short stature homeobox-containing gene (*SHOX*) encodes a transcription factor that is required for normal growth plate function and consequently mutations in *SHOX* cause short stature. Another example of an intracellular system that regulates growth plate chondrocyte function is the RAS-mitogen-activated protein kinase (MAPK) pathway. It is a signal transduction system that regulates cell proliferation and differentiation in many tissues. Ligand binding to tyrosine kinase receptors causes signal transduction involving phosphorylation of a series of proteins. Mutations that augment RAS-MAPK signaling lead to Noonan syndrome (NS), which includes short stature.⁶²

Growth Plate Senescence

Linear growth is extremely rapid in the human fetus and progressively slows during infancy and childhood. This deceleration is briefly interrupted by the pubertal growth spurt but then the deceleration resumes, causing growth to eventually cease. The decline in linear growth is caused by a developmental program intrinsic to the growth plates termed *growth plate senescence*. This developmental program includes a functional decline in chondrocyte proliferation and also a gradual involution of the growth plate with a decrease in the number of chondrocytes and a decrease in the height of the growth plate. Growth plate senescence advances until chondrocyte proliferation essentially ceases. At that point, the growth plate is no longer causing bone elongation, and the now-inert cartilage is remodeled into bone, an event termed *epiphyseal fusion*. Thus epiphyseal fusion is not the cause, but rather the effect, of growth cessation.⁶³ The bone age (usually assessed by a radiograph of the nondominant hand and wrist) serves as a radiological marker for growth plate senescence. As a consequence, the bone age provides an estimate of how far senescence has proceeded in a child and therefore how much of the child's growth potential has been expended and how much remains. For this reason, the bone age is useful for prediction of adult height.

Growth-inhibiting conditions, such as malnutrition, hypothyroidism, and GH deficiency, generally slow not only the growth rate, but also the rate of growth plate senescence. This delay in growth plate senescence is reflected by the delay in the bone age. From an evolutionary standpoint, this delay in growth plate senescence likely serves an important function in the malnourished child, allowing growth to slow to conserve nutrients, while conserving much of the growth potential for better times.

In contrast, estrogen accelerates growth plate senescence, explaining the advanced bone age and loss of adult height in children with precocious puberty and explaining the delayed bone age and increase in adult height of adolescents with hypogonadism, aromatase deficiency, and estrogen resistance. For example, in a man with biallelic mutations in estrogen receptor-alpha, linear growth continued at a slow rate well into adulthood, resulting in tall stature, and epiphyseal fusion occurred at 35 years of age.⁶⁴ This effect of estrogen on growth plate senescence also explains the potential therapeutic use of GnRH analogues⁶⁵ and aromatase inhibitors⁶⁶ to increase adult height.

Catch-Up Growth

Catch-up growth occurs in children following a period of growth inhibition.⁶⁷ Linear growth can be inhibited by many different conditions, including malnutrition, hypothyroidism, growth hormone deficiency, and glucocorticoid excess. If the condition persists long enough, the child's height standard deviation score (SDS) for age will decline (decreasing percentile on height curve). Often the growth-inhibiting condition will eventually resolve, for example, if food becomes more available, thyroid hormone or growth hormone replacement is initiated, or pharmacological glucocorticoids can be stopped. In this situation, the child's linear growth rate usually does not just increase to equal the normal growth rate for age. Instead, the child's growth rate exceeds normal, causing the height SDS to improve. This catch-up growth is often not complete in that the child's height SDS does not return all the way back to the value before the growth inhibition, and the adult height may still be somewhat compromised.

For some time, it was thought that catch-up growth results from a mechanism within the CNS that assesses the child's actual body size, compares this actual body size to

age-appropriate set point, and then adjusts the growth rate accordingly.⁶⁸ After a period of growth inhibition, this hypothesized mechanism would sense the height deficit and increase the growth rate to cause catch-up growth. However, more recent evidence suggests that the primary mechanism responsible for catch-up growth resides not in the CNS but rather in the growth plates and involves delayed growth plate senescence. Growth-inhibiting conditions slow growth plate senescence. When the growth-inhibiting condition resolves, the growth plates are less senescent than normal and therefore grow more rapidly than normal and for a longer period of time, causing catch-up growth.⁶⁹ In some conditions, the rate of human catch-up growth fits this hypothesis, but in others conditions the high initial rate of catch-up growth suggests the possibility of other contributing mechanisms.^{70,71}

Normal Variation in Stature

Childhood growth patterns and adult height are the result of interactions between genetic and environmental factors. Since Francis Galton's famous study in 1885, it has been known that a child's height is strongly correlated with the height of his or her parents. Later large-scale studies focusing on height variability between twins showed that environmental factors have greater importance during early childhood, whereas genetic factors have a more decisive influence on the height of adolescents and adults.⁷² Recently, the specific genes that control normal height variation have begun to be elucidated by genome-wide association studies (GWAS), which search for statistical associations between adult height and single nucleotide polymorphisms. These studies have identified common allelic variants (usually with a minor allele frequency >5%) that individually exert only a small phenotypic effect, but together determine much of the variability of height.⁷³ This approach was used to identify variants in more than 700 loci that have a significant influence on height. Each of these variants is responsible for approximately 1 mm in height variability per allele and in combination, explain approximately 25% of the heritability in adult height.^{74,75} In addition, 83 lower-frequency coding variants (minor allele frequency ≤1%) have been identified as being responsible for up to 2 cm of height per allele.⁷⁶

Clinical Evaluation of Growth

Measurement

Accurate measurement is the critical cornerstone of all growth evaluations. Although it is often dismissed as simple (after all, how many parents track their kids' growth on a wall in their homes?), two factors make it in fact challenging. First, because growth is defined as the change in length or height over a certain period of time, the two measurements at each end of that time interval must be performed according to identical technique; any deviations by one or the other measurer can introduce artifactual microaccelerations or microdecelerations into the calculated growth that become amplified errors when the velocity is annualized for analysis (Box 11.1). Second, intrinsic to identical technique is correct positioning each and every time, which can be challenging with an infant or child who is fidgety, anxious, bored, developmentally unable to understand or execute the instructions, or physically challenged in achieving and maintaining the correct position (e.g., because

of hypotonia, hypertonia, contractures, balance issues, leg length asymmetry, malformations, injury, or illness). Further compounding the difficulty in maintaining identical technique each and every time are both personnel issues (the same child is commonly measured by different staff members at serial visits because of the time that has lapsed or different locations of care) and equipment issues (which must be calibrated regularly and used appropriately). A final source of error can occur at the recording stage, when the measurement is entered into the medical record, such as transposing digits, other typographical error, or inadvertently recording the measurement in inches or centimeters when it had been measured in the other unit.

Standing height should be measured with a wall-mounted stadiometer. The child should be standing erect against the wall or backboard with shoes off, any interfering hair accessories removed, and the head in the Frankfurt plane (the lower margins of the orbits and the upper margins of the ear canals all lying in the same horizontal plane), that is, looking straight ahead. The head plate, which is lowered on top of the head for the measurement reading, must be firmly fixed such that it is perpendicular to the wall or backboard. Flip-up horizontal bars (floppy arms) on weighing scales, frequently used to measure height, are subject to great errors because of the child's slumping posture and considerable variation in the angle of the horizontal bar.

Recumbent length is similarly measured for infants and toddlers using a recumbent length board, composed of a firm platform, a fixed head plate, and a moveable footplate for reading the measurement. Two people are required for accurate length measurement: one to hold the head in the Frankfurt plane, looking straight up, and the second to keep the knees straight and bring the measuring board to the feet, which must also be secured perpendicular at the ankles. Many practitioners, measuring infants unassisted, lie the patient on the paper covering the examining table, mark off with a pen on the paper the positions of the head and feet, remove the patient, and then use a measuring tape to quantify the distance between the two pen markings. This common technique is highly inaccurate because of incorrect positioning of the infant, movement and crumpling of the paper, and failure to get perpendicular markings by the pen.

Despite well-established, generally accepted protocols for measuring children, studies have shown that it is frequently done improperly.^{77,78} Observation of 55 US primary care practices (44 pediatric, 11 family practice) found measurements were considered accurate (defined as ≤0.5 cm) only 30% of the time, and differed from measurements of the same children by the study investigators by an average of 1.3 cm.⁷⁹ Because the average error exceeded the difference between normal (5 cm/year) and subnormal (4 cm/year) growth velocities, measurement error likely contributes to both inappropriate investigation and/or subspecialist referrals of normally growing children and missed detection of true growth problems in others. However, this is readily corrected through training. Randomization of these same 55 practices to educational intervention and control groups found that measurement accuracy rates rose persistently in the intervention group at 3 months (55% vs. 37% in the control group) and 6 months (70% vs. 34% in the control group) later.⁷⁹ Although many busy primary care practices measure patients only once, triplicate measurements are taken (with the average recorded) as a means

BOX 11.1 Annualized Growth Velocity

$$\text{Annualized growth velocity (units/yr)} = \frac{(\text{height at time 2 minus height at time 1}) \times 12 \text{ months}}{\# \text{ months between times 1 and 2}}$$

of improving accuracy when precision is key (e.g., for children undergoing growth evaluations and treatment in endocrine clinics or participating in research studies).

With the proper equipment, experienced personnel, and a cooperative child, the standing height percentile can be assessed with considerable accuracy. In contrast, even with good measurement technique, the error in short-term growth velocity assessment is problematic.⁷⁷ The relative error (absolute error divided by the measurement) is far greater for growth velocity than for height primarily because the denominator for the growth velocity (the small difference between two successive measurements) is so much smaller than the denominator for the height. For example, for a 5-year-old child with an observed height at the third percentile, the 95% confidence interval spans the second to fourth percentile but the confidence interval for a 12-month height velocity spans the eighth to 52nd percentiles.⁷⁷ As a result, even with 1-year growth velocity, children can easily be misclassified as having a normal versus abnormal growth velocity.⁷⁷ Trying to measure growth velocity over periods less than a full year results in a relative measurement error that is even greater. Growth velocity, measured over a year, was found to have little diagnostic validity to identify children with isolated idiopathic GH deficiency or Turner syndrome.⁸⁰ Height velocity had to be measured over a 3-year period to achieve acceptable validity.⁸⁰ Thus overreliance on calculated growth velocity may be misleading. However, it is important for the clinician to identify children with poor height and/or weight gain, which can indicate an important underlying disorder. As an alternative to calculated growth velocities, declines in height and/or weight percentiles on a standard growth chart can provide valuable clues to a diminished growth rate.⁷⁷

Growth Charts

Because normal growth follows a predictable pattern, growth charts are constructed depicting this pattern as sequential centile curves showing the distribution of selected body measurements (usually length or height, weight, and weight-for-length or body mass index [BMI]) in children of a given country. Growth is fastest during the fetal period and gradually slows during infancy, with an approximate length increase of 25 cm over the first year of life and 10 to 15 cm over the second year. During infancy, children commonly cross length centiles (physiological rechanneling) as they transition from prenatal to postnatal growth determinants; maternal and pregnancy health and nutrition have a major impact on the former, whereas the latter normally aligns with the child's genetic height potential. After the second birthday, the linear growth rate continues to decline, but more gradually, and shifting centile channels is abnormal. With the onset of puberty, statural growth accelerates, resulting in the pubertal growth spurt, after which the decline in linear growth rate resumes, causing height gain to gradually grind to a halt. However, attainment of adult body composition continues in the transition period that follows.

The population selected for reference is important when judgments are made about the height and growth of an individual. Multiple growth charts have been constructed for US children in past decades, differing in the racial and socioeconomic composition of the reference populations upon which they were based. Growth charts for children of other countries are likewise available, reflecting the different height distributions of the local populations. The most recent and currently recommended US growth charts, published in 2000 by the Centers for Disease Control and Prevention (CDC; available at https://www.cdc.gov/growthcharts/cdc_charts.htm), are based on several cross-sectional data sources and introduced US pediatric

BMI charts. Because the CDC and other national charts are used for clinical judgment despite being reference charts (i.e., observationally describing how children in the populations grow), the World Health Organization (WHO) set out to create growth standard charts (i.e., depicting how healthy children *should* grow). The 2006 WHO growth charts (available at: https://www.cdc.gov/growthcharts/who_charts.htm) are based on longitudinal measurements from birth to age 2 years of 882 children from six different countries, representing racial diversity yet growing under "ideal" conditions, and cross-sectional data on other children after age 2 years. Thus the CDC recommends use of the 2006 WHO growth charts under age 2 years and the 2000 CDC growth charts after age 2 years in assessing growth of US children.⁸¹ One important distinction is that the WHO charts are based on breastfed infants versus the mostly formula-fed infants of the CDC charts, resulting in differently shaped weight curves that can alter the perceived rates of failure to thrive depending on the feeding modality of the infants assessed clinically.

Two special categories of growth charts are also helpful. Velocity charts plot annual increments rather than height (distance) and are tempo adjusted, allowing for the variation in timing and intensity of the adolescent growth spurt. These charts may be helpful to assess the growth patterns of children with slower or more rapid tempo of maturation ("early and late bloomers"), whose variations are effectively lost in the cross-sectional height charts that average growth across peers in different developmental periods.⁸² One limitation to such longitudinal growth charts is that their data were derived from relatively small, nonrepresentative population samples recorded a long time ago. A second limitation is that the large relative error in growth velocity measurements, discussed earlier, creates both uncertainty in the individual child's data and also in the reference data upon which the growth velocity charts were based. Thus based on studies of measurement error, Voss and colleagues concluded, "Although velocity charts are attractive in concept, they seem to be no more discriminating than height charts in practice, and may be clinically deceptive unless interpreted with great care."⁷⁷ Alternatively, growth charts that show height, rather than growth velocity, but are based on longitudinal data, may be helpful for children with slow or rapid tempo of maturation.⁸²

The second special category of growth charts includes specific charts for children with particular genetic syndromes.⁸³ These charts are used in essentially the same way as the charts for normal children. Deviation of growth from the appropriate disease-related growth curve suggests the possibility of a second underlying problem, such as acquired hypothyroidism in a patient with Down or Turner syndrome.

Body Proportions

The skeleton does not grow proportionally. The upper-to-lower body segment ratio starts at 1.7 at birth, indicating a trunk considerably longer than the lower extremities. However, after birth, the lower extremities grow more rapidly than the trunk, and the ratio drops to approximately 1.0 by 10 years of age. Early cessation of linear growth, such as that which occurs in precocious puberty, results in persistently childlike proportions (short limbs compared with the trunk), whereas prolonged growth, such as occurs in hypogonadism, creates a long-limbed habitus.⁸⁴ Limb to trunk proportions can be assessed by three different techniques, all incorporating standing height measurement for comparison. For the upper-to-lower segment ratio, the lower body segment is measured from the upper border of the pubic symphysis to the floor while the patient is standing straight. This lower segment length is subtracted from the standing height to determine the upper segment length and

then divided by the lower segment length to determine the ratio, which can be compared with reference data.⁸⁵ The second technique measures the arm span (between the tips of the middle fingers with the arms fully extended perpendicular to the torso, again with the child standing straight against the wall) for comparison with the standing height. Normally arm span is less than standing height before age 8 years, equal to height at ages 8 to 12 years, and greater than height above age 12 years.⁸⁵ The third technique measures the sitting height, which is divided by the standing height to yield the sitting

height index. The sitting height index is sensitive for detecting disproportionate growth of the trunk (vertebrae) versus the lower extremities and can be used to screen for specific causes of disproportionate short stature, such as SHOX deficiency and hypochondroplasia.⁸⁶ Excellent reference data are available from NHANES III^{87,88} (Table 11.2).

Disproportions can occur not only between the limbs and trunk but also in specific limb segments, categorizing patients into specific diagnostic groups. Rhizomelia refers to disproportionate shortening of the proximal limbs (humerus and

TABLE 11.2 Mean, Standard Deviation, and Percentiles of Sitting Height Index (Sitting Height/Stature \times 100) by Age for Males and Females of 2 to 90 Years

Age Group (years)	Mean Age (years)	Percentiles											
		N	M	SD	5th	10th	15th	25th	50th	75th	85th	90th	95th
MALES													
2.0–2.9	2.46	619	56.9	2.01	53.7	54.4	54.9	55.6	56.9	58.3	59.0	59.6	60.3
3.0–3.9	3.45	508	55.5	1.8	52.6	53.2	53.6	54.3	55.6	56.8	57.5	58.0	58.7
4.0–4.9	4.47	553	54.4	1.8	51.6	52.2	52.6	53.2	54.4	55.6	56.3	56.7	57.4
5.0–5.9	5.43	496	53.5	1.7	50.8	51.4	51.8	52.4	53.5	54.6	55.3	55.7	56.3
6.0–6.9	6.45	262	52.7	1.6	50.2	50.7	51.1	51.7	52.7	53.8	54.4	54.8	55.5
7.0–7.9	7.47	273	52.1	0.0	49.7	50.2	50.6	51.1	52.2	53.2	53.8	54.2	54.8
8.0–8.9	8.46	267	51.7	0.1	49.3	49.8	50.2	50.7	51.7	52.8	53.3	53.7	54.3
9.0–9.9	9.50	281	51.4	0.1	49.0	49.5	49.9	50.4	51.4	52.4	53.0	53.4	53.9
10.0–10.9	10.45	288	51.2	1.5	48.8	49.3	49.7	50.2	51.2	52.2	52.8	53.1	53.7
11.0–11.9	11.44	275	51.0	1.4	48.7	49.2	49.6	50.1	51.1	52.1	52.6	53.0	53.6
12.0–12.9	12.47	204	51.0	1.4	48.7	49.2	49.5	50.0	51.0	52.0	52.6	52.9	53.5
13.0–13.9	13.47	192	51.0	1.4	48.7	49.2	49.5	50.0	51.0	52.0	52.6	52.9	53.5
14.0–14.9	14.49	186	51.1	1.5	48.7	49.2	49.6	50.1	51.1	52.1	52.6	53.0	53.6
15.0–15.9	15.45	182	51.2	1.5	48.8	49.3	49.6	50.2	51.1	52.2	52.7	53.1	53.7
16.0–16.9	16.45	196	51.3	1.5	48.9	49.4	49.7	50.3	51.3	52.3	52.8	53.2	53.8
17.0–17.9	17.45	191	51.4	1.5	49.0	49.5	49.8	50.4	51.4	52.4	53.0	53.4	53.9
18.0–18.9	18.45	172	51.6	1.5	49.1	49.6	50.0	50.5	51.5	52.6	53.1	53.5	54.1
19.0–19.9	19.43	160	51.7	1.5	49.2	49.7	50.1	50.6	51.7	52.7	53.3	53.7	54.3
20.0–29.9	24.96	1620	52.3	1.5	49.8	50.3	50.7	51.2	52.2	53.3	53.8	54.2	54.8
30.0–39.9	34.72	1452	52.4	1.6	49.9	50.4	50.8	51.3	52.4	53.4	54.0	54.4	55.0
40.0–49.9	44.35	1195	52.2	1.5	49.9	50.4	50.8	51.3	52.3	53.4	53.9	54.3	54.9
50.0–59.9	54.89	835	52.1	1.5	49.8	50.3	50.7	51.2	52.2	53.3	53.8	54.2	54.8
60.0–69.9	64.83	1129	51.9	1.5	49.7	50.2	50.6	51.1	52.1	53.1	53.6	54.0	54.5
70.0–79.9	74.16	798	51.7	1.4	49.5	50.0	50.4	50.9	51.8	52.8	53.3	53.7	54.2
80.0–90.9	84.09	566	51.5	1.4	49.3	49.8	50.1	50.6	51.5	52.5	53.0	53.3	53.9
FEMALES													
2.0–2.9	2.45	584	56.9	1.9	52.9	53.5	54.0	54.7	55.9	57.2	57.9	58.4	59.1
3.0–3.9	3.46	586	55.3	1.8	52.1	52.7	53.2	53.8	55.0	56.2	56.9	57.4	58.1
4.0–4.9	4.43	528	54.1	1.6	51.5	52.1	52.5	53.1	54.3	55.4	56.1	56.5	57.2
5.0–5.9	5.46	543	53.2	1.6	50.9	51.5	51.9	52.5	53.6	54.7	55.4	55.8	56.4
6.0–6.9	6.47	273	52.5	1.6	50.4	51.0	51.4	52.0	53.1	54.2	54.8	55.2	55.8
7.0–7.9	7.44	268	52.1	1.4	50.1	50.7	51.0	51.6	52.7	53.7	54.3	54.7	55.3
8.0–8.9	8.47	247	51.8	1.5	49.8	50.4	50.7	51.3	52.3	53.4	54.0	54.3	54.9
9.0–9.9	9.43	273	51.7	1.5	49.6	50.1	50.5	51.0	52.1	53.1	53.7	54.1	54.7
10.0–10.9	10.43	260	51.6	1.5	49.5	50.0	50.3	50.9	51.9	52.9	53.5	53.9	54.4
11.0–11.9	11.46	288	51.6	1.4	49.4	49.9	50.2	50.8	51.8	52.8	53.3	53.7	54.3
12.0–12.9	12.46	221	51.7	1.5	49.3	49.9	50.2	50.7	51.7	52.7	53.3	53.6	54.2
13.0–13.9	13.45	227	51.8	1.5	49.3	49.8	50.2	50.7	51.7	52.7	53.2	53.6	54.2
14.0–14.9	14.47	216	52.0	1.5	49.4	49.9	50.2	50.7	51.7	52.7	53.3	53.6	54.2
15.0–15.9	15.47	189	52.1	1.5	49.4	50.0	50.3	50.8	51.8	52.8	53.3	53.7	54.2
16.0–16.9	16.46	224	52.2	1.5	49.5	50.0	50.4	50.9	51.9	52.9	53.4	53.8	54.3
17.0–17.9	17.45	213	52.4	1.4	49.6	50.1	50.5	51.0	52.0	53.0	53.5	53.9	54.4
18.0–18.9	18.43	186	52.5	1.4	49.8	50.3	50.6	51.1	52.1	53.1	53.6	54.0	54.6
19.0–19.9	19.48	191	52.6	1.3	49.9	50.4	50.8	51.3	52.3	53.2	53.8	54.2	54.7
20.0–29.9	24.91	1842	52.9	1.5	50.2	50.8	51.1	51.7	52.7	53.8	54.3	54.7	55.3
30.0–39.9	34.85	1837	52.8	1.5	50.5	51.0	51.4	51.9	52.9	53.9	54.5	54.9	55.5
40.0–49.9	44.28	1328	52.8	1.5	50.5	51.1	51.4	51.9	52.9	53.9	54.5	54.9	55.4
50.0–59.9	54.83	979	52.8	1.5	50.4	50.9	51.3	51.8	52.8	53.8	54.3	54.7	55.2
60.0–69.9	64.82	1110	52.3	1.5	50.1	50.6	50.9	51.4	52.4	53.4	54.0	54.3	54.9
70.0–79.9	74.46	893	52.0	1.5	49.5	50.0	50.4	50.9	51.9	52.9	53.5	53.9	54.4
80.0–90.9	84.45	599	51.1	1.5	48.8	49.3	49.7	50.2	51.2	52.2	52.8	53.2	53.8

M, Mean; SD, standard deviation.

(From Frisancho, A.R., *Anthropometric Standards: An Interactive Nutritional Reference of Body Size and Body Composition for Children and Adults*, The University of Michigan Press, Ann Arbor, MI: 2008. With permission.)

femur), *mezomelia* to disproportionately shortened middle portion of the limbs (radius/ulna and tibia/fibula), and *acromelia* to disproportionately shortened distal limbs (hands and feet). Thus every child who presents with a growth problem should be evaluated for disproportionality because this information helps to narrow the differential diagnosis, including the possibility of skeletal dysplasias.⁸⁹

Skeletal Maturation

Normal variation in rates of maturation among children and adolescents impacts growth prognosis, as children with more time to grow can achieve taller adult heights. An anteroposterior (AP) radiograph of the nondominant hand and wrist is used to determine the child's bone age as a quantitative determination of somatic maturation. The bone age assesses the degree to which the skeleton has ossified. In the human embryo, the skeleton first forms out of cartilage, and then later the cartilage is remodeled into bone. This process continues throughout childhood. In the long bones, this ossification begins as a primary ossification center in the middle of the bone shaft and then spreads outward. Later, secondary ossification centers form in the epiphyses and also spread outward. However, the primary and secondary ossification centers remain separated during childhood by a thin layer of cartilage, termed the *growth plate*, which is responsible for bone elongation and therefore height gain. In adolescence, as growth comes to a halt, the growth plate itself is finally ossified, an event termed *epiphyseal fusion*, leaving cartilage only at the articular surface. Because bone is more radiopaque than cartilage or soft tissues, a radiograph of the left hand and wrist reveals how far along the ossification has proceeded in an individual child. The image is then compared with published standards (Greulich and Pyle⁹⁰ or Tanner-Whitehouse [TW]⁹¹). There are separate sets of standards for boys and girls. If, for example, a patient's bone age image is most similar to the standard for a 9-year-old child, then the patient is said to have a bone age of 9 years. Recently, an automated method to determine bone age has been developed.⁹²

Just like other auxological measures, bone ages have normal ranges, based on age and gender. The normal ranges define the normal variance between bone age and chronological age of a child. Discordance among skeletal centers, with maturity levels differing between bone groups, can be a normal variant. Because bone age assignment is such a subjective measure, it is prudent for pediatric endocrinologists to make their own readings rather than simply rely on radiologists' reports. This will eliminate interreader variability between repeated studies, thereby improving the validity of longitudinally following bone age progression in a child.

The bone age mirrors the tempo of physical development of the child, particularly reflecting the progression of growth plate senescence, and indicates the remaining growth potential. Knee films can be used instead at very young ages when hand/wrist films are not yet informative because of developmentally appropriate lack of ossification. The bone age is also more closely associated than the chronological age with the timing of central puberty in some situations, for example, boys with congenital adrenal hyperplasia, familial male-limited precocious puberty, and idiopathic short stature.⁹³ However, surprisingly, the bone age does not predict pubertal onset better than does chronological age in normal children.^{94,95}

Even before obtaining a bone age, a clinician can surmise a child's rate of maturation by examining their teeth; the degree of dental maturation tends to correlate with skeletal maturation (i.e., delayed dentition can imply delayed bone age).⁹⁶

Skeletal maturation, like skeletal growth, is highly regulated by endocrine, nutritional, and genetic factors. In general,

BOX 11.2 Gender Adjusted Midparental Height Formulae

For males: [(mother's height + 13 cm^a) + father's height] divided by 2
For females: [(father's height - 13 cm^a) + mother's height] divided by 2

^aFor English units, 5 inches can be added or subtracted instead of 13 cm.

factors that positively regulate linear growth, such as thyroid hormone, GH, estrogen, and nutritional intake, also positively regulate skeletal maturation, whereas factors that negatively regulate growth, such as glucocorticoids in high concentration, also negatively regulate skeletal maturation.

Prediction of Adult Height

Adult height predictions are used to try to determine which children are growing toward a normal adult height. The "normal" range for adult height can be defined by the outer centiles demarcated on the country's growth chart, recognizing that the centiles selected for this purpose (e.g., 5th and 95th or 3rd and 97th) are arbitrarily chosen.

Because height is a highly heritable trait, a more specific target can be established from the heights of the child's biological parents. It is important to measure the parents' heights rather than rely on self-reports, which are often inaccurate. The two parents' heights can be combined using the formulae for gender-adjusted midparental height (Box 11.2), which adjust for the 13-cm (5-inch) difference between the mean adult male and female heights, and provide the midparental height ± 2 SD (1 SD would be about 5 cm, or 2 inches) as the "normal" adult height range for their offspring. However, the adjusted midparental height assumes that the child's height is inherited from the parents in a polygenic fashion, with multiple inherited polymorphisms contributing to the child's height. It does not apply to monogenic conditions. If, for example, one of the two parents is short because of a heterozygous mutation in *SHOX* and the other parent is of normal height, the offspring will tend to be either short, like the affected parent, or of normal stature, like the unaffected parent, not halfway in between. Even for polygenic inheritance, empirical data indicate that children of short parents tend not to be as short as their parents and tall children tend not to be as tall as their parents. For example, when the midparental height is -2 SD, the children's heights tend to be closer to -1 SD.⁹⁷

The simplest approach to adult height prediction is to assume that the child's height centile will remain fairly constant until adulthood. However, this method is not accurate and may fail if the child has an advanced or delayed bone age, a condition that affects growth, or an early or late timing of puberty.

There are three popular methods for predicting adult height that take skeletal maturation (i.e., bone age) into account, and all are gender specific. The most commonly used of the three methods, the Bayley-Pinneau prediction tables from 1952,⁹⁸ uses longitudinal data derived from healthy US children living in Cleveland to estimate the fraction of growth remaining at each bone age (by the Greulich-Pyle method⁹⁰). Adult height is predicted by combining the child's bone age with the height measured at the time of the radiographic study. The prediction tables and the prediction error are provided in the Greulich and Pyle Atlas.⁹⁰ The Greulich and Pyle bone age (read either manually or with an automated system) can also be used in a more sophisticated mathematical prediction model (currently available online), which appears to have a slightly greater accuracy than the Greulich and Pyle tables in children with idiopathic

short stature.⁹² The TW method, derived from British children, uses TW standards for bone age assessment, in which each bone in the hand and wrist is assessed individually.⁹¹ In addition to the bone age and measured height, this method factors in chronological age, parental heights, and in girls, the occurrence of menarche. The third method for predicting adult height is the Roche-Wainer-Thissen (RWT) method.⁹⁹ This method takes into account the weight or nutritional status of the child and uses recumbent length instead of standing height (calculated as 1.25 cm greater than standing height). The five predictor variables in the RWT method are recumbent length, weight, bone age, chronological age, and parental heights. An additional method of height prediction has more recently been developed that uses more sophisticated mathematical methods and may show better accuracy than previous methods. It is currently available online.⁹²

Unfortunately, even using bone age, height predictions are not highly accurate. The prediction error is considerable but does decrease with age. For example, using the Bailey-Pinneau tables for girls, the prediction can err for a 9-year-old child by approximately 7.4 cm or 3 inches (2 SD of measurement error), and by approximately 3 cm or 1.2 inches for a 13-year-old child. Furthermore, these prediction methods and the associated error estimates are based on data from healthy children. There may be systematic errors in the setting of illness or in idiopathic short stature. For example, these methods overpredict adult height in untreated precocious puberty, Turner syndrome, and primordial short stature.¹⁰⁰ Sources of inaccuracy in adult height prediction include the inaccuracy of the bone age estimation itself, the inability to predict pubertal onset and tempo, and the difficulty taking into account effects of disease. A small difference in bone age determination can lead to a great difference in adult height prediction, especially during the pubertal growth spurt. A computer simulation, comparison study of three methods for adult height prediction—Bailey-Pinneau, RWT, and Khamis-Roche (calculates adult height without bone age from the child's height, weight, and midparental height, using gender and age-specific coefficients)¹⁰¹—found poor overall agreement among the three methods ($\kappa = 0.21$ for boys and negative for girls) in predicting adult height under the 1.2nd percentile (the threshold for the US Food and Drug Administration [FDA]-approved indication of growth hormone treatment for idiopathic short stature).¹⁰² It is important for both clinicians and patient-families to understand the considerable limitations of height prediction.

Inheritance of Stature

Much of the normal variability of height between individuals is caused by a polygenic influence. Multiple genetic variants, both common and rare, each having a small or moderate effect on linear growth, combine to determine the individual's height.^{74,76} However, short or tall stature, especially when severe or associated with other abnormalities, can also be primarily monogenic, that is, caused by a single variant that has a strong effect on linear growth. These monogenic causes may be identified because they follow classic Mendelian inheritance patterns,¹⁰³ usually autosomal dominant or autosomal recessive and less often X-linked. Clinically, the inheritance pattern is investigated by eliciting a careful family history of the heights of the extended family members, which can be visualized with a written pedigree. For a more detailed discussion, see section on Diagnostic Approach to the Patient with Short Stature later in this chapter. When the pedigree suggests a polygenic inheritance pattern, the clinician can calculate the adjusted midparental height, which provides an estimate of the likely adult height of the child, based on the

heights of the two parents. This topic is discussed in more detail in a previous section of this chapter: Clinical Evaluation of Growth—Prediction of Adult Height

DISORDERS OF CHILDHOOD GROWTH

Many disorders affect childhood growth, particularly decreasing growth to cause short stature. Many of the major causes are discussed later. However, the list is not exhaustive. An extensive, organized list can also be found within the International Classification of Pediatric Endocrine Diagnoses (ICPED, <http://www.icped.org/>).¹⁰⁴

Short Stature

Short stature results from decreased growth plate chondrogenesis. The decreased chondrogenesis can be caused by factors intrinsic to the growth plate (primary growth impairment) or can be secondary to factors elsewhere in the body that secondarily affect growth plate chondrocytes (secondary growth impairment) (Fig. 11.4).

Primary growth plate impairment can involve abnormalities in paracrine signaling, cartilage matrix, or intracellular factors. In some conditions, the defect in growth plate chondrogenesis causes bones to be not only short but also malformed, in which case the condition is called a *skeletal dysplasia* or, more precisely, a *chondrodysplasia*, for example, ACH. Often, genetic defects that affect the growth plate also affect the development of other tissues, leading to a syndrome that includes not only short stature but also other congenital anomalies and organ dysfunction, for example, NS or pseudohypoparathyroidism 1A.

Secondary growth impairment can involve nutritional deficiencies, endocrine abnormalities, inflammatory disorders, and extracellular fluid abnormalities. Often these disorders are caused by a defect in some organ system other than the skeleton, such as the kidneys, pituitary, thyroid, or immune system. These disorders occurring elsewhere in the body then lead to an abnormal concentration of some molecules that are needed for normal growth plate chondrocyte function. These molecules can include steroid or polypeptide hormones, proinflammatory cytokines, or small molecules, such as phosphate or hydrogen ion.

Both primary and secondary growth impairment can be monogenic, oligogenic, polygenic, or nongenetic. Some conditions affect fetal growth, and thus the child is born small for gestational age. Other conditions affect only postnatal growth so that the child is born at a size appropriate for gestation age, and the short stature develops subsequently. Yet other conditions affect both prenatal and postnatal growth.

Causes of Short Stature

Secondary Growth Impairment.

Nutritional Deficiencies. Adequate nutrition is required to support optimal statural growth, and there is often a lag between weight deceleration/acceleration and height deceleration/acceleration. Malnutrition can result from insufficient dietary intake, malabsorption or some other gastrointestinal process, or a combination of the two.

Malnutrition is the most common cause of poor growth globally, with insufficient dietary intake (total calorie and/or protein-calorie malnutrition) often related to food insecurity and poverty.^{105,106} Malnutrition is also common in higher socioeconomic communities, when energy demands (e.g., increased because of illness, sports or activities) are not met by intake (e.g., because of unstructured meals, picky eating, fear

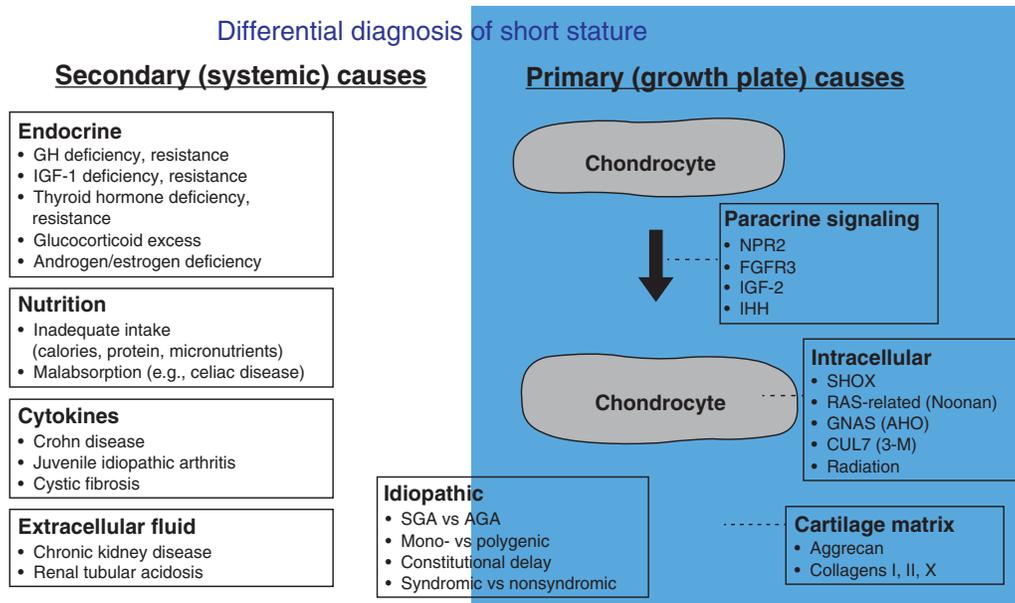


Fig. 11.4 The differential diagnosis of short stature. Short stature is caused by decreased growth plate chondrogenesis, which can result either from a local abnormality, within the growth plate itself, or a systemic abnormality, which then secondarily affects growth plate chondrocytes. The local causes include defects in autocrine/paracrine signaling, cartilage matrix, and intracellular regulatory systems. The systemic causes include endocrine, nutritional, cytokine, and extracellular fluid abnormalities. Idiopathic short stature is likely to be highly heterogeneous in etiology and include both systemic and local mechanisms. For each category, the diagram shows some examples of specific disorders and genes involved but does not present a complete list of known causes.

of obesity).¹⁰⁷ Decreased nutrient intake can result from oropharyngeal malformations (e.g., Pierre Robin sequence, cleft lip/palate), abnormal oral-motor function (e.g., pervasive developmental delay), as well as loss of appetite because of certain medications (e.g., stimulants for treatment of attention deficit hyperactivity disorder or chemotherapeutic agents). Eating disorders (e.g., anorexia nervosa) involve disturbed eating behaviors related to an altered body image or food avoidance because of phobias or sensory aspects of food.¹⁰⁸ Malabsorption can also cause malnutrition, and disorders such as celiac disease, IBD, or cystic fibrosis can present as isolated growth failure.^{109–111} Bone age and puberty are often delayed.

In addition to malnutrition, deficiency of certain micronutrients can impair statural growth. The frequent occurrence of concurrent deficiencies complicates the research and literature on any isolated micronutrient. Although iron deficiency is often found in children with nutritional growth stunting and should be corrected, multiple metaanalyses and systematic reviews of randomized controlled intervention trials of iron supplementation showed that iron therapy had no significant effect on growth in iron-replete children.¹¹² In contrast, human growth retardation (and male hypogonadism) from zinc deficiency was first reported by Prasad and colleagues in 1963,¹¹³ and several, but not all, systematic reviews show height and/or weight increase from zinc supplementation in children.¹¹² Zinc ion is required for activity of over 300 enzymes (zinc metalloenzymes), including DNA polymerase, ribonucleic acid (RNA) polymerase and thymidine kinase, which are important for nucleic acid and protein synthesis and cell division. Bone contains large amounts of zinc where it plays an important role in skeletal development, and zinc deficiency reduces IGF-1 production, cellular IGF-1 responsiveness, and growth response to GH treatment of children who are GH deficient.^{114,115}

Careful assessment of weight and height progression will identify children who are not gaining weight and growing

appropriately. Taking a detailed dietary history is essential to establishing this diagnosis, such as a 3-day diet diary for analysis of any macro- or micronutrient deficits. Treatment is, of course, nutritional repletion, which can involve patient-family education, behavior modification, dietary supplements and in severe cases, tube feedings, as well as treatment of any underlying gastrointestinal issues. However, it is important to have in mind, that several conditions that cause short stature are associated with low BMI (or have low BMI as a component of their phenotype), such as Silver-Russell syndrome (SRS), NS, and Bloom syndrome.

Endocrine Deficiencies

Growth Hormone Deficiency/Insensitivity.

Disorders of GH/IGF axis comprise a large and heterogeneous group of conditions with distinct phenotypes (Table 11.3). Clinical and hormonal evaluations are usually the cornerstones of diagnoses of these disorders.

Growth Hormone Deficiency. GH deficiency is a rare disorder with a prevalence of approximately 1 in 4000 during childhood. GH deficiency may be isolated (IGHD) or combined with other hormone deficiencies, when it is known as combined or multiple pituitary hormone deficiency (CPHD/MPHD). The condition may be congenital or acquired (Table 11.4).¹¹⁶ Early diagnosis and treatment is critical to ensure optimal final height outcomes. The diagnosis of GH deficiency is based on a combination of auxology, exclusion of other pathology leading to short stature, biochemical investigation of the GH-IGF-1 axis, and imaging of the hypothalamopituitary area.⁸⁰ More recently, a molecular diagnosis may be sought in those with “idiopathic” GH deficiency or MPH (see Table 11.1); however, the majority of patients with IGH deficiency or with CPHD remain to be explained in terms of etiology. Before evaluation of the GH-IGF-1 axis, other diagnoses, such as familial short stature, hypothyroidism, Turner syndrome, celiac disease, chronic illness,

TABLE 11.3 Phenotype Caused by Growth Hormone-Insulin-Like Growth Factor Axis Genetic Defects

	Gene	Inheritance	Growth Pattern	Hormone Measurement			Additional Features
				GH	IGF-1	IGFBP-3	
GH deficiency	multiple	AR/AD	varying severity/ postnatal	↓ to ↓↓↓	↓ to ↓↓↓	↓ to ↓↓↓	Midline defects, deficiency of other pituitary hormones
Bioinactive GH	<i>GH1</i>	AR/AD	varying severity/ postnatal	↑↑	↓↓	↓↓	Growth and IGF-1 improve after exogenous GH
GH insensitivity (GHI)	<i>GHR</i>	AR > AD	severe/postnatal	NI to ↑↑	↓ to ↓↓↓	↓ to ↓↓↓	Growth and IGF-1 do not improve after exogenous GH. Low GHBP in 70% of cases
GHI associated with immune dysfunction	<i>STAT5b</i>	AR > AD	severe/postnatal	↑↑	↓↓	↓↓	GHI phenotype with immune dysfunction and elevated PRL
Ternary complex defects	<i>IGFALS</i>	AR	mild/postnatal	NI to ↑	↓↓	↓↓↓	Laboratory abnormality disproportionate to mild short stature, (IGFBP-3 lower than IGF1 concentrations)
IGF1 defects	<i>PAPPA2</i>	AR	varying severity	↑↑	↑↑↑	↑↑↑	Mild microcephaly. Low free IGF-1 ^a
	<i>IGF1</i>	AR/AD	severe/pre- and postnatal	↑↑	↓↓↓↓	NI or ↑	Microcephaly, sensorineural deafness, developmental delay, intellectual disability
IGF2 defects	<i>IGF2</i>	ADp	severe/pre- and postnatal	NI or ↑	NI or ↑	NI or ↑	Phenotype resembling Silver Russell syndrome and low IGF-2 concentrations ^a
Bioinactive IGF-1	<i>IGF1</i>	AR	severe/pre- and postnatal	↑↑	↑↑↑↑	NI	Microcephaly, sensorineural deafness, developmental delay, intellectual disability, insulin resistance ^a
IGF insensitivity	<i>IGF1R</i>	AD > AR	varying severity/pre- and postnatal	NI to ↑↑	NI to ↑↑	NI to ↑↑	Variable clinical findings in the dominant forms. Microcephaly, developmental delay and intellectual disability may be present

^aPhenotype based on a limited number of patients/families.

The listed characteristics are typical of the associated disorders but may be absent in some children with these conditions. ↑, Increase; ↓, decrease; AD, autosomal dominant; ADp, autosomal dominant with paternal transmission; AR, autosomal recessive; GH, growth hormone; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; NI, normal; PRL, prolactin.

TABLE 11.4 Congenital and Acquired Causes of Growth Hormone Deficiency

1. CONGENITAL
- Genetic (see Table 11.2)
- Associated with structural defects of the brain
• Agenesis of the corpus callosum
• Septo-optic dysplasia
• Holoprosencephaly
• Encephalocele
• Hydrocephalus
- Associated with midline facial defects
• Cleft lip/palate,
• Single central incisor
• Cornelia de Lange Syndrome
• CHARGE syndrome
2. ACQUIRED CAUSES
Tumors in and around pituitary area, e.g., craniopharyngioma, germinoma
Metastatic tumors
Cystic lesions
Radiotherapy
Chemotherapy
Brain trauma
Inflammation/Infection, e.g., sarcoidosis, Langerhans cell histiocytosis, tuberculosis meningitis
Infiltration
Pituitary infarction
Psychosocial deprivation

such as Crohn disease or renal failure, and skeletal dysplasias should be excluded. Consensus guidelines on the diagnosis of GH deficiency in childhood were published in 2000,¹¹⁷ (<https://doi.org/10.1210/jcem.85.11.6984>) and suggest investigation into the following scenarios:

- 1) Severe short stature, defined as a height more than 3 SD below the mean.
- 2) Height more than 1.5 SD below the midparental height.
- 3) Height more than 2 SD below the mean and a height velocity over 1 year more than 1 SD below the mean for chronological age, or a decrease in height SD of more than 0.5 over 1 year in children over the age of 2 years.
- 4) In the absence of short stature, a height velocity more than 2 SD below the mean over 1 year or more than -1.5 SD sustained over 2 years, which may occur in GH deficiency presenting in infancy or in organic acquired GH deficiency.
- 5) Signs indicative of an intracranial lesion.
- 6) Signs of MPHID.
- 7) Neonatal symptoms and signs of GH deficiency.

The presentation in the neonatal period can include hypoglycemia, prolonged conjugated hyperbilirubinemia (usually with associated ACTH deficiency), and micropenis. Birth size is typically within the normal range, although there may be a reduction of approximately 10%, which occurs typically late in pregnancy. Growth velocity is reduced during the first year of life in children with severe GH deficiency with growth failure

manifesting by 6 months of age, but the phenotype evolves after 1 year of age in those with milder GH deficiency. The earliest manifestations are a reduction in height velocity followed by a reduction in height SDS adjusted for mean parental height SDS. The child's height SDS will ultimately fall below -2 SD, the time taken depending on the severity and duration of GH deficiency. A child with severe GH deficiency often has midface hypoplasia, hypotonia, a high-pitched voice, immature appearance, delayed dentition, thin sparse hair, slow nail growth, and truncal adiposity. GH deficiency is also associated with effects on cognition. In mice with GH deficiency, reduced spatial learning and memory has been reported whereas in untreated children with GH deficiency IQ, verbal comprehension and processing speed are reduced. In children and adults, the neurocognitive defects in GH deficiency are improved by GH therapy. With prompt recombinant human GH (rhGH) treatment, the outlook for final height is excellent. Other hormone deficiencies may require treatment; in particular those with structural abnormalities of the hypothalamopituitary region are more likely to evolve and develop other hormone deficiencies.

Causes of growth hormone deficiency.

Congenital disorders of hypothalamic development. Hypothalamopituitary dysfunction can arise from congenital malformations of the brain or hypothalamus or of the pituitary gland. Anencephaly results in a pituitary gland that is small or abnormally formed and frequently ectopic. Holoprosencephaly, resulting from abnormal midline development of the embryonic brain, is also typically associated with hypothalamic insufficiency. The clinical spectrum of holoprosencephaly can range from cyclopia to hypertelorism, accompanied by absence of the philtrum or nasal septum and midline clefts of the palate or lip. In these situations, the classic endocrine feature is diabetes insipidus (DI), often accompanied by GH, thyroid-stimulating hormone (TSH), and ACTH deficiencies.

Debate continues as to whether the incidence of GH deficiency is increased in cases of simple clefts of the lip and/or palate alone. Clearly, children with clefts who are growing abnormally require further evaluation, and both GH deficiency and CPHDs are more frequent in this cohort. SOD is a rare, congenital, heterogeneous anomaly with a prevalence ranging from 6.3 to 10.9 per 100,000.^{118,119} The condition is defined by the presence of any two of three features: midline forebrain defects, such as absence of the septum pellucidum and/or corpus callosum; optic nerve hypoplasia (ONH); and hypopituitarism caused by hypothalamopituitary maldevelopment. De Morsier, in 1956, described the postmortem findings of ONH and agenesis of the septum pellucidum and coined the term *SOD*, also known as *De Morsier syndrome*. Approximately 30% of patients with SOD manifest the complete clinical triad, 62% of patients have some degree of hypopituitarism, and 60% have an absent septum pellucidum.¹²⁰ The condition is equally prevalent in males and females. ONH may be unilateral or bilateral and may be the first presenting feature, with the later onset of endocrine dysfunction. Bilateral ONH is more common (88% as compared with 12% unilateral cases). In addition, there appears to be little correlation between the size of the optic nerve and its visual function. Neuroradiological abnormalities are present in up to 75% to 80% of patients with ONH. Pituitary hypoplasia may manifest as endocrine deficits varying from isolated GH deficiency to panhypopituitarism. There has been some suggestion that abnormalities of the septum pellucidum and hypothalamopituitary axis on neuroimaging can predict the severity of endocrine dysfunction. A decrease in growth rate because of GH deficiency is the most common feature, with hypoglycemia and polyuria and polydipsia being less common. Either sexual precocity or failure to develop in puberty may occur. Abnormal

hypothalamic neuroanatomy or function and DI may be a feature. The endocrinopathy evolves with a progressive loss of endocrine function over time. The most common endocrinopathy is GH deficiency followed by TSH and ACTH deficiency. Gonadotropin secretion may be retained in the face of other pituitary hormone deficiencies. Neurological deficit is common, but not invariably so, and deficits range from global retardation to focal deficits, such as epilepsy or hemiparesis. Other neuroanatomic abnormalities include cavum septum pellucidum, cerebellar hypoplasia, schizencephaly, and aplasia of the fornix. An association between SOD and other congenital anomalies, such as digital abnormalities, is not uncommon.

The etiology of SOD has remained largely unknown until recently. Both genetic and environmental factors have been implicated in the etiology of the condition.^{121,122} Mutations in genes implicated in forebrain, eye, and pituitary development account for a small number of patients with SOD, and these genes include *HESX1*, *SOX2*, *OTX2*, and *TCF7L1*.^{1,123} Environmental agents, such as viral infections, vascular or degenerative changes, and exposure to alcohol or drugs have also been implicated in the etiology of SOD. The condition presents more commonly in children born to younger mothers and clusters in geographic areas with a high frequency of teenage pregnancies. The overall frequency of genetic mutations in SOD has, however, been low suggesting that mutations in other known or unknown genes may contribute to this complex disorder.

Recently, the Sonic Hedgehog (SHH) signaling pathway has been implicated in more complex disorders of pituitary development. Mutations within SHH are associated with holoprosencephaly.¹²⁴ Gli transcription factors are involved in hedgehog signal transduction, and heterozygous loss-of-function mutations in *GLI2* have been identified in patients with holoprosencephaly.¹²⁵ Phenotypic penetrance is variable. In several affected individuals with *GLI2* mutations, pituitary gland function was abnormal, accompanied by variable craniofacial abnormalities. Other features included postaxial polydactyly, single nares, single central incisor, and partial agenesis of the corpus callosum. More recently, mutations in *GLI2* have been associated with hypopituitarism in the absence of any midline defects.¹²⁶

SOX2 and *SOX3* are members of the SOX (SRY-related high mobility group [HMG] box) family of transcription factors and are early markers of progenitor cells; their expression is down-regulated as cells differentiate. Initially *SOX2* mutations had been associated with bilateral anophthalmia, severe microphthalmia, learning difficulties, esophageal atresia, and genital abnormalities. However, further phenotypic characterization has revealed the presence of anterior pituitary hypoplasia, hypogonadotrophic hypogonadism, and variable GH deficiency, often with accompanying phenotypes, such as hippocampal abnormalities, corpus callosum agenesis, esophageal atresia, hypothalamic hamartoma, and sensorineural hearing loss.^{127,128} Although, in the majority of patients, the magnetic resonance imaging (MRI) reveals a small anterior pituitary, in occasional cases, the pituitary is enlarged and remains so for years.¹²⁹

Normal hypothalamopituitary development is critically dependent upon the dosage of *SOX3*; over- or underdosage can lead to hypopituitarism or infundibular hypoplasia. In humans, *SOX3* duplications or mutations are associated with variable phenotypes, including either IGHD or panhypopituitarism. There is variable developmental delay, and MRI usually reveals a small anterior pituitary, an ectopic/undescended posterior pituitary, and dysgenesis of the corpus callosum.¹³⁰ Deletion of *SOX3* has recently been associated with a persistent craniopharyngeal canal in association with hypopituitarism.

Trauma of the brain and/or hypothalamus. Traumatic brain injury (TBI) has been recognized as a cause of acquired hypopituitarism in a number of adult studies. Data on pediatric patients are still sporadic, but there is a growing awareness that hypopituitarism after TBI is underdiagnosed with possible negative effects on growth and development.¹³¹ Although the pituitary gland is protected within the sella turcica, the rich vascular network of the hypothalamus and pituitary and the structure of the pituitary stalk make it vulnerable to the effects of traumatic brain injury. The hypothalamus and pituitary have a complex vascular supply consisting of long hypophyseal vessels and a rich network of portal capillaries that surround the pituitary and infundibulum. The pathophysiology of hypopituitarism related to TBI is not clearly defined, but it is thought that it is the result of direct trauma or of vascular injury resulting in ischemia and infarction. This is supported by the anatomic findings of autopsies following head trauma, which include anterior lobe necrosis, pituitary fibrosis, hemorrhage, infarction, or necrosis of the pituitary stalk.¹³²

It is of note that the peripheral layer of anterior pituitary cells, under the capsule, receive arterial blood from the capsule and not from the system of portal veins, and this may explain why these cells and those in a small area adjacent to the posterior lobe are the only surviving cells in cases of pure anterior lobe necrosis.¹³³ Somatotrope cells are located in the wings of the pituitary gland, their vascular supply comes from portal vessels, and they are vulnerable to the disruption of blood supply after head injury. On the other hand, ACTH and TSH secreting cells are located in the medial portion of the pituitary and receive blood supply from portal vessels and the anterior pituitary artery. This may explain why GH deficiency is the most common deficiency seen after TBI. Hormone deficiencies may be identified in the first days to weeks posttrauma (acute phase) or may develop over time (late effect). As there is overlap between the symptoms and signs of hypopituitarism and those of neurological-psychological sequelae of TBI, it is possible that late-evolving or partial deficiencies can remain undiagnosed for years. It is not surprising, therefore that, in different studies, the time to diagnosis ranges from 1 to 40 years. It would appear that the majority of patients show a degree of pituitary hormone dysfunction in the first days after TBI (53%–76%); there is, however, wide variation in the reported hormone responses, which reflects the differences in patient selection and time of testing. All anterior pituitary hormones can be affected. Pituitary hormone deficiencies presenting in the acute phase are usually transient, but they may persist or appear and evolve over time. In adults, the incidence of permanent hypopituitarism ranges between 23% and 69% depending on the study. The GH axis is the most frequently affected (10%–33%), followed by the gonadal (8%–23%), adrenal (5%–23%), and thyroid (2%–22%) axes. The prevalence of permanent DI varies between 0% and 6%.^{134–137}

Until recently, there were only sporadic reports of hypopituitarism following TBI in children, but prospective studies designed to better characterize this disorder in the pediatric and adolescent population are in progress. The incidence of hypopituitarism is reported to range from 10% to 60% and, although this is lower in children as compared with adults, it is not uncommon.^{138–140} In general, the long-term outcome of TBI seems to be more favorable in children. Patients with hypopituitarism after head injury may have no clinical signs and symptoms suggestive of this disorder and prompt diagnosis requires a high degree of suspicion. A consensus guideline on the screening of patients post-TBI suggests that all patients who had TBI, regardless of its severity, should undergo baseline endocrine evaluation 3 and 12 months after the event or discharge from intensive treatment unit.¹⁴¹ For children and adolescents, an

algorithm for endocrine assessment and follow-up has also been suggested.¹³¹ However, a recent study has reported that permanent hypopituitarism is uncommon after severe brain injury in young children when stringent clinical criteria for pituitary insufficiency are used. Routine testing of pituitary function, as suggested for adults, may not be necessary in children and may lead to a high number of abnormal test results.

Perinatal trauma to the brain, hypothalamus, pituitary stalk, or anterior pituitary may result in isolated GH deficiency or in multiple deficiencies of the anterior pituitary, as may child abuse. Many published series of patients with GH deficiency indicate an increased incidence of birth trauma, such as breech deliveries, extensive use of forceps, prolonged labor, or abrupt delivery. Debate continues as to whether GH deficiency is the consequence of difficult delivery or merely reflects the perinatal consequences of fetal pituitary insufficiency.

Inflammation and infiltration of the brain and/or hypothalamus. Inflammation of the brain (meningitis or encephalitis resulting from bacterial, viral, or fungal infections) may result in hypothalamic/pituitary insufficiency, with most cases being reported as isolated case reports. Despite the bias in the selection of cases, the incidence of endocrine deficiencies likely depends on the virulence of the infectious organism, the severity and localization of the disease, and the immune status of the host. In a study of 19 adult patients who were investigated 10 to 56 months following CNS infection, 21% had ACTH deficiency and 11% had gonadotropin deficiency, whereas there was no GH deficiency or DI reported.¹⁴² Hypopituitarism has been reported following infection by a variety of agents, including group B streptococcus, *Haemophilus influenzae*, and *Mycobacterium tuberculosis*. The hypothalamus and/or pituitary may also be involved in sarcoidosis. Sarcoidosis is a multisystem granulomatous disease of unknown etiology, which clinically affects the CNS in 5% to 10% of cases.¹⁴³ The effects of sarcoidosis on the hypothalamopituitary axis are the result of infiltration by granulomatous tissue. On MRI, the lesion may infiltrate the hypothalamus and pituitary and cause thickening of the pituitary stalk, and it usually enhances with gadolinium. The most frequently reported endocrine abnormality is DI, which is reported in 25% to 50% of patients with neurosarcoidosis.^{143,144} This is followed by hyperprolactinemia although anterior pituitary dysfunction with hypogonadism has also been reported.¹⁴⁵ Sarcoidosis of the nervous system has a poor prognosis, but long-term remissions have been reported with high-dose intravenous pulse methylprednisolone therapy. Hormonal defects of less than 1-year duration may respond to steroid treatment, but longer standing deficits usually persist.¹⁴⁵

Hypophysitis is an inflammation of the pituitary gland that can be either primary or secondary and can result from infection, systemic disease, or irritation from adjacent lesions. This inflammatory process mimics, clinically and radiologically, tumors of the pituitary area. There are three histological types of primary hypophysitis: lymphocytic, granulomatous, and xanthomatous. Lymphocytic hypophysitis is the most common type; it involves the anterior pituitary and may infiltrate the infundibulum and posterior lobe. Lymphocytic hypophysitis occurs mainly in young women and is associated with pregnancy or the presence of autoimmune diseases, including Hashimoto thyroiditis, Graves disease, type I diabetes, and systemic lupus erythematosus.¹⁴⁶ There are rare case reports of hypophysitis in children and adolescents, and, in most cases, diagnosis has been made only after biopsy and histological examination, although in clinical practice, this is rarely performed. In many cases, hypophysitis presented with DI and hypopituitarism and preceded the diagnosis of an intracranial tumor, such as germinoma. In other cases, it can present with

DI and hypogonadotropic hypogonadism. Once the diagnosis is established, management is generally conservative, unless there are signs of increased intracranial pressure or optic nerve compression.

Langerhans cell histiocytosis (LCH) is characterized by clonal proliferation and accumulation of abnormal dendritic cells that can affect either a single site or many systems causing multiorgan dysfunction.¹⁴⁷ In children, the median age of diagnosis ranges between 1.8 and 3.4 years. LCH infiltrates the hypothalamopituitary area in 15% to 35% of patients with subsequent development of at least one pituitary hormone deficiency.¹⁴⁸ In a multicenter French national study of 589 pediatric patients with LCH, 145 patients (25%) had pituitary dysfunction. In 60 patients, pituitary involvement was already present at the time of diagnosis and in 20 of them it was the first manifestation of the disease. Patients at high risk of pituitary involvement seem to be those with multisystem disease involving skull and facial bones, mastoid, sinuses, and mucous membranes (i.e., gums, ear, nose, and throat region). Furthermore, compared with patients without pituitary involvement, patients with pituitary involvement have a higher rate of relapse (10% at 5 years vs. 4.8% at 5 years), and a higher incidence of neurodegenerative LCH.¹⁴⁹

DI is the most frequently reported permanent consequence of LCH and the most common endocrinopathy; almost all patients with pituitary involvement have DI, which usually presents early in the course of the disease, within the first 3 to 5 years, and occasionally may precede the diagnosis of LCH. Children with LCH and DI may also have anterior pituitary hormone deficiencies, with most deficits developing in the 6 years after the diagnosis of DI. The second most common endocrinopathy is GH deficiency, which occurs in 14% of all patients with LCH and in more than 40% of patients who have pituitary involvement.¹⁴⁸ In the vast majority of patients, GH deficiency is associated with DI, with a median interval of 2.9 to 3.5 years between the diagnosis of DI and development of GH deficiency.^{150,151} Isolated GH deficiency, or the association of GH deficiency with other anterior pituitary hormone deficiencies, occurs less commonly.

Pituitary MRI findings in patients with LCH include thickening of the pituitary stalk, suggestive of the infiltrative process, enhancing changes in the pituitary gland and hypothalamus, and absence of the bright signal of the posterior pituitary in T1-weighted images, caused by the loss of the phospholipid-rich antidiuretic hormone secretory granules. The latter is an invariable feature of patients who develop DI.^{152,153} Although, at the time of diagnosis of DI, 75% show a thickened pituitary stalk, only 24% have persistent stalk thickening after 5 years. These changes are variable and do not correlate with treatment or with clinical recovery as DI persists in all cases. The role of MRI in predicting the development of anterior hormone deficiencies is uncertain. It has been reported that patients who will become GH deficient are more likely to have a smaller anterior pituitary, while the size of the stalk and posterior pituitary are not significantly different.

Long-term follow-up of patients with LCH has shown that the already established hormone deficiencies cannot be reversed by treatment.

Tumors of the brain and/or hypothalamus. Tumors of the CNS are a major cause of hypothalamic insufficiency. This is especially true for midline brain tumors, such as germinomas, meningiomas, gliomas, colloid cysts of the third ventricle, ependymomas, and gliomas of the optic nerve. Although metastasis from extracranial carcinomas is rarely found in children, hypothalamic insufficiency may result from local extension of craniopharyngeal carcinoma or Hodgkin disease of the nasopharynx. Pituitary adenomas represent less than 3% of supratentorial tumors in childhood and about 3.5% to

6% of all surgically treated pediatric pituitary tumors. In most cases, they are hormonally active, arising from any of the five cell types of the anterior pituitary, and may produce prolactin (prolactinomas, 52%), ACTH leading to Cushing disease (corticotropinomas 33.3%), GH (somatotropinomas 8%), or more rarely TSH (thyrotropinomas). Nonfunctioning pituitary adenomas are rare in children (2.7%) compared with adults where they represent almost 20% of pituitary adenomas. Although the majority of childhood pituitary adenomas are prolactinomas presenting in adolescence, corticotropinomas are the most common tumors in prepubertal children.¹⁵⁴ Pituitary adenomas occur in isolation or may be part of a genetic syndrome, such as multiple endocrine neoplasia type 1 (MEN1), McCune-Albright syndrome (MAS), or Carney Complex. Their pathogenesis is not clear but there is increasing evidence that dysregulation in hormone receptor signaling, changes in molecules that regulate cell cycle or are important for adhesion to extracellular matrix, as well as changes in growth factors, may be implicated.¹⁵⁵ Their clinical presentation results from pituitary hormone hypersecretion or deficiencies, disruption of growth and sexual maturation, and pressure effects. On MRI, pituitary adenomas show slow uptake of gadolinium and appear as hypoenhancing lesions that may displace the pituitary stalk.

Optic gliomas. Tumors of the optic pathway represent 4% to 6% of all pediatric intracranial tumors and among these the most common are optic gliomas (65%). Most optic gliomas are low-grade lesions with favorable prognosis if treated optimally. Gliomas confined to the optic nerve have a predilection for females (60%–70%) and are associated with neurofibromatosis type 1 (NF-1) in more than half of cases, whereas 38% are sporadic. Children with sporadic gliomas were more likely to manifest increased intracranial pressure, decreased visual activity, and more commonly documented endocrine complications. The most frequent symptoms at presentation are visual defects (diminished vision, optic atrophy, strabismus, nystagmus, proptosis), ataxia, and precocious puberty. Because of their close anatomic relation to the hypothalamus and pituitary, dysregulation of the hypothalamopituitary axis is common and is caused either by the tumor itself or secondary to treatment. Premature sexual maturation is a frequently presenting symptom whereas the most common defect following cranial irradiation is GH deficiency.¹⁵⁶ NF-1-associated optic gliomas can also cause GH excess and excessive growth.¹⁵⁷

Cystic lesions. Cystic lesions in the pituitary area include Rathke's cleft cysts, arachnoidal cysts, and cystic adenomas and craniopharyngiomas. Rathke's cleft cysts are benign cystic remnants of Rathke's pouch. They are usually small (<5 mm), asymptomatic, and are found in almost 20% of routine autopsies.¹⁵⁸ They consist of well-differentiated columnar or cuboidal epithelial cells and the content of the cyst varies. Rathke's cleft cysts can grow gradually and become symptomatic, especially if they have suprasellar extension. Symptoms include headache, visual defects, and pituitary dysfunction ranging from increased prolactin to pituitary hormone deficiencies. Differential diagnosis from other cystic lesions in the area is not always easy, as on MRI the cyst fluid shows variable signal intensities and therefore cysts can appear as hypo- or hyperdense. Almost 50% of Rathke's cleft cysts show rim enhancement. Recurrence of the cyst after treatment is rare (2 of 14 patients in one series) and it is recommended that treatment should include both fluid drainage and cyst wall removal to avoid relapse.

Arachnoid cysts consist of a collection of cerebrospinal fluid (CSF)-like fluid surrounded by a wall made of arachnoid structures. They are mainly suprasellar, with only rare cases being intrasellar. Suprasellar cysts are usually diagnosed following nonendocrine symptoms, such as neurological deficits,

macrocephaly, and visual symptoms. Because of the proximity of the lesion to the hypothalamopituitary area, arachnoid cysts may cause central precocious puberty, amenorrhea, and hyperprolactinemia, in addition to thyrotropin, ACTH, or GH deficiency.

Craniopharyngiomas may result in hypothalamic dysfunction (discussed later).

Irradiation of the brain and/or hypothalamus. Cranial irradiation represents an important cause of hypothalamic/pituitary dysfunction. Irradiation may directly impair hypothalamic and pituitary function, and it is not always easy to discriminate between damage at each of these levels. In addition, thyroidal and gonadal function may also be directly affected if total body irradiation or craniospinal irradiation is administered. The degree of pituitary impairment is related to the dose of radiation received. Low doses typically result in isolated GH deficiency. With higher doses, multiple pituitary hormone deficiencies are observed. Two to 5 years after irradiation, 100% of children receiving more than 3000 cGy over 3 weeks to the hypothalamic-pituitary axis showed subnormal GH responses to insulin-provocative tests.

The degree of pituitary deficiency observed increases with time since irradiation. Children who test normally at 1-year postirradiation may still develop pituitary deficiencies at later times. Even when serum GH concentrations after provocative testing are normal, measures of spontaneous GH secretion may indicate an impairment. As little as 1800 cGy has been shown to affect spontaneous GH secretion in pubertal children.

Decreased GH secretion may be further complicated by the impact of irradiation on spinal growth, which can result in short stature and skeletal disproportion. Surprisingly, cranial irradiation can also result in precocious puberty—with the consequence that epiphyseal fusion occurs at an earlier age than is ideal from the perspective of maximizing growth. GnRH analogues may be used to delay pubertal progression. The endocrinologist observing the child who has received craniospinal irradiation must consider these three factors: evolving hypopituitarism, decreased spinal growth potential, and early puberty and premature epiphyseal fusion. Care must be taken to maximize the growth potential of the patient without causing skeletal disproportion and without delaying puberty excessively or allowing growth to terminate too early.

Genetic abnormalities resulting in combined pituitary hormone deficiency. The molecular basis of SOD and other midline conditions has been previously discussed.

Recessive mutations in *LHX3* have been identified in several consanguineous families, in addition to a single patient who was found to be compound heterozygous for two missense mutations within the gene. These patients usually present with multiple anterior pituitary hormone deficits, including ACTH deficiency, which was initially thought to be spared.¹⁵⁹ Pituitary morphology is variable between patients with *LHX3* mutations: most patients have a hypoplastic anterior pituitary with a normal posterior pituitary and midline structures^{1,6} conversely, an enlarged anterior pituitary has also been reported in a patient that was not evident in a previous MRI scan performed 10 years earlier.¹⁶⁰ In addition, a patient with a hypointense lesion in the anterior pituitary consistent with a microadenoma has also been described.¹⁶¹ The majority of patients with *LHX3* mutations reported to date have also exhibited a short rigid cervical spine with limited neck rotation and trunk movement. Again, the skeletal phenotypes can vary, and a single patient with normal neck rotation and no other syndromic features has been reported. In addition, most patients with *LHX3* mutations manifest sensorineural deafness,¹⁵⁹ the severity of which is also highly variable and can range from profound to very mild and may be missed in some cases. A direct role may be implicated here because *LHX3* is expressed in specific regions

of the inner ear in a pattern highly conserved between humans and mice, and it is likely to have a role in cochlea hair cell development.¹⁵⁹

Heterozygous mutations in *LHX4* have also been reported, with all patients exhibiting GH deficiency and associated short stature on presentation, again with variable additional endocrine deficits and extrapituitary abnormalities. Initially, a heterozygous intronic mutation that abolishes normal splicing of *LHX4* was reported by Machinis and colleagues¹⁶² in a three-generation family segregating in a dominant and fully penetrant manner. The probands presented with short stature and were found to be GH, TSH, and ACTH deficient, with anterior pituitary hypoplasia, an undescended posterior pituitary, and absent pituitary stalk on MRI. Other affected family members presented with short stature associated with IGHD and a normal posterior pituitary. Additional manifestations included a poorly formed sella turcica and pointed cerebellar tonsils. Several mutations have been subsequently described and are clearly variable in terms of penetrance and phenotype. Recently, a novel homozygous mutation in *LHX4* was associated with a severe recessive phenotype leading to a lethal form of hypopituitarism, suggesting that the variable penetrance observed in the majority of heterozygous mutations may indicate the presence of a second genetic abnormality that has not been detected to date.

The gene encoding *PROP1* is involved in the early determination and differentiation of multiple anterior pituitary cell lineages. Abnormalities of *PROP1* result in CPHD, characterized by variable degrees of deficiency of GH, prolactin, TSH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and occasionally ACTH. The first mutations in *PROP1* were identified in human patients with hypopituitarism characterized by GH, TSH, and prolactin (PRL) deficiencies in addition to reduced gonadotrophins and failure to enter spontaneous puberty.¹⁶³ Subsequently, *PROP1* mutations have been identified in patients from over 21 different countries, implicating *PROP1* mutations as the most common genetic cause of CPHD accounting for approximately 50% of familial cases, although the incidence in sporadic cases is much lower.¹⁶⁴ Affected individuals exhibit recessive inheritance, and the majority of mutations identified involve the DNA binding homeodomain. The mutations in *PROP1* identified to date include nonsense, missense, frameshift, intronic, and deletion mutations. The majority of the mutations are predicted to result in complete loss of function by ablating DNA binding and transcriptional activation, although some missense mutations retain partial activity. By far the most common mutation, accounting for 50% to 72% of all familial *PROP1* mutations in multiple unrelated families, is a 2-bp deletion within exon 2 resulting in a frameshift (often referred to as *p.S109X*). This mutation likely represents a mutational hot spot within the gene, rather than a common founder mutation, and combined with the c.150delA mutation, accounts for more than 90% of all known *PROP1* mutations.^{1,6}

Homozygosity for mutations in *PROP1* is typically associated with a deficit of GH, TSH, PRL, and gonadotrophins, although the time of onset and severity of hormone deficiency varies. Most patients present with early-onset GH deficiency and growth retardation; however, normal growth in early childhood has been reported in a patient who attained normal final height without GH replacement therapy. Patients may present with gonadotropin deficiency with the evolution of other hormone deficiencies later in life. TSH deficiency is also highly variable and has been reported as the initial presenting symptom in some cases, whereas other patients show delayed onset. The frequency of ACTH deficiency increases with age. Most patients exhibit normal ACTH and cortisol concentrations in early life; however, patients as young as 6 years have been described with

cortisol deficiency, emphasizing the necessity for complete and continuing clinical assessment of patients with *PRO1* mutations. The spectrum of human gonadotropin deficiency is extremely variable in patients with *PRO1* mutations, ranging from early hypogonadism with a micropenis and undescended testes and complete lack of pubertal development to spontaneous, albeit often delayed, onset of puberty with subsequent deficiency of gonadotropins, requiring hormone replacement therapy.

The pituitary morphology in patients with *PRO1* mutations is unpredictable; most cases show a hypoplastic or normal-sized anterior pituitary gland on imaging, with a normal pituitary stalk and posterior lobe, although some reports have documented an enlarged anterior gland. Longitudinal analyses of anterior pituitary size over time have revealed that several patients with an enlarged anterior gland at initial scanning in childhood show spontaneous regression and involution, so that MRI in older patients often demonstrates anterior pituitary hypoplasia, although the size of the pituitary can wax and wane during this time.¹⁶⁴ The pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe or Rathke's pouch remnant in the cleft, although the underlying mechanism for the mass remains unknown.

The evolving nature of hormone insufficiencies in patients with *PRO1* mutations suggests a progressive decline in the anterior pituitary axis, so such patients require regular monitoring for the development of hormone deficits that may not be apparent on initial presentation. The highly variable nature of the phenotype associated with *PRO1* mutations, even between siblings within the same family carrying identical mutations, again implicates unidentified genetic modifiers playing a role in the severity and onset of disease pathogenesis.

POU1F1 is the human homologue of the mouse gene *Pit1* and encodes a transcription factor involved in the activation of *GH* and *PRL* genes, regulation of the TSH- β promoter, and specification, proliferation, and survival of the corresponding cell lineages. Mutations within *POU1F1* (*PIT1*) (reviewed in¹⁶⁵) are generally associated with *GH*, *PRL*, and *TSH* deficiencies with variable anterior pituitary hypoplasia. Deficiencies of *GH* and *PRL* are generally complete and present early in life, whereas *TSH* deficiency can be highly variable. The majority of cases present with early *TSH* deficiency; however, in some cases, hypothyroidism occurs later in childhood. MRI of patients with *POU1F1* mutations demonstrates a small or normal-sized anterior pituitary gland with a normal posterior pituitary and infundibulum, but with no extrapituitary abnormalities. More than 28 different mutations in *POU1F1* have been described to date; the majority of these show recessive inheritance, including a complete gene deletion, as well as splice site mutations, whereas a small number are dominant mutations. Of these, the amino acid substitution p.R271W is the most frequent, having been identified in several unrelated patients from a variety of different ethnic backgrounds. The p.R271W substitution results in production of a protein that remains capable of binding to DNA but acts as a dominant negative inhibitor of transcription.

Studies of *POU1F1* in CPHD patient cohorts suggest that the incidence of mutations in cases of sporadic CPHD is quite low (approximately 3%–6%), whereas the incidence among familial patients with hypopituitarism is much greater (25%). Overall, screening studies suggest that abnormalities of *POU1F1* are a less common cause of CPHD than are abnormalities of the *PRO1* gene.¹

Haploinsufficiency of the homeobox gene *RIEG* results in Rieger syndrome, an autosomal-dominant disorder that involves abnormal development of the anterior chamber of

the eye, teeth, and umbilicus—with an occasional association with *GH* deficiency.

Genetic abnormalities of GH production and/or secretion resulting in isolated GH deficiency. It has been reported that up to 30% of patients with *IGH* deficiency have an affected parent or sibling. In addition to combined pituitary hormone deficiency, such as that caused by abnormalities of *PRO1* and *POU1F1* described previously,¹¹⁶ four Mendelian forms of *IGH* deficiency have been reported.

Isolated GHDIA results from deletions (6.7- 7.0- 7.6-, and 45-kb deletions have been reported) or mutations of the *GH1* gene that totally block *GH* synthesis or secretion. Transmission of isolated GHDIA is autosomal recessive, and patients have profound congenital *GH* deficiency. Because *GH* has never been produced by the patient, even in fetal life, patients are immunologically intolerant of *GH* and typically develop anti-*GH* antibodies when treated with pituitary-derived or recombinant DNA-derived *GH*, although development of growth-attenuating antibodies appears to be less frequent with the newer synthetic *GH* preparations.

When antibodies prevent a patient from responding to *GH*, recombinant (rh)IGF-1 therapy should be considered. The less severe form of autosomal-recessive *GH* deficiency (isolated GHDIB) is likely to also be the result of mutations or rearrangements of *GH1*, presumably resulting in a *GH* molecule that retains some function but is perhaps unstable. In addition, mutations in *GHRHR* also lead to GHD1B. To date, however, most patients with presumed isolated GHDIB have not demonstrated an alteration of *GH1* and *GHRHR* and the cause of their *GH* deficiency remains unclear. These patients generally respond well to *GH* therapy, and development of clinically significant anti-*GH* antibodies is unusual.

Isolated GHDII is transmitted as an autosomal dominant. Such patients usually have abnormalities of *GH1* that function in a dominant negative manner. The most common causes of this disorder appear to be splice site and intronic mutations that inactivate the 5'-splice donor site of intron 3, resulting in skipping of exon 3 with generation of the 17.5-kDa isoform of *GH* that exerts a dominant-negative effect on the secretion of the 22-kDa molecule. Transgenic mice that overexpress the 17.5-kDa isoform have a defect in the maturation of the *GH*-containing secretory vesicles and display anterior pituitary hypoplasia with the development of other hormone deficiencies. The 17.5-kDa isoform is retained in the endoplasmic reticulum, misfolded, and disrupts the secretory pathway. Similar mutations identified in humans are also associated with GHDII and an evolving endocrinopathy.¹¹⁶

Missense mutations that affect *GH* secretion and/or action can also cause autosomal dominant IDH deficiency. Patients with the p.Arg183His mutation have impaired release of *GH* as, at the cellular level, the secretory granules that contain the mutant Arg183His protein are not exocytosed as effectively as those containing the wild-type hormone.¹⁶⁶ Other mutations (for example, p.Pro89Leu) can cause more profound and early disturbances in the secretory pathway by alteration of the orientation of the *GH* helices and an effect on the correct folding of the molecule.

Patients with autosomal dominant *IGH* deficiency show considerable variation in the severity of *GH* deficiency. They present with low but detectable serum *GH* concentrations, variable height deficit, and can show anterior pituitary hypoplasia on MRI (38%–50%).^{167,168} Data on pedigrees with the Arg183His or e3+1G>A mutations highlight the fact that patients with the same mutation can vary considerably in height (≤ -4 SDS to normal) and even attain normal adult height without treatment. Patients with splice site mutations are thought to be more severely affected than those with missense mutations¹⁶⁹; however, patients with the IvS3+1 or

IvS3+2 splice site or the p.Pro89Leu mutations can develop additional pituitary hormone deficiencies, including ACTH, prolactin, TSH, or gonadotropin deficiency.¹⁷⁰ This evolving phenotype is unpredictable and dictates the need for lifelong follow-up of affected individuals. Another intriguing observation is that, even in patients with a genetic cause (for example, p.Glu32Ala), GH deficiency seems to reverse when they are retested at the end of growth, in the transition period before transfer from pediatric to adult services. However, this effect is temporary, and is observed in patients tested at the time of transition, and who should not be discharged from follow-up.

No convincing cases of mutations of the *GHRH* gene in humans have been identified. Abnormalities of the gene for the *GHRHR*, on the other hand, have been found in a number of pedigrees. *GHRHR* is a seven transmembrane domain receptor. Its expression requires the presence of the *POU1F1*. Homozygous or compound heterozygous mutations have been reported in *GHRHR* (missense, nonsense, splice site, deletions, or regulatory mutations) leading to type IB IGH deficiency. *GHRHR* mutations are identified in about 10% of patients with familial recessive IGH deficiency,¹⁷¹ and in about 3% of a selected cohort of patients IGH deficiency.¹⁷² Mutations are more likely to be identified in consanguineous pedigrees or patients from certain ethnic backgrounds, such as from the Indian subcontinent and Brazil.

Children with mutations in *GHRHR* have severe GH deficiency and short stature, but midfacial hypoplasia, neonatal hypoglycemia, and microphallus, as found in those with recessive *GHI* mutations, are rare. The anterior pituitary may rarely be normal; in the majority it is hypoplastic. The posterior pituitary and the pituitary stalk are normal. Partial loss of function mutations may be associated with a relatively mild GH deficiency phenotype. Overall, mutations in genes regulating GH secretion are rarely identified, suggesting that novel genes remain to be identified.¹⁷³

Isolated *GHDIII* is transmitted in an X-linked manner. Little is known about the etiology of this condition, although rare mutations in *SOX3* can be associated with IGHD with or without learning difficulties.

Congenital abnormalities of the pituitary. A number of reports have described the association of “idiopathic” GH deficiency with an ectopic neurohypophysis. MRI findings have been described in several series of patients with idiopathic GH deficiency. Abrahams and colleagues studied 35 patients with idiopathic GH deficiency and found that those with MRI findings could be divided into two groups: 43% had an ectopic neurohypophysis (neurohypophysis located near the median eminence), absent infundibulum, and an absence of the normal posterior pituitary bright spot, and 43% had a small anterior pituitary as an isolated finding or combined with an ectopic neurohypophysis.¹⁷⁴ All in all, an ectopic neurohypophysis was found in 87% of cases with multiple pituitary hormone deficiencies but in only 10% of cases with IGH deficiency.

In other studies, however, high-resolution MRI findings of one or more of the following have been suggested to be sensitive and/or specific indicators of hypopituitarism: a small anterior pituitary, attenuated pituitary stalk, and ectopic posterior pituitary. In one study, pituitary abnormalities were found in 80% of patients with IGH deficiency and 93% with MPHD. In patients whose peak GH concentration was less than 3 ng/mL, 90% had abnormal MRI findings—compared with 39% of those with GH concentrations 3 ng/mL or more.¹⁷⁵ In another study, the stalk was abnormal in 90% of patients with IGHD and was absent in 96% of patients with MPHD.¹⁷⁶ Thus MRI abnormalities are common in children with IGH deficiency and MPHD and are closely associated with the severity of GH deficiency. Patients with structural abnormalities will need lifelong follow-up in adulthood owing to the risk of

developing other pituitary hormone deficiencies. Patients with an ectopic posterior pituitary, however, can also show reversal of their GH deficiency on retesting at the time of transition from pediatric to adult services or in adulthood.^{177,178}

Tumors involving the pituitary. Many of the tumors that affect hypothalamic function also directly impact pituitary secretion of GH. In particular, craniopharyngiomas comprise a major cause of pituitary insufficiency. These tumors arise from remnants of Rathke’s pouch, the diverticulum of the roof of the embryonic oral cavity that normally gives rise to the anterior pituitary. Some consider this tumor a congenital malformation because it is believed to be present at birth, gradually growing over the ensuing years and decades.^{179–181} The tumor arises from rests of squamous cells at the junction of the adenohypophysis and neurohypophysis. As it enlarges, it forms a cyst that contains degenerated cells and it may calcify but does not undergo malignant degeneration. In children, they represent 5% to 15% of intracranial tumors and are the most common neoplasm of the hypothalamopituitary area, accounting for up to 80% of tumors in this location. Their incidence in the United States is 1.3 per million per year and almost 28% affect children younger than 14 years. There is a bimodal peak in incidence: the first peak occurs in children between 5 and 14 years and the second peak in adults older than 50 years. They can, however, be diagnosed at any age and have even been reported in the neonatal period.

At presentation, most craniopharyngiomas have a combined intra- and suprasellar location (74.2%) and almost half have hypothalamic involvement (51.6%). A smaller percentage is exclusively suprasellar (22.6%) or confined within the sella turcica (6%–3%). Almost one-third invade the floor of the third ventricle and may cause obstructive hydrocephalus.¹⁸² In pediatric patients, craniopharyngiomas are predominantly cystic (56.7%), multicystic (16.7%), predominantly solid (13.3%), purely solid (10%), or purely cystic (3.3%). The cystic fluid is viscous and rich in cholesterol and the incidence of calcification is much higher in children (83.3%) compared with adults.¹⁸³

There are two main histological types: the most common is the adamantinomatous type and consists of epithelial neoplastic cells that resemble those found in lesions of the jaw. The papillary type is much rarer and is found almost exclusively in adults. Although craniopharyngiomas are histologically benign, they can extend from their initial site, develop papillae, and invade vital surrounding tissues, including the hypothalamus and optic chiasm. This attachment makes their complete excision difficult, if not impossible, and contributes to tumor recurrence and morbidity.

Craniopharyngiomas are typically sporadic tumors. There have been, however, rare case reports of affected family members suggesting recessive inheritance. Overactivation of the *Wnt* signaling pathway appears to play a role in craniopharyngioma development; activating mutations in beta-catenin, a downstream component of the *Wnt* signaling pathway, have been identified in adamantinomatous craniopharyngiomas.¹⁸⁴

Clinical signs and symptoms of craniopharyngiomas can arise at any age, from infancy to adulthood, but most typically in midchildhood. The most common presentation is with symptoms of increased intracranial pressure (up to 75%), such as headaches, vomiting, or oculomotor abnormalities. Impaired vision is common. Visual field defects may result from compression of the optic chiasm, and papilledema or optic atrophy may be observed. Visual and olfactory hallucinations have been reported, as have seizures and dementia.

It is estimated that 70% to 80% of children have evidence of endocrine deficiencies at presentation and that growth failure is observed in 32% to 52% of children before diagnosis. Low concentrations of IGF-1 have been reported in 80% of children at time of diagnosis. GH deficiency is the most common

hormone deficiency, documented in 75% to 100% of those tested before treatment. It is followed by ACTH (20%–70%) and TSH (3%–30%) deficiencies. Compression of the pituitary stalk or damage to hypothalamic dopaminergic neurons results in elevated PRL concentrations, observed in 8% to 20% of children at diagnosis. The incidence of DI at presentation varies between 10% and 29% of patients, depending on the study. In adolescence in particular, craniopharyngiomas may present with delayed puberty or pubertal arrest. Rare presentations include precocious puberty and syndrome of inappropriate anti-diuretic hormone secretion.

Lateral skull films often demonstrate enlargement or distortion of the sella turcica, frequently accompanied by suprasellar calcification(s). Nevertheless, some children with craniopharyngiomas will have normal plain films, and alternative radiological techniques are recommended. Computed tomography (CT) is a sensitive technique for identification of small amounts of calcification or cystic abnormalities. MRI is probably the most sensitive technique. Craniopharyngiomas appear as mass lesions in the sellar and/or suprasellar area that may extend to the hypothalamus and invade the third ventricle. Adamantinomatous craniopharyngiomas are predominantly cystic and the cystic portion of the lesion appears hyperintense in T1 and T2 images. The solid part of the tumor shows areas of high and low signal intensity that represent areas of calcification and hemosiderin deposits. In the majority of cases (58%–76%), the size of craniopharyngiomas, as estimated by MRI or CT, has been reported to be 2 to 4 cm, whereas it is smaller than 2 cm in 4% to 28% cases and more than 4 cm in 14% to 20%.

The management of craniopharyngiomas is complex, controversial, and it is best achieved by a multidisciplinary approach. Aims of the treatment are to relieve acute signs and symptoms of compression (raised intracranial pressure, threatening visual failure); to preserve hypothalamic function, thus reducing later morbidity and mortality; and to provide long-term control and prevent recurrence. What has been evident from different studies is that the extent of surgical resection is probably the most important factor that influences the recurrence of craniopharyngioma. In patients who had surgery only, the 10-year recurrence free survival rate was 83% after total removal, 50.5% after subtotal removal and 15.6% after partial removal. Tumor recurrence usually occurs in the first 5 years and is relatively rare thereafter. However, even after complete resection confirmed radiologically, relapses occur in up to 15% to 25% of patients. In older series, however, when patients were treated with radical and repeated surgical resections, mortality was high (25%–50%) and hypothalamic, visual, and cognitive morbidity occurred in the vast majority (75%), especially in craniopharyngiomas with suprasellar or retrochiasmatic extension.¹⁸⁵

Guidelines for the multidisciplinary management of children with craniopharyngioma have recently been published.^{186,187} It is now suggested that patients can be categorized into two risk groups with respect to management and prognosis. The good risk group includes older children with small tumors (2–4 cm) and no hypothalamic syndrome or hydrocephalus. Younger children, with larger tumors (>2–4 cm) and hypothalamic syndrome or hydrocephalus are in the poor risk group. Complete radical resection, with or without adjuvant radiotherapy, is suggested for the good risk group, whereas limited surgery and immediate or delayed radiotherapy is the treatment of choice for the poor risk group. The recent availability of proton beam therapy is a valuable addition to the therapeutic armamentarium.¹⁸⁸

Idiopathic pituitary growth hormone deficiency. As discussed previously, many of the disease processes that affect hypothalamic regulation of GH secretion also impact pituitary function. Given current diagnostic limitations, it is not always possible to completely discriminate between hypothalamic and pituitary

dysfunction. Furthermore, it is likely that many cases of so-called idiopathic GH deficiency will be found to have a molecular basis for the disorder. Indeed, in the past 5 years we have seen an explosion of information concerning genes critically involved in pituitary somatotrope differentiation and function.

At this time, however, a clear cause for pituitary GH deficiency is often not identified—hence the term *idiopathic*. An incidence of pituitary GH deficiency in 1:60,000 live births was reported from the United Kingdom. A more recent survey of 48,000 Scottish schoolchildren has indicated an incidence as high as 1:4000, whereas the best estimate available in the United States population is at least 1:3480.

It is likely, however, that childhood GH deficiency is an overdiagnosed condition. In particular, the diagnosis of later onset idiopathic IGH deficiency should always be questioned. Although one may argue that destructive or inflammatory lesions of the hypothalamus or pituitary may only affect GH secretion, that IGH deficiency caused by a mild mutation/deletion of the *GHRHR* gene or *GHI* gene may appear late, or that CPHD may first present with what appears to be IGH deficiency, such circumstances appear to be rare. In the absence of anatomic abnormalities evident on imaging studies and/or biochemical evidence of CPHD, the diagnosis of acquired isolated idiopathic GH deficiency demands careful and thorough documentation. Indeed, recent data suggest that retesting of such patients may be associated with an early reversal of the diagnosis.^{189,190} The overdiagnosis of GH deficiency likely arises from the poor specificity of provocative GH testing, particularly in prepubertal children.^{191–193}

Bioinactive growth hormone. A small number of patients with a defect in the *GHI* gene that generates a biologically inactive but immunoreactive GH molecule have been reported.^{194,195} These patients have a common phenotype and are characterized by severe short stature (usually height SDS <−3) and very low concentrations of IGF-1 and IGFBP-3, but normal or elevated GH concentrations (basal or after a stimulation test). Patients may present with mild clinical characteristics, such as prominent forehead and saddle nose, that are typical of GHI. Contrary to what would be expected in a patient with GHI, these patients showed some improvement in growth rate and IGF-1 levels with exogenous GH treatment. Missense mutations in *GHI*, which are inherited in an autosomal dominant or recessive fashion, generate a mutated GH that is capable of binding to the receptor, but cannot properly activate signal transduction by the GHR.

Growth hormone insensitivity.

GHI is a genetic disorder that is defined as the inability to respond to endogenous and exogenous GH with appropriate growth and metabolic effects. The classical form of GHI, also known as *Laron syndrome*, is characterized by severe short stature that manifests postnatally and is associated with dysmorphic features, such as craniofacial disproportion with a relatively small face, depressed nasal bridge, high-pitched voice, truncal obesity, and micropenis, a phenotype similar to that seen in patients with complete congenital GH deficiency (Fig. 11.5).¹⁹⁶ Typically, GHI patients show very low concentrations of IGF-1, IGFBP-3, and ALS, but elevated basal and stimulated GH concentrations. In most cases, GHI is caused by a fully penetrant autosomal recessive mutation in the *GHR*. The majority of these mutations are located in exons that encode the extracellular domain of *GHR* and disrupt the ligand-binding domain and/or impair transport of the receptor to the plasma membrane.¹⁹⁷ For these reasons, patients with classic GHI often show low or undetectable GHBP levels because GHBP is generated by proteolytic cleavage of the extracellular GHR domain. However, mutations elsewhere in the



Fig. 11.5 Phenotype of a patient with severe growth hormone deficiency or insensitivity: frontal bossing, depressed nasal bridge, midfacial hypoplasia, and truncal obesity.

GHR gene may result in normal or elevated GHBP concentrations.¹⁹⁸

After characterization of complete forms of GHI, several cases with a milder or otherwise atypical GHI phenotype were described.^{197,199} The clinical spectrum of partial GHI may vary between patients with typical laboratory findings of GHI who lack classic facial and dysmorphic features associated with Laron syndrome, and patients who are initially classified as having ISS. Usually, partial GHI patients are heterozygous for dominant-negative *GHR* mutations or present a biallelic defect involving hypomorphic *GHR* mutations.¹⁹⁹ Some residual *GHR* activity is retained in both situations, which causes a variety of partial phenotypes. In addition, GHBP is frequently detectable, and even elevated, in some patients with partial GHI.

Usually the diagnosis of patients with severe GHI is not difficult, and molecular studies add little other information to the clinical assessment. Conversely, the absence of a typical phenotype raises a challenge for establishing the diagnosis of partial GHI; hence the use of genetic studies that analyze *GHR* is a valuable molecular diagnostic tool.²⁰⁰

It is noteworthy that GH pharmacogenetic studies have suggested that a single *GHR* polymorphism, the presence or absence of *GHR*-exon 3, was associated with a small but significant modulation of growth response during recombinant human GH therapy.^{201,202} In addition, a whole-genome association study has demonstrated that a *GHR* nonsense variant, which causes severe GHI in the homozygous state, results in a reduction of 4.2 cm in height in heterozygotes,²⁰³ indicating

that *GHR* variants also play an important role in height variability in a polygenic model.

Growth Hormone Insensitivity Associated With Immune Dysfunction. There is a distinct group of patients with GHI that exhibit an associated immune dysfunction, mainly characterized by lymphocytic interstitial pneumonia, eczema, and autoimmune disease.²¹ These patients harbor biallelic mutations in the *STAT5B* gene. Patients with biallelic *STAT5B* defects have a hormonal profile similar to patients with homozygous defects in *GHR*. *STAT5B* is a critical molecule involved in *GHR* signal transduction, mediating the growth-promoting actions of the *GHR*. The immune dysregulation resulting from *STAT5B* mutations can be explained by the fact that other molecules use the *STAT5B* signaling pathway, including some ILs. The defect in IL-2 action is particularly important because this IL participates in the activation of T lymphocytes and in the development of regulatory T lymphocytes.²⁰⁴ Elevated concentrations of PRL are a distinctive feature of biallelic *STAT5B* mutations, because *STAT5B* signaling is required for negative feedback of PRL production. Such patients also show moderate lymphopenia, with a marked reduction of natural killer and T cells, featuring a notable impairment in T-regulatory cells, which contribute to mucosal immunity and host defense against intracellular pathogens.²¹ Individuals carrying heterozygous loss-of-function *STAT5B* mutations have a reduced height (approximately 3.9 cm) and lower IGF-1 and IGFBP-3 levels than noncarrier controls.²⁰⁵ Although these individuals are usually within the normal height range, this finding supports the hypothesis that heterozygosity of rare pathogenic variants contributes to normal height heritability.⁷⁶

Recently, patients carrying dominant-negative heterozygous *STAT5B* variants were reported to have postnatal growth failure, eczema, and elevated immunoglobulin E but not severe immune disease,²⁰⁶ expanding the range of clinical and genetic presentations associated with aberrant *STAT5B* genotypes. Moreover, there are some reports of patients with partial GHI and immune dysfunction who harbor *STAT3* gain-of-function variants that are associated with diminished *STAT5B* transcriptional activity.²⁰⁷

Insulin-Like Growth Factor-1 Deficiency/Resistance.

Acid-Labile Subunit Deficiency. Homozygous defects in the gene encoding the insulin-like growth factor acid-labile subunit (*IGFALS*) cause severe ALS deficiency and an absence of ternary complex formation (IGFs/ALS/IGFBP-3).²⁰⁸ Patients with ALS deficiency are characterized by mild to moderate short stature, undetectable or very low serum concentrations of ALS, and very low serum concentrations of IGF-1 and IGFBP-3, with IGFBP-3 being more affected than IGF-1. Some of these patients also present with variable reduction in birth size, delayed onset of puberty, variable degrees of insulin insensitivity, and/or low bone mineral density. It was assumed that growth restriction in these patients is mild, despite the marked reduction in IGF-1 and IGFBP-3 concentrations, because the relative preservation of circulating free IGF-1 concentrations²⁰⁹ and/or expression of locally produced IGF-1.²⁰⁸ *IGFALS* defects are autosomal recessive traits and patients can be either homozygous or compound heterozygous for ALS mutations. Heterozygous carriers have a mild reduction in height (approximately 1 SDS) in relation to their noncarrier relatives.¹⁹⁹

Defects in the Proteolytic Cleavage of IGFBPs (*PAPPA2* Gene). *PAPPA2* is a protease highly specific for cleavage of IGFBP-3 and -5. Cleavage of these IGFBPs releases IGF-1 from the ternary complex (IGFBP-3 and ALS) and allows free IGF-1 to act on target tissues. To date, only two families have been reported with homozygous mutations in the *PAPPA2* gene.²⁸ Affected members of these families had an increased amount of IGF-1 bound in ternary complexes and decreased free or bioactive IGF-1. These patients were clinically characterized

by progressive growth failure, microcephaly, and thin bones. They also had a marked elevation of total IGF-1, IGF-2, IGFBP-3, IGFBP-5, and ALS concentrations, but low free IGF-1. GH is also elevated in these patients, probably because of decreased negative feedback caused by the reduction in free IGF-1.

Primary Insulin-Like Growth Factor Deficiency.

IGF1 defects. A small number of patients with pathogenic variants in *IGF1* have been described to date,^{58,210} approximately half of whom harbor homozygous variants and an autosomal recessive inheritance pattern. These patients are characterized by severe intrauterine and postnatal growth failure, microcephaly, sensorineural deafness, variable intellectual deficits, and reduction in vertebral bone mass. Patients show undetectable concentrations of IGF-1 and elevated basal and stimulated GH concentrations but have normal concentrations of IGFBP-3 and ALS. Insulin resistance has also been observed in these patients (secondary to GH excess), as well as partial gonadal dysfunction and obesity. Patients homozygous for less disruptive *IGF1* mutations⁵⁸ or harboring heterozygous *IGF1* mutations²¹⁰ have significantly short stature, with or without microcephaly or intellectual disability, and low or low-normal IGF-1 concentrations. This milder clinical presentation, particularly in patients with heterozygous *IGF1* variants, makes diagnosis difficult without molecular genetic investigations.

Bioinactive Insulin-Like Growth Factor-1. There is a report describing a patient homozygous for an *IGF1* mutation that results in formation of an immunoreactive but biologically inactive polypeptide.²¹¹ This abnormal IGF-1 molecule is the product of a missense variant in the *IGF1* gene and has a 90-fold reduced affinity for its receptor. In this study, the affected patient showed severe pre- and postnatal growth failure, microcephaly, sensorineural deafness, intellectual disability, and osteoporosis, a phenotype very similar to that seen in patients with severe IGF-1 deficiency. GH concentrations were very elevated in response to insulin-induced hypoglycemia and IGF-1 was also markedly elevated.

Insulin-Like Growth Factor Insensitivity. IGF1R defects have been identified in patients born small for gestational age (SGA) who did not show catch-up growth.²¹² Patients with biallelic (homozygous or compound heterozygous) *IGF1R* mutations have a homogeneous clinical phenotype that is similar to the phenotype of patients with homozygous *IGF1* mutations. In contrast with the rarity of biallelic *IGF1* and *IGF1R* mutations, heterozygous *IGF1R* variants are relatively frequent and manifest considerable clinical variability. The extent of growth restriction is variable as are the degrees of delay in psychomotor and mental development with absence or presence of microcephaly. Patients with *IGF1R* mutations also show delayed bone age and IGF-1, IGFBP-3, and GH concentrations that range from normal to high.²¹²

There are also reports of patients with abnormalities in chromosome 15, in which the *IGF1R* gene is deleted. Phenotypes displayed by these patients depend upon the type and extent of the abnormality. As a rule, patients with biallelic *IGF1R* defects or heterozygous nonsense mutations have a more severe clinical presentation than patients heterozygous for missense mutations, reflecting a spectrum related to the residual activity of IGF-1.²¹² Up to 2% of children born SGA without catch-up growth may have heterozygous defects in *IGF1R*.²¹³ *IGF1R* haploinsufficiency has been observed even among patients born at an appropriate size for gestational age, and is usually classified as idiopathic short stature.²¹⁴ Because of significant clinical variability, diagnosis of IGF insensitivity based only on clinical and laboratory findings is not possible, reinforcing the importance of molecular genetic diagnosis for these patients.

Hypothyroidism and Thyroid Hormone Resistance.

Hypothyroidism. Many of the clinical features characteristic of adult myxedema are lacking in pediatric patients with acquired hypothyroidism. Although it is widely believed that the most common and prominent manifestation of chronic acquired hypothyroidism is growth failure,²¹⁵ several studies have shown that growth failure is often a relatively uncommon manifestation of hypothyroidism, as the diagnosis is usually made relatively early, before significant growth failure sets in.^{216,217} Hypothyroidism may present with short stature, excess weight gain, and a suboptimal growth velocity. Classical features, such as constipation, dry skin, hair loss, delayed deep tendon reflexes and coarse facies may be either absent or mild. Postnatal growth retardation may also be observed in the infant with congenital hypothyroidism, but the development of newborn screening programs for hypothyroidism have generally resulted in prompt diagnosis and treatment of such patients in the Western world. In acquired hypothyroidism, growth retardation may take several years to become clinically evident. However, once present, growth failure is typically severe and progressive.

Although chronic hypothyroidism is generally characterized by delayed puberty, precocious puberty and even premature menarche can occur in hypothyroid children—an entity called *VanWyk-Grumbach syndrome*. However, in males, the testes are usually enlarged with relatively minimal virilization possibly because of predominant FSH secretion. In some females with severe primary hypothyroidism, large recurrent ovarian cysts may manifest. Skeletal age is usually markedly delayed.

Confirmation of the diagnosis of primary hypothyroidism is usually straightforward. Serum concentrations of free thyroxine (FT4) are reduced, and TSH concentrations are elevated. The presence of antithyroid antibodies is consistent with a diagnosis of Hashimoto thyroiditis, the most common cause of acquired hypothyroidism in the Western world. Isolated secondary and tertiary hypothyroidism, caused by TSH and thyroid-releasing hormone deficiency (respectively), are very rare causes of acquired hypothyroidism.

Replacement therapy with levothyroxine is associated with a period of rapid catch-up growth. Despite this gratifying response, however, accelerated growth often does not result in restoration of full growth potential—largely because of the rapid increase in skeletal age during the first 18 months of treatment, often with rapid progression through puberty. In the study of Rivkees and associates, children with severe growth retardation caused by long-standing hypothyroidism, who were treated at an initial mean chronological age of 11 years, had adult heights approximately 2 SD below the means for sex. These final heights were significantly lower than midparental heights or initial predicted adult heights based on data of Bayley and Pinneau. The deficit in adult stature correlated significantly with the duration of hypothyroidism before initiation of treatment.

Thyroid Hormone Resistance.

RTHbeta. TR β -mediated resistance to thyroid hormone (RTH) in humans has an incidence of approximately 1 in 40,000 and arises because of point mutations in the *TRHB* gene,²¹⁸ which encodes thyroid hormone receptor beta (TR β). Individuals with RTH β exhibit a characteristic biochemical profile (raised FT4, free triiodothyronine [FT3] with unsuppressed TSH) because of refractoriness within the TR β dependent negative feedback signaling system, which regulates the hypothalamopituitary-thyroid axis. This results in the axis equilibrating around a new higher “set point” with elevated FT4 and FT3 concentrations.

Clinical manifestations of RTH β are highly variable and the majority of individuals are relatively asymptomatic. Associated clinical features may be attributable to differential TR isoform expression in different organs such that tissues predominantly

expressing TR β (liver, kidney, hypothalamus, pituitary) exhibit resistance to thyroid hormone, whereas tissues expressing predominantly TR α (skeletal and cardiac muscle, bone, brain), remain sensitive to the elevated circulating concentrations of thyroid hormones and so may exhibit relative thyrotoxicosis. Thus RTH subjects have increased heart rate compared with euthyroid controls, which may reflect the predominance of TR α in myocardium, and increased resting energy expenditure probably mediated by the predominance of TR α in skeletal muscle. Children with RTH β may present with growth retardation or failure to thrive and attention deficit hyperactivity disorder may also be present. Goiter is also common.

Rarely, homozygous *THRB* point mutations or deletions have been described in association with a more severe phenotype including marked intellectual impairment, hearing loss, dysmorphic features (bird-like facies, pigeon breast, winged scapulae), deaf-mutism, and color blindness.²¹⁹

RTH α . RTH occurs as a result of heterozygous, dominant negative, loss-of-function mutations in the *THRA* gene,²²⁰ which encodes thyroid hormone receptor alpha (TR α). It presents with tissue-specific hypothyroidism in tissues predominantly expressing the TR α isoform and near-normal thyroid function tests because TR α is the predominant isoform regulating thyroid hormone negative feedback in the hypothalamus and pituitary gland. Patients may exhibit characteristic broad facies with hypertelorism, a flattened nose, prominent tongue, and thick lips and are macrocephalic, perhaps because of delayed fontanelle closure. An excessive number of skin tags have been noted in many cases.²¹⁹

Growth retardation is often prominent, in keeping with the crucial role of TR α for normal skeletal maturation and is commonly predominantly lower segmental. Radiological features of skeletal dysplasia in affected children may include wormian bones (disordered, intramembranous ossification), epiphyseal dysgenesis (disordered, endochondral ossification), and delayed dentition.^{220–223} Constipation is common and may be severe.

TR α is the predominant TR isoform in brain and affected individuals usually exhibit neurocognitive deficits, including delayed milestones (motor, speech) in childhood, impaired motor coordination and slow initiation of movement, manifesting as dyspraxia or broad-based gait, and slow speech. IQ is variably reduced.

The most common biochemical abnormalities include low/low-normal T4 and high/ high-normal T3 concentrations, a subnormal T4/T3 ratio, and variably reduced reverse T3, which may reflect altered metabolism of thyroid hormones.

Levothyroxine therapy has been beneficial in some childhood cases, improving growth, alleviating constipation, and improving motor development and wellbeing. TSH concentrations suppress readily on treatment with elevation of FT3 to supraphysiological levels, which does raise the possibility that chronic excess TH exposure in thyroxine-treated RTH patients might lead to unwanted toxicities in normal TR α -containing tissues, such as liver or bone.

Glucocorticoid Excess (Cushing Syndrome).

Glucocorticoid excess has a profound effect on skeletal growth whether the cause of Cushing syndrome is hypersecretion of ACTH, a primary adrenal tumor, or glucocorticoid therapy. Glucocorticoids appear to slow growth primarily through a direct action on the growth plate.²²⁴ GH secretion is typically normal and serum concentrations of IGF peptides and IGF-BPs are not generally affected. This concept also explains the observation that treatment with GH cannot completely overcome the growth inhibiting effects of excess glucocorticoids.

If the glucocorticoid excess resolves, catch-up growth occurs but is often not complete such that the adult height may be diminished.²²⁵ The longer the duration and the greater the intensity of glucocorticoid excess, the less likely the patient will

experience complete catch-up growth. It is therefore important to limit exposure to excess glucocorticoids as much as the underlying condition being treated will allow. The characteristic signs of Cushing syndrome (such as truncal obesity, decreased muscle mass, striae, easy bruising, thin skin, hypertension, and osteoporosis) are well known. Adrenal tumors secreting large amounts of glucocorticoids frequently also produce excess androgens, which may mask the growth inhibitory effects of glucocorticoids, but also advance skeletal maturation.

Children with Cushing syndrome may lack many of the clinical signs and symptoms associated with the disorder in adults and may present primarily with an acceleration in weight gain accompanied by a deceleration in linear growth. Thus a growth chart showing increasing weight percentiles and decreasing height percentiles suggests Cushing syndrome. In contrast, exogenous obesity is associated with normal or even accelerated skeletal growth.

Sex Steroid Exposure.

Both premature and excess sex steroid exposure, as well as delayed puberty may impact the height of a child.

Premature/Excess Sex Steroid Exposure.

Causes include premature sexual maturation because of gonadotrophin-dependent and -independent causes, adrenal hyperandrogenism because of adrenarache, virilizing adrenal tumors or congenital adrenal hyperplasia (CAH), ovarian and testicular tumors, and human chorionic gonadotropin hormone-producing tumors. Excess production of sex steroids will lead to an increase in growth rate with tall stature initially, later followed by early fusion of epiphyses and eventual short stature. For example, undertreatment or late diagnosis of CAH results in rapid growth with initial tall stature, with early growth cessation and epiphyseal fusion and short stature in adulthood. Estrogen accelerates linear growth in part by stimulating GH secretion, but estrogen also acts on the growth plate to accelerate growth plate senescence and thus decrease the remaining growth potential.^{226,227} Androgen appears to have a direct, stimulatory effect on growth at the growth plate.³⁹

Delayed Puberty.

Pubertal delay because of constitutional delay of growth and puberty is one of the most common causes of short stature in the peripubertal years. This is a variant of normal puberty that appears to be more common in males and is characterized by delayed maturation of the hypothalamic-pituitary-gonadal (HPG) axis with a consequent lack of gonadal sex steroid secretion and sexual development. Short stature with reduced GH secretion, a delayed skeletal age, and a low concentration of IGF-1 may be associated with a reduced growth velocity, as GH secretion is highly dependent on sex steroid production during adolescence. The condition is benign and self-limiting but may be associated with considerable psychological distress and poor self-esteem.

Inflammatory Disorders.

Chronic inflammatory disorders, such as JIA, IBD, and cystic fibrosis, can impair linear growth causing short stature. The underlying mechanisms are probably complex; the growth impairment may be mediated by proinflammatory cytokines, including TNF- α , IL-1-beta, and IL-6, undernutrition, and glucocorticoid therapy and may involve both direct effects on growth plate chondrocytes and indirect effects mediated by endocrine abnormalities⁵¹ (see previous sections on Regulation Of Linear Growth, Cytokine Regulation of Linear Growth, Nutritional Regulation of Linear Growth, and Glucocorticoids).

JIA is a heterogeneous group of disorders, which, as the name implies, share the common features of persistent joint inflammation, onset in childhood, and a poorly understood etiology. Categories of JIA differ in their clinical presentation, including number of joints involved, systemic inflammatory

manifestations, and the presence of psoriasis.²²⁸ Treatments include nonsteroidal antiinflammatory drugs, intraarticular and systemic glucocorticoids, methotrexate, and a variety of anticytokine therapies. Impaired linear growth is more common in systemic and polyarticular JIA but may also occur in oligoarticular JIA.²²⁸ Short stature is uncommon at initial clinical presentation but becomes more frequent in subsequent years.²²⁹ Adult height can be adversely affected. Decreased circulating IGF-1 is reported in slowly growing children with JIA.²²⁸ Decreased GH has also been reported, but the interpretation of these findings is less straightforward and could be complicated by glucocorticoid treatment and poor test specificity.²²⁸ Small randomized trials suggest that GH treatment increases growth rate²²⁸ and may increase adult height.²³⁰ Larger, long-term, controlled studies in specific JIA subtypes are needed to establish efficacy and safety.²²⁸ It is hoped that recent advances in antiinflammatory treatment will improve long-term outcomes, including stature.

IBD includes ulcerative colitis, in which the inflammation is limited to the mucosal layer of the colon, and Crohn disease, in which the inflammation is transmural and can involve any part of the gastrointestinal tract. Significant growth impairment is more common in Crohn disease. Some children may present to medical attention with poor growth as the predominant manifestation, rather than marked symptoms of gastrointestinal disease.²³¹ Screening laboratory tests, such as hemoglobin, platelet count, albumin, C-reactive protein (CRP), and erythrocyte sedimentation rate are often, but not always, abnormal at presentation.²³² Effects on adult height are mild in most patients.^{228,233} The mechanisms underlying the decreased growth may involve nutritional deficiencies, inflammation, and glucocorticoid therapy. Circulating IGF-1 concentrations can be decreased.²³⁴ Decreased GH has also been reported, but the interpretation is complicated by poor test specificity. Randomized short-term trials suggest that GH treatment can increase the linear growth rate.^{235,236} Long-term trials are needed to determine safety and efficacy.

Disorders Involving Abnormal Extracellular Fluid and/or Failure of Specific Organ Systems.

Abnormalities in a variety of organ systems, such as the kidney, liver, heart, and lungs can impair childhood growth. Many of these disorders affect the content of the extracellular fluid, an aqueous solution that contains many different solutes including inorganic ions, nutrients, proteins, dissolved gases, and metabolic waste. Abnormalities in the extracellular fluid can slow linear growth both through direct effects on growth plate chondrogenesis and indirect effects involving nutritional and endocrine factors.

In children with chronic kidney disease, short stature is common, and adult height is often affected.²³⁷ Short stature is more prevalent with more severe renal impairment and with earlier age of onset.^{238,239} The etiology is thought to be multifactorial. Poor nutritional intake caused by anorexia often contributes to the growth failure, as can glucocorticoid use.²³⁹ There is evidence that the mechanisms can include GH insensitivity and decreased IGF bioavailability because of increased concentrations of IGF-BPs.^{240,241} In children with chronic kidney disease, approaches to address the growth impairment include improving nutritional intake, optimizing dialysis, and, when possible, minimizing glucocorticoid use.^{238,239} Recombinant GH treatment increases growth rate in children with chronic renal insufficiency and is used therapeutically in select patients^{237,238} (see section on Treatment of Short Stature).

Short stature also occurs in children with renal tubular acidosis. In these disorders, hyperchloremic metabolic acidosis results from impaired acid excretion by the kidney. Impaired linear growth is seen both in distal and proximal renal tubular acidosis.^{242,243}

Decreased linear growth is also found in children with hyperphosphatemia, including those with X-lined hypophosphatemic rickets, which involves elevated FGF-23 concentrations²⁴⁴ and also hereditary hypophosphatemic rickets with hypercalciuria, which is caused by defective renal sodium-phosphate cotransporters.²⁴⁵ In these disorders, the growth plates show abnormalities typical of rickets. There is evidence that low phosphate concentrations in the extracellular fluid directly impair growth plate chondrocyte differentiation, contributing to the abnormal linear growth.²⁴⁶

Congenital heart disease frequently impairs childhood growth.²⁴⁷ Growth impairment occurs both in cyanotic and noncyanotic disorders. Undernutrition, involving both anorexia and increased nutritional needs, is thought to be a major contributing factor.²⁴⁷⁻²⁴⁹

Psychosocial Deprivation.

Psychosocial deprivation growth failure is a disorder of growth associated with emotional deprivation and/or a pathological environment that disrupts normal parent-child attachment. It can affect children of all ages; failure to thrive is usually seen in infancy, whereas toddlers and older children are frequently depressed and often manifest bizarre behaviors surrounding food and eating (such as drinking from toilets and dish pans, eating from garbage cans and pets' dishes, and binging). Delayed statural growth and puberty, growth arrest lines in long bones, and temporary widening of cranial sutures have been reported.²⁵⁰ Although malnutrition is commonly associated, there is some evidence suggesting that psychosocial deprivation may cause growth stunting despite an adequate supply of nutrients. For example, Widdowson described two German orphanages run by women of opposite personalities. Despite similar dietary intakes, children under the care of the unpleasant, aggressive, nonnurturing woman did not thrive, whereas those under the care of the woman with opposite personality traits grew well.²⁵¹

Suppressed GH secretion, not related to disturbed sleep, has been reported in some cases,²⁵² but this finding is confounded by the poor specificity of GH testing.¹⁹¹ The hallmark of this condition is reversibility of the growth stunting and GH secretory abnormalities (when found) upon removal from the hostile environment and placement into a nurturing one.²⁵²⁻²⁵⁴ Lower serum concentrations of certain neuroendocrine markers (melatonin, serotonin, β -endorphins, and adrenocorticotrophic hormone) were found in children with social deprivation, particularly those with growth failure, than in controls or children with failure to thrive.²⁵⁵

Early placement of children suffering from social deprivation into a nurturing environment is critical not only for recovery of height and weight gain, but also for cognitive improvement, and catch-up in height serves as a positive predictor of cognitive recovery.²⁵⁶ A study of socially deprived Romanian children randomized to foster versus ongoing institutional care, age 5 to 32 months at baseline, found a mean increase of 12.6 points (SD, 4.7 points) in verbal IQ for each incremental increase of 1 SD in height Z-score between baseline and 42 months.²⁵⁶ Even with growth recovery, children who had suffered early deprivation may still face persistent abnormalities of the hypothalamic-pituitary-adrenal system; early puberty; increased risk of metabolic syndrome in adulthood; and cognitive, behavioral, and emotional sequelae.²⁵⁷

Primary Growth Impairment.

Disorders Involving Autocrine/Paracrine Factors.

Growth plate chondrocytes secrete multiple factors that act locally in a paracrine fashion on nearby chondrocytes and an autocrine fashion on the secreting cell. These factors, many of which are polypeptides, serve to coordinate the proliferation and differentiation of cells in the different zones of the growth plate (see section on Autocrine/Paracrine Regulation of Linear

Growth earlier). Consequently, mutations in genes that are involved in this local intercellular communication can impair growth plate chondrogenesis, causing short stature, or sometimes enhance growth plate chondrogenesis, causing tall stature. If the abnormal growth plate chondrogenesis affects not only bone length but also bone shape, malformation ensues, and the condition is termed a *chondrodysplasia*.⁵³ The discussion that follows provides some examples of growth disorders related to paracrine signaling defects, focusing especially on those that can present as isolated short stature without an obvious chondrodysplasia.

Disorders Involving Insulin-Like Growth Factor-2. *IGF2* is an imprinted gene that is paternally expressed prenatally and, in several tissues, postnatally. DNA hypomethylation in the region of the *IGF2* gene results in reduced paternal *IGF2* expression and presents clinically as SRS (see section on Imprinting Disorders—Silver-Russell syndrome for details), which affects both pre- and postnatal growth in humans.²⁵⁸ Recently, several patients with pathogenic *IGF2* gene variants associated with intrauterine and postnatal growth restriction were described.²⁵⁹ Typically, only those who inherited the variant through paternal transmission were affected, a finding consistent with the imprinting of the *IGF2* gene. These patients also had clinical signs compatible with SRS, such as low birth weight with normal head circumference, triangular face, and micrognathia. Some patients presented with delayed psychomotor development. Hormonally, the patients had low concentrations of IGF-2, but normal to elevated IGF-1, IGFBP-3, and GH concentrations. It has also been suggested that heterozygous pathogenic variants in *HMGA2* and *PLAG1* genes can cause similar phenotypes. *PLAG1* activates *IGF2* through a promoter and *HMGA2* is an upstream regulator of *PLAG1*. Both genes were associated with growth abnormalities in mice models and in GWAS metaanalyses of height in humans.²⁶⁰

Disorders Involving C-Type Natriuretic Peptide Signaling. C-type natriuretic peptide (CNP) acts on its receptor, NPR2 (also called *NPRB* and *GC-B*) to stimulate growth plate chondrogenesis. Consequently, mutations in either the ligand or the receptor can impair linear growth. Biallelic (homozygous or compound heterozygous), loss-of-function mutations in *NPR2* cause a chondrodysplasia with short stature, termed *acromesomelic dysplasia, Maroteaux type*. Monoallelic (heterozygous) loss-of-function mutations in *NPR2* can present as isolated short stature, often with an increased sitting to standing height ratio.²⁶¹ Similarly, heterozygous mutations in *NPPC*, which encodes CNP, cause autosomal dominant short stature with a tendency to small hands.²⁶²

Conversely, gain-of-function mutations in *NPR2* can cause tall stature that may be accompanied by scoliosis, arachnodactyly, and particularly long great toes.⁵⁴ Overexpression of CNP appears to cause a similar phenotype.²⁶³

Disorders Involving Fibroblast Growth Factor Signaling. FGFs, acting through *FGFR3*, negatively regulate growth plate chondrogenesis. Consequently, gain-of-function mutations in *FGFR3* impair linear growth. The severity of the phenotype varies depending on the mutation, including (in decreasing order of severity) thanatophoric dysplasia, ACH, and hypochondroplasia, all of which include short stature that disproportionately affects the extremities as compared with the trunk.

ACH is the most common chondrodysplasia. The vast majority of patients with ACH have the same point mutation in *FGFR3* because of a high mutation rate at that base pair. It is inherited in an autosomal dominant fashion, but, because of the high mutation rate, most cases are sporadic, arising from de novo mutations. ACH results in rhizomelic short limbs, a large head, and midface hypoplasia.²⁶⁴ Impaired growth of

the foramen magnum can result in cervical cord compression, which can be lethal.

Hypochondroplasia has a milder phenotype than ACH and can present with short stature, brachydactyly, large head, and limited elbow extension.²⁶⁵ Diagnosis involves clinical features, radiological skeletal features, and molecular genetic testing. Some patients with clinical and radiological features of hypochondroplasia may not have mutations in *FGFR3*, indicating locus heterogeneity.²⁶⁵

Milder gain-of-function mutations have been reported to present as proportionate short stature.⁵⁵

Conversely, monoallelic, loss-of-function mutations cause tall stature with camptodactyly, arachnodactyly, scoliosis, and hearing loss, termed *CATSHL syndrome*.^{56,266}

Disorders Involving Parathyroid Hormone-Related Protein and Indian Hedgehog Signaling. PTHrP and IHH form a local negative feedback loop in the growth plate that controls chondrocyte proliferation and particularly hypertrophy. PTHrP acts through PTH1R, the same receptor that PTH acts through. Interestingly both loss-of-function and gain-of-function mutations in PTH1R impair growth plate chondrogenesis, causing skeletal dysplasias—Blomstrand lethal chondrodysplasia and Jansen metaphyseal chondrodysplasia, respectively.⁶⁰

PTH1R is a seven-transmembrane-domain receptor, which signals in part through Gs-alpha. Heterozygous mutations in *GNAS*, the gene encoding Gs-alpha, cause Albright hereditary osteodystrophy (AHO), which is characterized by short stature, subcutaneous ossifications, and variable brachydactyly (particularly with shortened distal phalanges and fifth, fourth and third metacarpals).²⁶⁷ The short stature develops with age because of early slowing and cessation of linear growth, accompanied by an advanced radiograph bone age and early epiphyseal closure. AHO occurs with either paternal or maternal inheritance of the mutation. When the heterozygous mutations in *GNAS* is inherited on the maternal allele, the AHO findings can be accompanied by resistance to PTH, gonadotropins, TSH, and GHRH, as well as obesity as part of pseudohypoparathyroidism 1A (PHP1a). These additional features occur with maternal inheritance because *GNAS* is imprinted, such that it is normally preferentially expressed by the maternal allele in some tissues. The role of GH treatment in PHP1a is under investigation.²⁶⁷ When the mutation is paternally inherited, the patient shows only AHO, a condition termed *pseudopseudohypoparathyroidism (PPHP)*. Mutations in protein kinase cyclic adenosine monophosphate-dependent type I regulatory subunit alpha (PRKAR1A), which lies downstream in Gs-alpha signaling, cause acrodysostosis which has features similar to AHO and PHP1a.²⁶⁸

Heterozygous mutations in *IHH* cause autosomal dominant short stature as part of brachydactyly type A1²⁶⁹ or can cause short stature with nonspecific skeletal abnormalities. The latter condition is characterized by mild disproportionate short stature often with shortening of the middle phalanx of the fifth finger but not classic features of brachydactyly type A1.⁶¹

Disorders Involving Cartilage Extracellular Matrix. Growth plate chondrocytes secrete a cartilage matrix, which is composed of many different proteins, including multiple types of collagen, and proteoglycans. Mutations in genes encoding these proteins and proteoglycans can impair growth plate chondrogenesis and therefore linear growth. The clinical manifestation can be a chondrodysplasia. For example, *COL10A1* encodes collagen X, which is a major component of the growth plate cartilage matrix, and heterozygous mutations in *COL10A1* cause metaphyseal chondrodysplasia, Schmid type. Similarly, heterozygous mutations in cartilage oligomeric matrix protein can present as pseudoachondroplasia or the milder multiple epiphyseal dysplasia-1, both of which cause short stature.

Mutations in genes encoding components of the cartilage extracellular matrix may also have a milder phenotype, presenting, not as a chondrodysplasia, but instead as short stature without an obvious chondrodysplasia. For example, *ACAN* encodes aggrecan, a proteoglycan that is an important component of cartilage matrix. Homozygous mutations can cause a severe chondrodysplasia with extreme short stature termed *spondyloepimetaphyseal dysplasia*, aggrecan type. Heterozygous mutations can cause a milder skeletal dysplasia or can present clinically as isolated short stature without obvious signs of a chondrodysplasia.²⁷⁰ The condition can be inherited as an autosomal dominant trait. Typically, the bone age is advanced, and growth cessation occurs at an early age. Affected family members may develop early-onset osteoarthritis and intervertebral disc disease because of the cartilage matrix abnormality.

Disorders Involving Intracellular Factors and/or Fundamental Cellular Processes

Genetic defects in several intracellular pathways or fundamental cellular processes result in a large and heterogeneous group of short stature syndromes. These syndromes often show distinct phenotypes reflecting multiple mechanisms of growth impairment, some of which are not completely elucidated.²⁷¹ A discussion of representative disorders in this category, chosen for their frequency or importance as models of disease, is included in this text, but bear in mind that this is a large group of disorders that includes hundreds of distinct conditions.

Defects in *SHOX*. The *SHOX* is located inside the pseudoautosomal region 1 (PAR1) of the sex chromosomes X and Y. Genes located within PAR1 escape X-inactivation; hence two copies of *SHOX* are expressed in both males and females. *SHOX* encodes a transcription factor that is required for normal limb development and growth plate function, especially for the forearm and lower legs.²⁷² Monosomy of chromosome X, in patients with Turner syndrome, leads to *SHOX* haploinsufficiency and partially explains the short stature and some of the skeletal features observed in these patients (*cubitus valgus* and the bowing and shortening of forearms and lower legs) (see [Chapter 17 on Turner syndrome](#) for details). Furthermore, heterozygous mutation in *SHOX* coding or regulatory regions cause *SHOX* haploinsufficiency and is associated with a spectrum of short stature phenotypes, including Leri-Weill dyschondrosteosis (LWD) and short stature without any specific skeletal features. LWD is a dominant inherited skeletal dysplasia that is characterized by disproportionate short stature, mesomelic limb shortening, and Madelung deformity ([Fig. 11.6](#)). In addition, LWD patients often have a high-arched palate, *cubitus valgus*, *genu varum*, muscular hypertrophy, and a tendency to have an increased BMI. Heterozygous *SHOX* defects are identified in 70% to 90% of patients with LWD and in 2% to 15% of children classified as idiopathic short stature. Homozygous *SHOX* defects are associated with Langer mesomelic dysplasia, which is a rare and severe form of short-limb dwarfism. The estimated prevalence of *SHOX* deficiency is 1:1000, and for this reason, it is considered one of the main monogenic causes of short stature.

The chromosomal location of *SHOX*, in a region with a high number of repeat sequences, is predisposed to deletions, which are responsible for 80% of cases of *SHOX* haploinsufficiency. Most of the remaining 20% are caused by single nucleotide mutations. Molecular genetic investigation of a *SHOX* defect is indicated in patients and families with LWD and in children classified as ISS because molecular diagnosis of *SHOX* defects permits the identification of the etiology of short stature. Such diagnoses have therapeutic implications because hGH treatment is effective in improving adult height.²⁷³ Both phenotypes, LWD and ISS, can be observed among affected members of the same family; for this reason the presence of



Fig. 11.6 Madelung deformity observed in patients with Leri-Weill dyschondrosteosis associated with *SHOX* haploinsufficiency: presence of dorsal subluxation of the distal radioulnar joint. X-ray image of hand and wrist with triangularization of the distal radial epiphysis; “V-shaped” proximal carpal row (or pyramidalization) and early epiphyseal growth arrest in the medial part of the distal radius, resulting in bowing of distal radius.

a relative with LWD in a family with autosomal dominant short stature increases the probability of a *SHOX* defect. In addition, the presence of disproportionate short stature, evidenced by a sitting height index (sitting height/total height) SDS over 2, without evidence of skeletal dysplasia, is a simple and useful tool for selecting ISS children for *SHOX* molecular genetic screening.⁸⁶ Because the sensitivity of the sitting height index is greater in adults than children, it is also useful to measure the sitting height index of short parents of a short child.

Noonan Syndrome and the *RAS*opathies. NS is a relatively common genetic disorder, with an estimated prevalence of 1 in 1000 to 1 in 2500 live births with an equal male and female prevalence.²⁷⁴ It has an autosomal dominant inheritance pattern, with near complete penetrance and marked clinical heterogeneity. Approximately one-third of patients diagnosed with NS have an affected parent, with the remainder of patients harboring de novo mutations. NS is characterized by distinctive facial dysmorphic features ([Fig. 11.7](#)), congenital heart defects (most commonly pulmonary valve stenosis, hypertrophic cardiomyopathy, and septal defects), chest wall abnormalities (*pectus carinatum* and/or *excavatum*), and growth retardation. Patients with NS may also show mild to moderate developmental delays, learning disabilities, kyphoscoliosis, and lymphedema, frequent easy bruising, and bleeding tendency. Individuals with NS have a mildly increased risk of developing malignancies (mostly juvenile myelomonocytic leukemia, other myeloproliferative disorders, and brain tumors) during childhood.²⁷⁵ Unilateral or bilateral cryptorchidism is frequently observed in affected boys. Although birth weight and length are usually within normal ranges, most infants with NS have feeding difficulties that can lead to failure to thrive.



Fig. 11.7 Facial features of a patient with Noonan syndrome. The same patient is shown, first at the age of 8 years (left) and 17 years (right). Typical characteristics of Noonan syndrome are observed: mild hypertelorism, ptosis, downslanting palpebral fissures, low-set posteriorly rotated ears, and *pectus excavatum*.

Growth retardation develops after birth and is accompanied by reduced IGF-1 concentrations, but usually normal GH secretion,²⁷⁶ although GHD has been described in some patients. Boys and girls with NS typically have delayed onset of puberty, which results in a prolonged adolescent growth period. Mean adult height for patients with NS is approximately -2 SDS,²⁷⁷ and it was recently recognized that patients with NS show a lean body phenotype and resist weight gain during childhood and adult life.^{276,277}

Mutations in more than 15 genes acting in the RAS/MAPK pathway cause NS, as well as other rare syndromes with characteristics that overlap with NS, including cardiofaciocutaneous syndrome, Costello syndrome, NS with multiple lentiginos (formerly called *LEOPARD syndrome*), and Noonan-like syndrome with loose anagen hair.²⁷⁶ These syndromes represent a group of genetic conditions known collectively as *RASopathies*. The molecular defects associated with NS and other *RASopathies* lead to RAS/MAPK pathway hyperactivation, which is responsible for the pleiotropic effects observed in these disorders.

Diagnosis of NS should primarily be based on clinical findings. Classical facial features or typical cardiac malformation generally trigger a suspicion for NS, especially when associated with developmental delay and/or short stature. The molecular diagnosis of NS can be established by a multigene panel or whole exome sequencing (WES).²⁷⁸ Serial single-gene testing can be considered if panel testing is not feasible and should start with protein-tyrosine phosphatase nonreceptor-type 11 (*PTPN11*) sequencing because 40% of NS patients harbor heterozygous pathogenic variants in this gene. *PTPN11* pathogenic variants are more likely to be found when pulmonary stenosis is present, and mutations at codons 61, 71, 72, and 76 are associated with juvenile myelomonocytic leukemia.²⁷⁸ It is noteworthy that several studies investigating children with idiopathic short stature by multiple gene-targeted sequencing identified patients who were heterozygous for pathogenic variants in *PTPN11*, reflecting the mildest end of the clinical spectrum of NS.²⁷⁹

Disorders of *GNAS* Inactivation. Guanine-nucleotide binding protein (G protein) subunit alpha S (Gs-alpha) is a key protein that mediates the intracellular signaling of many hormone receptors by activation of adenylyl cyclase. Gs-alpha is one of the transcripts generated from the *GNAS* locus, a complex, imprinted locus that produces multiple transcripts using alternative promoters and alternative splicing. Gs-alpha shows biallelic expression in most cells (e.g., chondrocytes), but in some cells (e.g., pituitary somatotropes, proximal renal tubular cells, thyroid epithelial cells, gonadal cells), it is preferentially expressed from the maternal allele.²⁸⁰

Heterozygous inactivation of *GNAS* is responsible for two main phenotypes: PHP and PPHP.²⁶⁷ Patients with PHP show

resistance to PTH in proximal renal tubular cells and consequent hypocalcemia and hyperphosphatemia, despite high PTH concentrations. Resistance to other hormones can be observed, mainly TSH, gonadotropins, and GHRH. In addition, patients with PHP can also present with early-onset obesity and cognitive impairment. PHP can be caused by inactive mutations in *GNAS* coding regions on the maternal allele (PHP1a) or epimutation (e.g., abnormal methylation patterns), which decrease the expression of maternal Gs-alpha (PHP1b).²⁶⁷ Short stature with skeletal involvement is present in patients with PHP1a and PPHP, because of a decrease in PTHrP action through the PTH receptor (PTH1R) resulting from Gs-alpha deficiency. Patients with PPHP carry mutations in the paternal *GNAS* allele and hence do not show PTH resistance in renal tubular cells where the maternal allele is expressed. The skeletal phenotype observed in patients with PHP1a and PPHP is termed *AHO*. *AHO* is characterized by a rounded face, short stature, premature closure of growth plates, brachydactyly, and ectopic ossifications.²⁶⁷

In most children with PHP1a and PPHP the height percentile declines with age, and the bone age is usually advanced.²⁶⁷ Consequently, most patients with PHP1a and with PPHP have short stature by the time of adulthood. In both conditions, resistance to PTHrP in the growth plate likely plays an important role, and, in PHP1a, GHRH resistance may also contribute. In contrast, most patients with PHP1b are of normal stature.

It is recommended that molecular diagnosis of *AHO* should include DNA sequencing, followed by copy number variation (CNV) analysis of the *GNAS* locus for patients with PHP and PPHP. Methylation analysis of the *GNAS* locus is recommended for PHP patients with negative genetic test results.^{267,268}

3-M Syndrome. 3-M syndrome is an autosomal recessive disorder characterized by pre- and postnatal growth retardation, normal head circumference, facial dysmorphism (round face with a large forehead, hypoplastic midface, full eyebrows, short nose with anteverted nares, prominent mouth, and pointed chin), mild skeletal changes (short neck, hyperlordosis, tall vertebral bodies, and prominent heels), and normal intelligence.^{281,282} Hypogonadism and hypospadias have been reported in some male patients. To date, biallelic loss-of-function mutations in three genes (*CUL7*, *OBSL1*, or *CCDC8*) have been implicated in 3-M syndrome, but it is expected that mutations of other genes within the same pathway might be involved in patients with no identified molecular defect. *CUL7* is a ubiquitin ligase and is involved in the degradation of a number of proteins, such as p53, which regulate the cell cycle, IGF-1 signaling, and chondrocyte proliferation. 3-M syndrome is part of the differential diagnosis of SRS (see section on Imprinting Disorders—Silver-Russell Syndrome for details),

and should be considered in patients with typical skeletal findings and/or with consanguineous parents. Molecular diagnosis of 3-M syndrome is preferably established by multigene panel or WES because of genetic heterogeneity.²⁸³

DNA Damage Repair Syndromes. As a group, DNA damage repair syndromes are characterized by genetic instability, growth failure, intellectual disability, increased risk of cancer, and features of accelerated aging.²⁸⁴ These are rare conditions, but Bloom syndrome²⁸⁵ and Fanconi anemia²⁸⁶ are two important differential diagnoses for children born SGA that do not show catch-up growth. Both are autosomal recessive disorders associated with prenatal onset of growth retardation and microcephaly. Bloom syndrome is classically associated with telangiectatic erythema of the midface in a butterfly distribution, with photosensitivity and signs of immunodeficiency (i.e., increased susceptibility to pneumonia, bronchiectasis, and chronic lung disease). Fanconi anemia should be suspected when patients have a thumb deformity (hypoplasia or aplasia), and signs of bone marrow failure (anemia, neutropenia, and/or thrombocytopenia). In addition to growth impairment and low BMI, both conditions are also associated with other endocrine abnormalities. Hypergonadotropic hypogonadism is commonly observed in both conditions. In addition, patients with Fanconi anemia are also at markedly increased risk for diabetes mellitus, dyslipidemia, and thyroid dysfunction.²⁸⁶ Diagnosis of both Bloom syndrome and Fanconi anemia can be established through a specialized cytogenetic test for chromosomal abnormalities or by molecular genetic testing for biallelic pathogenic variants in an associated gene.^{287,288}

Primordial Dwarfisms. Primordial dwarfism is the term for a group of disorders characterized by extreme, global pre- and postnatal growth failure, marked microcephaly, and varying degrees of intellectual disability.^{289,290} It comprises rare recessive genetic diseases, such as Seckel syndrome, microcephalic osteodysplastic primordial dwarfism (MOPD), and Meier-Gorlin syndrome. Each of these syndromes has specific clinical features and is caused by defects in specific genes. The genetic defects associated with these syndromes compromise cell division and include abnormalities in genes that participate in fundamental cellular processes.²⁹⁰ For example, Seckel syndrome type I is caused by defects in the *ATR* gene, a master regulator of a DNA damage response signaling cascade, which is an essential regulator of genomic integrity. MOPD type II is caused by defects in the pericentrin gene (*PCNT*), which encodes a core centrosomal protein that is essential for mitotic spindle formation.

Imprinting Disorders.

Imprinting disorders are a group of congenital disorders caused by molecular changes that affect imprinted chromosomal regions and genes.²⁹¹ Imprinting is an epigenetic phenomenon in which genes are expressed in a parent-of-origin-specific manner—either expressed from the maternal or the paternal allele, not biparentally. Genomic imprinting involves DNA or histone methylation that does not alter the genetic code. Although imprinted genes may undergo mutations that affect protein function (e.g., see *IGF2* defects), in addition, their expression can be affected epigenetic changes, including alterations in DNA methylation (epigenetic mutation or epimutation) or inheritance of both alleles from the same parent (uniparental disomy).²⁹¹ SRS, Temple syndrome, and Prader-Willi syndrome (PWS) are examples of growth disorders that involve imprinted genes and can be caused by epimutations.

Silver-Russell Syndrome. SRS is a well-recognized cause of pre- and postnatal growth retardation. Typical patients are born SGA with preserved head circumference (relative macrocephaly) and do not undergo spontaneous catch-up growth in



Fig. 11.8 Phenotype of a patient with Silver-Russell syndrome: low-body mass index, triangular-shaped face, and a broad forehead with relative macrocephaly.

postnatal life (height SDS < -2 after the second year of life). In addition, patients with SRS usually have protruding foreheads, feeding difficulties with low body mass indices, and body asymmetry (Fig. 11.8).²⁵⁸ Other common features observed in patients with SRS are triangular face, micrognathia, crowded or irregular teeth, fifth finger clinodactyly, low muscle mass, and excessive sweating; males may also exhibit hypospadias. Hypoglycemia may also be a feature and necessitate overnight feeding.

SRS is often caused by imprinting abnormalities involving the chromosomal region 11p15.5.²⁹² This locus contains the *IGF2* gene, which is expressed exclusively from the paternal allele during fetal life in most embryonic tissues. Hypomethylation of the *H19/IGF2* differentially methylated region is identified in 40% to 60% of patients with SRS, whereas only 1% of patients carry CNVs in this region. Interestingly, patients with 11p15.5 epimutation frequently have multilocus imprinting disturbances, although the clinical significance of this phenomenon remains unclear.²⁵⁸ Maternal uniparental disomy involving this region [UPD(11p)m], although possible, has rarely been described. All these molecular defects lead to a decrease in paternal *IGF2* expression, which contributes to growth restriction. Less than 10% of patients with SRS carry maternal UPD of an imprinted locus on chromosome 7 [UPD(7q)m] that harbors two imprinted genes: one maternally expressed, *GRB10* (7p12.1), and another paternally expressed *MEST* (7q32).^{258,291}

There is extensive overlap in clinical phenotypes associated with SRS-related (epi)genotypes. Body asymmetry, fifth finger clinodactyly, congenital anomalies, and marked prenatal growth impairment are more frequently observed in patients with 11p15.5 hypomethylation, whereas neurocognitive problems are more frequent in patients with UPD(7q)m.^{258,293} Molecular tests are useful to confirm clinical diagnoses and are usually assessed by methylation-specific multiplex ligation-mediated polymerase chain reaction (PCR) amplification (MS-MLPA), which is cost-effective and enables parallel analysis of copy number and DNA methylation.²⁵⁸ Molecular karyotype analysis (single nucleotide polymorphism [SNP]-array or comparative genomic hybridization [CGH]-array) should be carried out in patients in which the diagnosis of SRS is considered but mUPD7 and 11p15 hypomethylation have been excluded.²⁵⁸

Prader-Willi Syndrome. PWS is an imprinting disorder characterized by neonatal hypotonia, failure to thrive in early infancy,

global developmental delay, short stature, and rapid-onset obesity during childhood with high body fat mass and low muscle mass.^{294,295} Patients typically also have some degree of intellectual disability, behavioral disorders, and small hands and feet. Patients with PWS have an increased incidence of sleep apnea and sudden death. In addition, PWS is associated with hypothalamic dysfunction causing hypogonadotropic hypogonadism, reduced GH secretion, and an increased risk of central adrenal insufficiency.^{294,295}

PWS is caused by a lack of expression of paternally inherited genes in the region of chromosome 15q11.2-q13.^{295,296} Several genes in this locus that contribute to the PWS phenotype are expressed exclusively from the paternal allele. The majority of patients (70%) have a deletion of the paternal allele, whereas one-quarter have maternal uniparental disomy [UPD(15q)m]. Imprinting defects that silence paternal genes in the PWS locus have also been observed in PWS patients but are less common.^{295,296} Although a DNA methylation analysis by MS-MLPA that is consistent with PWS is sufficient for diagnosis, identification of the underlying genetic mechanism is necessary for appropriate genetic counseling.²⁹⁶

Evidence that GH deficiency may contribute to the short stature and body composition abnormalities in PWS has motivated clinical trials of GH therapy for this disorder. These trials suggest that GH treatment of children with PWS increases linear growth, lean body mass, physical function, and decreases fat mass. As a result, GH treatment for PWS was approved by the FDA. However, final height may be compromised because of the relatively frequent occurrence of premature adrenarche. Because children with PWS are at increased risk of sleep apnea and sudden death and the possibility that GH might exacerbate this risk, clinical and polysomnographic evaluation for sleep-related disordered breathing is recommended.

Temple Syndrome. Temple syndrome is an imprinting disorder that is considered in the differential diagnosis of SRS and PWS because of phenotypic overlaps.²⁹⁷ It is mainly caused by maternal uniparental disomy of chromosome 14 [UPD(14)m] but can also be caused by epimutation and/or microdeletion affecting a paternal imprinting region on chromosome 14q32.2. Typical patients with Temple syndrome are born SGA with relative macrocephaly and do not experience catch-up growth during childhood. These individuals usually show hypotonia and feeding difficulties during infancy but develop truncal obesity later in childhood. Other features that overlap with SRS or PWS include frontal bossing, prominent forehead, small hands and feet, and mild intellectual disability. Precocious puberty and metabolic abnormalities, such as overweight/obesity, early-onset glucose intolerance/type 2 diabetes, and hyperlipidemia, are important distinguishing characteristics of patients with Temple syndrome.²⁹⁸ These metabolic associations are attributed to haploinsufficiency of *DLK1*, a paternally expressed gene located at 14q32.2, which acts as a negative modulator of adipocyte differentiation.

Chromosomal Abnormalities and Copy Number Variation. Chromosomal abnormalities constitute a well-established cause of growth disorders that was first identified after the development of classical cytogenetic techniques, such as karyotyping.²⁹⁹ Karyotyping allowed for identification of the molecular basis of Turner and Down syndromes as complete or partial X monosomy and trisomy of chromosome 21, respectively. The knowledge derived from these pioneering studies was responsible for inclusion of genetic testing in the routine evaluation of children with growth disturbance. It is accepted that karyotyping should be ordered in any female with unexplained growth failure.³⁰⁰ The clinical features of Turner syndrome are described in Chapter 17.

After the development of fluorescence in-situ hybridization and array-based techniques, it became feasible to identify an

increasing number of small, unbalanced structural rearrangements that are below the resolution limit of the light microscope. These submicroscopic deletions and duplications involve a single gene or can involve a set of genes in close proximity and thus can cause contiguous gene syndromes. The patients' phenotype is determined by the combination of phenotypes associated with each gene present in the unbalanced chromosome segment. There are numerous examples of contiguous gene syndromes associated with growth disorders,²⁷¹ such as Williams-Beuren syndrome, caused by 7q11.23del, and DiGeorge syndrome, caused by 22q11.2del. The prevalence of pathogenic CNV in children with short stature of unknown etiology reportedly ranges from 10.4% to 15.5%, being more frequent in patients with developmental delay, intellectual disability, or additional major malformations.³⁰¹ However, both in research studies and in clinical practice, it is often difficult to determine whether a particular CNV is pathogenic or benign and coincidental.

Idiopathic Growth Impairment. Often children present to an endocrinologist with short stature of postnatal onset, and an etiology cannot be identified. In these idiopathic conditions, there are clues that the underlying unknown etiologies are likely to be extremely diverse. Some idiopathic short stature is inherited in a pattern that suggests a polygenic inheritance, whereas others show a monogenic recessive or monogenic dominant pattern. Some short stature is proportionate, affecting different bones in the body similarly, whereas some is disproportionate, often with growth of the extremities affected more than growth of the spine. In some children, the child appears completely healthy and normal whereas other children have dysmorphic features, developmental delay, or other health problems, suggesting that an underlying genetic change may affect more than just growth plate chondrocytes. In some children with idiopathic short stature, the weight is affected more than the height, suggesting that the decreased linear growth is secondary to decreased nutrition, perhaps involving reduced appetite.³⁰² In some children with short stature of unknown etiology, body length was already below normal at birth, suggesting that linear growth was impaired in the fetus. In other children, the birth size was normal, and the short stature developed at some point during childhood, suggesting that the linear growth impairment only became manifest in postnatal life.

What might be the etiologies? We can speculate that the etiology of the decreased growth plate chondrogenesis in these children may fall into the same categories as the known etiologies of short stature. Some may be endocrine, for example, GH or IGF-1 effects that are too subtle to be detected by our current tests. Others may involve local paracrine/autocrine signaling systems, cartilage matrix, or intracellular regulatory mechanisms. Some may be mild forms of known skeletal dysplasias or dysmorphic syndromes, but with manifestations too subtle for clinical recognition. Others are likely caused by genes whose effect on growth plate chondrogenesis is currently unknown.

Following traditional classification schemes, short stature of postnatal onset and short stature of prenatal onset are discussed separately in the following sections.

Idiopathic Short Stature of Postnatal Onset, Including Constitutional Delay. In many children with short stature of postnatal onset, the etiology cannot be determined despite a careful medical history, physical examination, and laboratory studies. In some of these children, both parents, the siblings, and other family members are short, suggesting a polygenic inheritance. Presumably, these children have inherited multiple sequence variants each of which has a mild, negative effect on linear growth. Some of these variants are likely to be common polymorphisms that have been identified by recent GWAS.⁷⁴

In other children, the short stature appears to be inherited primarily from one parent, and a careful family history suggests an autosomal dominant inheritance. In these children, the short stature primarily arises from a single genetic variant. Some dominant causes, such as mutations in *SHOX* or *NPR2* are known, but it is likely that there are many monogenic causes yet to be discovered.

Some children with short stature show a marked delay in physical maturation, with a delayed bone age, a delayed onset of puberty, and continuation of linear growth to a later age than other children. Professor James Tanner, a pioneer in the field of childhood growth, used the musical term *tempo* to describe this overall pace of maturation, which varies between different children. The term *constitutional delay* of growth and puberty is used to describe children with a delayed tempo. This term can conflate two conditions—constitutional delay that has a clinical onset at puberty and constitutional delay that has a clinical onset in early childhood.

Some children have a delay of pubertal onset in which the primary cause appears to be a delay in the activation of the HPG. Often there are other family members with a similar delay in pubertal activation and sometimes family members with hypogonadotropic hypogonadism. These children typically grow normally in the prepubertal years. After the normal age of pubertal onset, a transient delay in sex steroid secretion in these children causes their linear growth percentile to decrease. This decrease in percentile results in part from the fact that other children undergo a pubertal growth spurt, causing the normal percentile curves to pull away from the delayed child. However, even if one ignores the upward deflection of the normal range, the linear growth rate of the child with delayed puberty tends to decline from his or her prior prepubertal rate, presumably because of progressive growth plate senescence without the countervailing accelerating effect of sex steroids. In these children, once puberty starts at a late age, a pubertal growth spurt occurs. Although the magnitude of the pubertal growth spurt is typically diminished compared with a child with earlier pubertal onset, the overall prolonged period of growth allows for a normal or even tall adult stature.

In other children with constitutional delay the delay appears to have a prepubertal onset. These children are short throughout childhood and have a delayed bone age even before puberty. Some of these children may be short in childhood but, because of the delayed cessation of growth in adolescence, will end up at a normal adult height. In other children with this form of constitutional delay, the adult stature may be somewhat low. In these children, a primary lesion affecting the HPG axis could not account for the condition. One possible contributing factor to explain the decreased growth, bone age delay, and pubertal delay is diminished appetite.³⁰²

Short Stature of Unknown Etiology and of Prenatal Onset. In some children with short stature, body size was already diminished at birth, that is, the child was born SGA. Various definitions of SGA have been used. However, a consensus statement defines SGA by a weight and/or length less than -2 SDS.³⁰³ A history of being born SGA indicates a process that diminishes fetal growth because of fetal, placental, or maternal abnormalities. Fetal causes include genetic disorders (such as single gene defects, imprinting defects, deletions/duplications, and aneuploidy—see discussions earlier in chapter), fetal infection (such as cytomegalovirus and toxoplasmosis), and multiple gestations. Placental causes include confined placental mosaicism, abruptio placenta, placenta previa, and other conditions associated with uteroplacental insufficiency. Maternal causes include preeclampsia; hypertension; pregestational diabetes mellitus; and systemic maternal renal, cardiac, pulmonary, hematological, and rheumatological disorders, as well as maternal use of alcohol

or cigarettes. However, in many infants born SGA, the underlying etiology remains unknown.³⁰⁴

If the SGA child continues to fall further away from the normal range postnatally, then the underlying etiology likely involves a fundamental process that affects both fetal and postnatal growth, for example, some chondrodysplasias. Genetic disorders that cause both marked fetal and postnatal growth impairment are referred to as *primordial dwarfisms*. Typically, the growth impairment affects multiple tissues, including the CNS, resulting in a relatively small head size. Often, in addition to diminished growth, there are congenital anomalies. This category is heterogeneous and includes recognized genetic syndromes, such as Seckel syndrome, microcephalic osteodysplastic primordial dwarfism types I and II, and Meier-Gorlin syndrome.³⁰⁵ Multiple genetic defects have been identified, involving fundamental cellular functions, for example, DNA replication, DNA repair, RNA splicing, and centrosome formation.²⁹⁰ SRS is characterized by decreased fetal and postnatal growth, which can affect body size asymmetrically. A triangular-shaped face, prominent forehead, and specific minor anomalies are often found (see Primary Growth Impairment—Imprinting Disorders earlier).

However, in most children born SGA, the growth rate after birth exceeds normal, causing catch-up growth. Much of this catch-up growth occurs during the first year of life and approximately 90% of term SGA children attain a stature greater than -2 SDS by 2 years of age.³⁰³ However, the adult height of individuals born SGA is decreased by approximately 1 SDS compared with the normal population.³⁰³ Catch-up growth tends to be more gradual and less complete in preterm SGA children. For children born SGA whose height fails to catch up into the normal range, treatment with recombinant GH increases linear growth and is approved for this use in both the United States and Europe.

There is evidence that individuals born SGA tend to show more insulin resistance and adiposity than those born at normal size. The effects appear to be greater in those who were SGA but gained weight rapidly during the first 3 months of life.³⁰⁶ There is also evidence that girls born SGA have a tendency to earlier menarche, although usually still within the normal range, and are also more likely to have premature adrenarche,³⁰³ although not all studies support these conclusions.³⁰⁷

Diagnostic Approach to the Patient With Short Stature

Although short stature itself is not a disease, growth failure can be a sensitive sign of an underlying health problem. The diagnostic evaluation should first try to distinguish healthy variant from pathological growth patterns and, for the latter, identify the underlying cause and treat them accordingly.

Time Course. The onset of growth faltering can be a very helpful clue for narrowing the differential diagnosis. To ascertain the time course accurately, the clinician must have the child's growth records available, both for length/height and weight, preferably beginning at birth. Because these records are of such value to the diagnostic evaluation, the parents should be urged to obtain a complete set. If the data are not already plotted on appropriate length/height and weight growth charts, the clinician should do so. The clinician then visually evaluates the overall pattern of growth to determine when the growth rate was subnormal, that is, when the child's height or weight was crossing percentiles downward. Often the growth curve will show some irregularities resulting from measurement error, and the experienced clinician learns to smooth the data in the mind's eye.

If the child's height and/or weight were subnormal at birth (SGA), the implication is that the growth rate was below

normal in utero and therefore that a condition interfering with growth was already present in fetal life. If the growth curves then show recovery after birth, the overall pattern suggests a condition that was present in utero but then resolved, such as a maternal or placental disorder. In contrast, if the SGA child continues to show slow growth postnatally, the pattern suggests an intrinsic growth problem, such as a genetic syndrome or skeletal dysplasia.³⁰⁸ Similarly, defects in IGF-1 action, including *IGF-1* gene defects, *IGF1R* gene defects, as well as *IGF-2* gene defects and SRS, cause combined pre- and postnatal growth failure. If the child is born at a normal size but then grows slowly during early postnatal life, the clinician should suspect a congenital disorder that affects postnatal but not fetal growth, including congenital GH deficiency or GH insensitivity syndrome. Onset of growth faltering during childhood suggests an acquired problem, for example, IBD, a tumor causing pituitary dysfunction, or a medication that interferes with growth. The astute clinician can sometimes find a correspondence between the onset of slow growth on the growth chart and some event in the child's history, such as starting a growth-suppressing medication or the onset of an illness. Such a temporal correspondence suggests a causal relationship. Onset of growth slowing during adolescence may also result from delayed puberty.

Inheritance Pattern. A careful family history, ideally including the heights of parents, siblings, grandparents, aunts, uncles, and cousins, can be highly informative. Traditionally, the family history is used to categorize the short stature as either familial or nonfamilial. However, this categorization is confusing in terms of the underlying genetic etiology. Familial short stature conflates monogenic short stature (primarily because of a mutation in a single gene, either dominant or recessive) and oligo/polygenic short stature. Nonfamilial short stature, despite the label, can still be genetic (see later).

Therefore it is more helpful to use the pedigree to help distinguish monogenic (primarily autosomal dominant and autosomal recessive) causes of short stature from oligo/polygenic causes. In some cases, the short stature appears to be inherited from an affected parent and an affected grandparent, suggesting a dominantly inherited defect, for example *SHOX* deficiency or a heterozygous *NPR2* mutation. Less often, short stature occurs in two affected siblings with unaffected parents, suggesting a recessive disorder, such as GH insensitivity syndrome or 3-M syndrome. In other cases, the child is uncharacteristically short compared with all the other family members. This apparently sporadic short stature could be caused by an acquired problem or by a genetic defect that is autosomal recessive, de novo dominant, or (if the patient is male) X-linked recessive. If the parents are of truly midnormal stature or even tall, the condition is less likely to be polygenic. In other families, multiple family members are short, in a pattern that cannot be explained by a monogenic inheritance, suggesting an oligogenic or polygenic inheritance. These conditions are generally benign.

In practice, the pedigree may not clearly distinguish between a monogenic and polygenic inheritance, in part because of assortative mating, that is, the tendency of short individuals to mate with other short individuals. As a result, families may have components of both monogenic and oligo/polygenic short stature. When there is a wide spread in parental heights, a child may take after one or the other parent's height channel rather than the calculated average, and with random assortment of genes at each and every conception, it is impossible to predict a priori which parent that is.

Careful Medical History and Physical Examination. With the differential so broad, a careful and comprehensive history is critically important. Multigenerational history of adult heights and pubertal timing, as well as diseases that run in

the family, is needed to set the stage. The family history should identify family members with delayed puberty, which would suggest constitutional delay of growth and puberty, as well as those with early puberty, which can also be a heritable factor affecting a child's growth pattern. In addition, the clinician should ask about family history of medical illnesses that have a hereditary component and might affect growth, such as hypothyroidism.

Prenatal and birth history, particularly birth weight and length and gestational age, help identify conditions that affect fetal growth. Detailed questioning should be used to screen for subtle symptoms of systemic illnesses, for example, gastrointestinal disease, CNS tumors, GH deficiency or other pituitary disease, hypothyroidism, Cushing syndrome, and eating disorders. Medications should be ascertained. History of neonatal jaundice or hypoglycemia, CNS malformations (especially SOD and holoprosencephaly), history of head trauma or neurological insult, and history of neoplasia, especially when treated with cranial irradiation, should raise suspicion for possible GH deficiency. For adolescents, the age of thelarche, pubarche, menarche, and voice change, as appropriate, are needed to place the growth pattern within the context of pubertal timing. Social and nutritional histories sometimes make the diagnosis.

The physical examination serves particularly to screen for dysmorphic syndromes and systemic illnesses. The clinician should look carefully for dysmorphic features that might point to a syndrome, such as Silver-Russell, Turner, Noonan, Down, Prader-Willi, or Williams syndrome, or *SHOX* haploinsufficiency. Comprehensive physical examination should include funduscopy, visual fields by confrontation, and thyroid palpation. GH deficiency may be associated with midline defects (especially central maxillary incisor), nystagmus (presenting sign of SOD), and papilledema, and in boys, micropenis, mid-face hypoplasia, abdominal adiposity, and high-pitched voice. Similarly, signs of hypothyroidism should be sought.

Evaluation of dental age may provide insight into a child's skeletal maturation. There are wide variations in the time of eruption of primary and secondary teeth, which may be affected by local and environmental factors, such as jaw size, position of the unerupted teeth, and premature loss of deciduous teeth. Children with GH deficiency or untreated hypothyroidism usually have significantly delayed dentition or abnormal teeth (hypodontia, usually of the maxillary incisors). Mild delays in dental progress may occur in constitutional growth delay. Delays in dentition are often associated with delayed closure of the fontanels and delayed bone age.

Anthropometrics. Accurate anthropometric measurements should be performed (see section on Measurement earlier in this chapter) including height, weight, head circumference, arm span, and sitting height or lower segment. Abnormal body proportions can be a valuable clue for the diagnosis of a chondrodysplasia. Many chondrodysplasias affect the growth plates of the extremities more than those of the vertebrae, resulting in an increased sitting height index, increased upper-to-lower segment ratio, and short arm span compared with the height. Conversely, a disproportionately short trunk can reflect conditions that adversely affect the vertebrae, such as scoliosis, spinal irradiation, or certain chondrodysplasias. When disproportionate short stature is suspected, further measurements of the various limb segments should also be made to distinguish rhizomelic, mesomelic, and acromelic conditions. The diagnosis of a specific chondrodysplasia can often be made by expert interpretation of skeletal radiographs complemented with specific biochemical and genetic tests that target the specific causal mutations.⁸⁹

The relative gain in weight compared with gain in height is important to consider in every child undergoing a growth

BOX 11.3 Body Mass Index

$$\text{Body mass index (BMI)} = \frac{\text{weight (kg)}}{(\text{height in meters})^2}$$

evaluation, even those without any gastrointestinal symptomatology, to exclude a nutritional cause of growth faltering. BMI (Box 11.3) is calculated for children over age 2 years, and weight-for-length can be used from birth to age 5 years; both are plotted on their respective growth charts (available at https://www.cdc.gov/growthcharts/cdc_charts.htm). Although BMI or weight-for-length “below the curves” is an obvious sign of severe malnutrition, growth faltering can result from milder cases of malnutrition that plot within the “normal” ranges. Drop across major centiles of BMI or weight-for-length and/or a drop in weight-for-age centiles that precedes the drop in height-for-age centiles should alert to a possible underlying nutritional problem. In contrast, when the height is affected similarly to the weight or more than the weight (resulting in a normal to mildly increased BMI), one should suspect disorders in which the growth plates are affected more than the growth of other tissues, for example, GH deficiency, hypothyroidism, and chondrodysplasias. When the height percentile decreases but the weight percentile increases, glucocorticoid excess should be considered.

Prenatal malnutrition can result in a birth size characterized by SGA or IUGR. Infants exposed to early fetal malnutrition have low birth weight and are symmetrically small (proportionate IUGR). Undernutrition in late pregnancy results in an asymmetrically growth-restricted infant whose head circumference is preserved by a physiological adaptation (brain-sparing phenomenon).

Bone Age. Although the bone age is helpful in assessing a patient’s growth potential, it provides only limited information as to the underlying cause of the short stature. A bone age can be delayed because of constitutional delay, many systemic or endocrine diseases impairing growth, chronic glucocorticoid treatment, or malnutrition. A bone age consistent with the chronological age can occur in polygenic familial short stature, some monogenic causes of isolated short stature, some syndromic causes, and some chondrodysplasias. An advanced bone age, which occurs uncommonly in children with short stature, points to specific genetic disorders, such as heterozygous *ACAN* mutations, *AHO*, and *SHOX* deficiency.

Screening Laboratory Investigations. Not all children with short stature need a screening laboratory investigation. For example, in a child who has been growing steadily just below and parallel to the lower limit of normal for stature, who appears otherwise healthy in the medical history and physical examination, and who has a pedigree suggesting polygenic short stature, the yield of laboratory investigation would be very low. A second example would be a child with a condition, such as a genetic syndrome, that readily explains the short stature. However, when the pattern of growth, medical history, physical examination, or pedigree are concerning for an occult pathological etiology (see sections on Time Course, Inheritance Pattern, Careful Medical History and Physical Examination, and Anthropometrics), then a laboratory investigation may be warranted.³⁰⁹

Recommended laboratory screening tests may include a complete blood count, erythrocyte sedimentation rate, creatinine, glucose, electrolytes, bicarbonate, liver function tests, calcium, phosphate, albumin, celiac screen, FT4, TSH, IGF-1 and, in younger children, IGFBP-3.^{309,310} A karyotype or microarray

should be performed in girls with unexplained short stature to exclude a diagnosis of Turner syndrome³¹¹ and in short boys with associated genital abnormalities. A radiograph of the left hand and wrist should be obtained for a bone age evaluation by an expert to estimate the remaining growth potential. Malformations on this radiograph may sometimes point to a skeletal dysplasia. An advanced bone age in a child with short stature suggests specific genetic etiologies, such as *ACAN* mutations.²⁷⁰ A skeletal survey should be considered in children with suspicion for a skeletal dysplasia, such as those with disproportion or those with a family history of disproportionate short stature.

There is evidence that such laboratory screening approaches have a low yield in a population of children below the third percentile for height, negative history, review of systems, and normal physical examination,³¹² again emphasizing that these laboratory tests can be reasonably omitted in some children, as discussed earlier.

Measurement of Insulin-Like Growth Factor-1 Concentrations in Growth Disorders. Serum IGF-1 concentrations are greatly influenced by chronological age, degree of sexual maturation, and nutritional status.³¹³ As a result, use of normative values based on both age and pubertal stage is critical. For example, using a normal range for Tanner I children is problematic because normal IGF-1 concentrations rise substantially with age even before puberty. Conversely, using the normal range for 14-year-old boys as normative data for a prepubertal patient of that age is also problematic because the patient will have lower IGF-1 concentrations because of lower sex steroids. Therefore a practical approach is to use age-specific normative data for children of prepubertal age and to use pubertal stage-specific normative data for children of pubertal age.

Serum IGF-1 concentrations are commonly used as a screening test for GH deficiency. This use is based on the well-established physiological observation that GH stimulates circulating IGF-1 concentrations. Consequently, IGF-1 concentrations are, on average, lower in children with GH deficiency than in normal children or children with non-GH-deficient short stature. However, there is extensive overlap between the serum IGF-1 concentration in GH-deficient and non-deficient children both because GH-deficient children can have serum IGF-1 within the lower portion of the normal range and because non-GH-deficient children with short stature can have serum IGF-1 below the normal range. Thus serum IGF-1 appears to show both incomplete sensitivity and specificity for GH deficiency.^{314–316} However, studies in this area are complicated to interpret because we do not have a gold standard test for GH deficiency, but instead rely on provocative GH tests, which are also problematic (see section Follow-Up Laboratories and Imaging later). As a practical matter, serum IGF-1 concentrations may help direct the evaluation toward or away from GH deficiency but should be interpreted with caution. Low IGF-1 concentrations can also be seen in other conditions, for example, malnutrition, GH insensitivity, *IGF1* mutations, and ALS deficiency. High IGF-1 concentrations can occur in some causes of short stature, for example, *IGF1R* mutations, in which the IGF-1 concentrations tend to be above the average for age unless the patient is malnourished, and *PAPPA2* mutations.

Because GH stimulates not only IGF-1 but also IGFBP-3 production, serum IGFBP-3 can similarly be used as a screening test for GH deficiency. Blum and associates³¹³ have suggested that radioimmunoassay determination of serum concentrations of IGFBP-3 might be more specific (but less sensitive) than IGF-1 assays in the diagnosis of GH deficiency and may be useful in young children in whom the normal concentrations of IGF-1 are low and therefore difficult to distinguish from GH deficiency.

Follow-Up Laboratories and Imaging. After screening laboratories (random blood draw) narrow the differential diagnosis, follow-up functional testing may be needed. The most commonly performed functional tests are provocative GH tests (also called *GH stimulation tests*) to diagnose GH deficiency. Because of its pulsatile secretion during deep sleep, random GH levels are generally uninformative except in the neonatal period, before the sleep entrainment develops.³¹⁷ Measurement of circulating concentrations of IGF-1 and IGFBP-3 may be helpful but are generally not definitive, as discussed earlier. Thus a group of provocative (stimulation) tests has been developed in which GH concentrations are serially measured via an in-dwelling intravenous catheter after administration of pharmacological agents identified as GH secretagogues (Table 11.5). These tests should be performed in the fasting

TABLE 11.5 Provocative Growth Hormone Tests Used to Diagnose Growth Hormone Deficiency

Stimulus	Dosage and procedure	Sampling for GH, min
Exercise	Forced climbing of steps Cycle ergometer, 2 W/kg BW (10 min)	0, 15–20 after start
Arginine HCl	0.5 g/kg BW (max, 30 g) (infused IV 10% arginine HCl in 0.9% NaCl at a constant rate over 30 min)	–30, 0, 15, 30, 45, 60, 90, 120
Ornithine HCl	12g/m ² BSA (infused IV 6.25% ornithine HCl in 0.9% NaCl at a constant rate over 30 min)	–30, 0, 15, 30, 45, 60, 90, 120
Insulin (regular)	0.1–0.05 IU/kg BW IV	–30, 0, 15, 30, 45, 60, 90, 120
Arginine HCl-insulin	See arginine test (insulin is injected [see earlier] 60 min after administration of arginine)	–30, 0, 15, 30, 45, 60, 80, 90, 105, 120, 150
Clonidine (2-[2,6-dichlorophenyl] amino-2-imidazoline)	0.15 mg/m ² BSA orally	–30, 0, 30, 60, 90, 120, 150
Glucagon	0.03 mg/kg BW (IM or SC) (may be combined with propranolol, 0.75 mg/kg BW orally 2 h before glucagon)	0, 60, 90, 120, 150, 180
L-Dopa (L-3,4-dihydroxyphenylalanine)	< 15 kg BW: 125 mg <35 kg BW: 250 mg >35 kg BW: 500 mg (taken orally)	–30, 0, 30, 60, 90, 120
GHRH (1–40) (1–44) (1–29)NH ₂	1 g/kg BW IV bolus	–30, 0, 15, 30, 45, 60, 90, 120

BSA, Body surface area; BW, body weight; GHRH, GH-releasing hormone. IM, intramuscular; IV, intravenously; SC, subcutaneously. From Ranke MB. Diagnosis of GH Deficiency and GH Stimulation Tests. In: Ranke MB, ed. *Diagnostics of Endocrine Function in Children and Adolescents*. 3rd Ed. Basel, Switzerland: Karger; 2007:107–128. Table 3.

state and hypothyroidism, if present, should be corrected before testing. Because of the high frequency of false “failure” results on any provocative test, a conventional standard has developed that diagnosis of GH deficiency requires inadequate peak GH concentrations on two different provocative GH tests (which can be performed in sequence on the same day). The generally accepted diagnostic GH threshold is 10 mcg/L.

Unfortunately, even the combination of two provocative GH tests, as commonly performed, has poor test specificity. Marin and colleagues performed provocative GH testing (exercise and arginine-insulin) on normal children and found that the majority of prepubertal, healthy children had a peak GH response less than 10 mcg/L on all three tests. The lower limit of the normal range was approximately 2 mcg/L. These data suggest that GH stimulation testing results in a very large number of false positive diagnoses.¹⁹¹ The test specificity improved with increasing Tanner pubertal stage. There is also evidence that GH response and therefore test specificity decreases with BMI, even within the normal BMI range¹⁹³ and children with obesity have particularly low GH concentrations. Overdiagnosis of GH deficiency has potential adverse consequences, including the psychological effects of incorrectly labeling a child as having a disease, the triggering of additional testing, such as pituitary MRI and pituitary function tests, a failure to pursue other testing to find the actual cause of growth failure, and taking away the family’s ability to make an informed decision about whether to use GH treatment in a child with non-GH-deficient short stature. The latter concern may be partially addressed by informing the family about the high rate of false positive stimulation tests. The poor specificity of provocative GH tests in children also may explain why isolated childhood GH deficiency frequently is not confirmed when the patient is retested as a young adult.^{318,189}

Sex steroid priming before provocative GH testing augments the ability of the secretagogues to elicit a GH response and improves test specificity. In the study by Marin and colleagues cited earlier, when prepubertal children received estrogen priming before the provocative GH testing and when a diagnostic threshold of 7 mcg/L was used, then provocative GH test showed acceptable specificity.¹⁹¹ Similar studies suggest that estrogen priming improves specificity while retaining adequate sensitivity, but test sensitivity is difficult to assess, particularly for partial GH deficiency, because we lack a gold standard for the diagnosis to which other tests can be compared.¹⁹² In studies supporting estrogen priming, priming was used even in young children; even though high estrogen concentrations are nonphysiological at a young age, the estrogen served as a useful adjunctive pharmacological stimulus. Despite these data, GH stimulation testing is commonly performed without sex steroid priming.

Because of the significant limitations of provocative GH tests, the Pediatric Endocrine Society Guidelines for GH treatment recommended “against reliance on GH provocative test results as the sole diagnostic criterion of GH deficiency,”³¹⁹ but rather suggested that the test results should be interpreted in the context of the patient’s growth pattern, risk factors for GH deficiency, and other diagnostic test results, such as IGF-1 and IGFBP-3 concentrations, bone age, and pituitary imaging (see later).

Similar logic was used in creating the IGF-1 generation test to diagnose primary IGF-1 deficiency (PIGFD). In this case, GH is used as the secretagogue, and the serum IGF-1 response is measured to identify those patients whose low random IGF-1 concentration results from GH insensitivity. Such patients would be expected to similarly fail to increase their IGF-1 production (and hence, growth) in response to GH therapy and might be better served with recombinant IGF-1 treatment instead. However, this test too is fraught with limitations; although multiple test protocols have been proposed, no

standard IGF-1 generation test protocol with a highly sensitive and specific IGF-1 threshold has been established.³¹⁹

MRI of the pituitary, with and without contrast, is standard of care for all patients diagnosed with GH deficiency. The goal is to screen for pituitary malformations and for neoplasms. However, the majority of cases of IGH deficiency remain idiopathic, in part because imaging can capture only gross structural defects, whereas GH deficiency can arise from cellular and molecular defects that cannot be visualized by MRI. In addition, the likely high rate of false positive diagnoses of GH deficiency may contribute to the low yield of findings on imaging. In patients with GH deficiency, additional pituitary hormone deficiencies should be sought.

Genetic Testing. Genetic tests are being progressively introduced into clinical practice for investigating patients with growth disorders. Current genetic testing is primarily able to identify the etiology only in children with monogenic, not polygenic, growth disorders. Patients with severe short stature (height SDS < -3), from consanguineous parents or families with clear autosomal dominant short stature, with evidence of a skeletal dysplasia or with a complex phenotype (syndromic condition) are more likely to have a monogenic genetic condition.¹⁰³ Even in children with monogenic growth disorders, genetic testing often fails to uncover the etiology. This frequent failure occurs in part because the genetic etiology of growth disorders is highly heterogeneous and many of the involved genes remain to be discovered. In addition, the genetic testing may reveal a variant in a gene known to be responsible for the growth disorder, but there may not be sufficient evidence to determine whether that particular variant is pathogenic.

There are three main scenarios in which a genetic test for children with short stature should be requested (Fig. 11.9). The first is when there is a clinical suspicion of a specific genetic condition in a patient. In this situation, genetic tests serve to confirm a suspected diagnosis. A molecular confirmation of the diagnosis has a more obvious impact on genetic counseling and patient care when the phenotype does not allow an unambiguous diagnosis. For example, for a 2-year-old patient with a phenotype typical of ACH, the genetic test could be postponed. In contrast, for a patient at the same age that is suspected to have NF-1, but who does not fulfill the diagnostic criteria because of their young age, a positive genetic test could evince valuable information, with immediate consequences in the follow-up evaluation. In patients with a recognizable condition that is caused primarily by mutations in a single gene, sequencing that single candidate gene is feasible. However, in

conditions with genetic heterogeneity or involving large genes, the use of a targeted sequencing panel or exome sequencing may be preferable.

The second scenario involves patients with syndromic phenotypes that do not fit any recognized condition. For these patients, molecular genetic investigation using a genome approach, such as by molecular karyotyping (SNP array or array-CGH)³⁰¹ and/or WES^{103,320} can identify a causative genetic variant in a significant number of cases.

The third scenario applies to patients with isolated short stature, in other words, to healthy children with height SDS < -2 and normal laboratory and radiological investigation. Certainly, this scenario comprises the majority of children evaluated for growth impairment. Many of these children have parents with short stature, supporting the presence of a genetic factor in their height. It has long been thought that such children have a polygenic etiology,^{76,321} but recent studies have demonstrated several monogenic causes in patients initially classified as ISS³²² (Table 11.6). A proportion of these children are on the mild end of the phenotypic spectrum of a known syndromic disorder associated with short stature,³²³ such as NS. Furthermore, numerous studies have associated specific genes mainly involved in growth plate regulation, such as *SHOX*,⁸⁶ *ACAN*,²⁷⁰ *NPR2*,^{54,324} *NPPC*,²⁶² and *IHH*,⁶¹ which result in isolated short stature phenotypes. Patients with short stature caused by defects in these genes may also show nonspecific radiological findings (Fig. 11.10), such as advanced bone age or abnormalities in the metacarpals or phalanges. Each of these genes accounts for a small fraction of short stature cases ($\leq 1\%$ – 2%), but may be significantly higher in cases of familial short stature.³²⁴ In children with ISS, the absence of specific clinical findings makes recognition difficult without a molecular genetic study and makes the candidate gene approach impracticable. For these reasons, a multiple-gene testing approach using next-generation sequencing is preferable for investigation of ISS. The choice to use WES or gene-panel analysis depends on the availability of facilities and cost-benefit evaluations.³²⁵ Even when using WES, analysis should prioritize genes already associated with the isolated short stature phenotype and genes associated with syndromic conditions that exhibit well-documented phenotypic variability (see Table 11.6). Although a conclusive diagnosis cannot be made for most patients with ISS after broad genetic investigation, a positive result will have a significant impact for patients and their families.³²⁶ It is expected that molecular genetic screening using a panel of genes or exome analysis will become increasingly valuable in the diagnostic evaluation of children with isolated short stature^{322,326} because, over time,

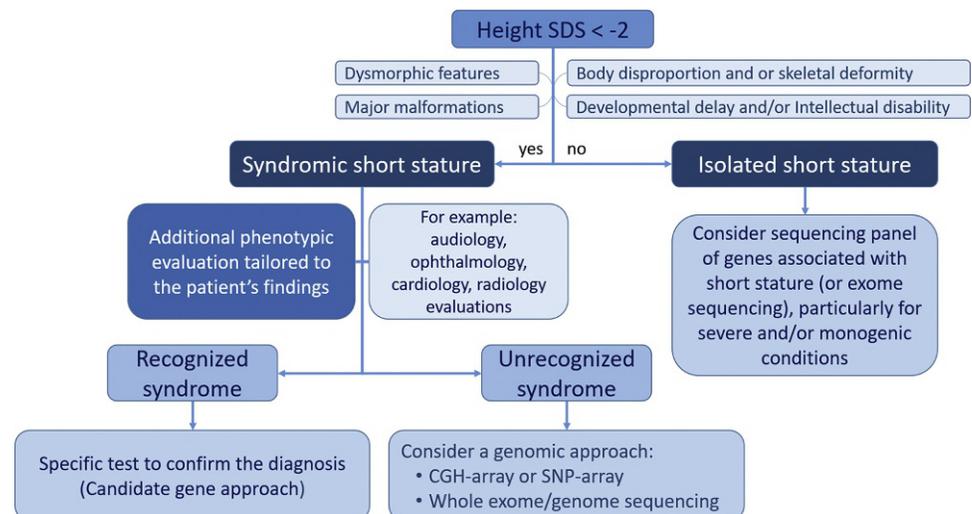


Fig. 11.9 Overview of the molecular genetic investigation of children with short stature.

TABLE 11.6 Genes Associated With Isolated Short Stature Phenotype, Usually Initially Classified as Idiopathic Short Stature

Gene	Inheritance Pattern	Frequency ^a	Evidence of Association ^b	Observation	Ref
GENES INVOLVED IN GH-IGFs AXIS					
<i>GH1</i>	AD	NA	Limited		386
<i>GHSR</i>	AD/AR	NA	Limited	It may be associated with GH deficiency in the same family	13
<i>GHR</i>	AD	NA	Limited	Mild GH insensitivity phenotype (Low IGF-1 and GHBP levels)	387
<i>STAT5B</i>	AD	NA	Limited	Mild GH insensitivity phenotype with eczema	206
<i>IGFALS</i>	AR	NA	Strong	Severe IGF-1 and IGFBP-3 deficiency out of proportion to the mild height deficit	388
<i>IGF1</i>	AD	NA	Limited		210
<i>IGF1R</i>	AD	NA	Moderate	Majority born SGA and elevated IGF-1 concentrations either basal or during rhGH therapy	389
<i>PAPPA2</i>	AR	NA	Limited	Elevated IGF-1 and IGFBP-3 concentrations	28
GENES INVOLVED IN GROWTH PLATE DEVELOPMENT					
<i>SHOX</i>	AD	1%–16%	Strong	Mild body disproportion, sometimes with family members with typical LWD	390
<i>ACAN</i>	AD	1.4%–2.1%	Strong	Advanced bone age	391
<i>NPPC</i>	AD	NA	Limited	Mild brachydactyly	262
<i>NPR2</i>	AD	1.2%–3.4%	Strong	Short metacarpals in some patients	392
<i>FGFR3</i>	AD	NA	Limited	Normal body proportion in contrast with hypochondroplasia phenotype	55
<i>IHH</i>	AD	1.6%	Moderate	Shortening of middle phalanges of 2 nd and 5 th fingers in some patients	61
GENE INVOLVED IN RAS-MAPK PATHWAY					
<i>PTPN11</i>	AD	1.5%	Limited	Clinically unrecognized mild forms of Noonan syndrome	279

^aFrequency observed in studies that evaluated unselected children with isolated short stature (idiopathic short stature or nonsyndromic small for gestational age).

^bStrength of evidence that changes in this gene are associated with the isolated short stature phenotype³⁹³ (many of these genetic changes have been more clearly associated with syndromic short stature).

AD, Autosomal dominant; AR, autosomal recessive; GH, growth hormone; GHBP, GH binding protein; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IHH, Indian hedgehog; LWD, Leri-Weill dyschondrosteosis; NA, not available; rhGH, recombinant human GH; SGA, small for gestational age.

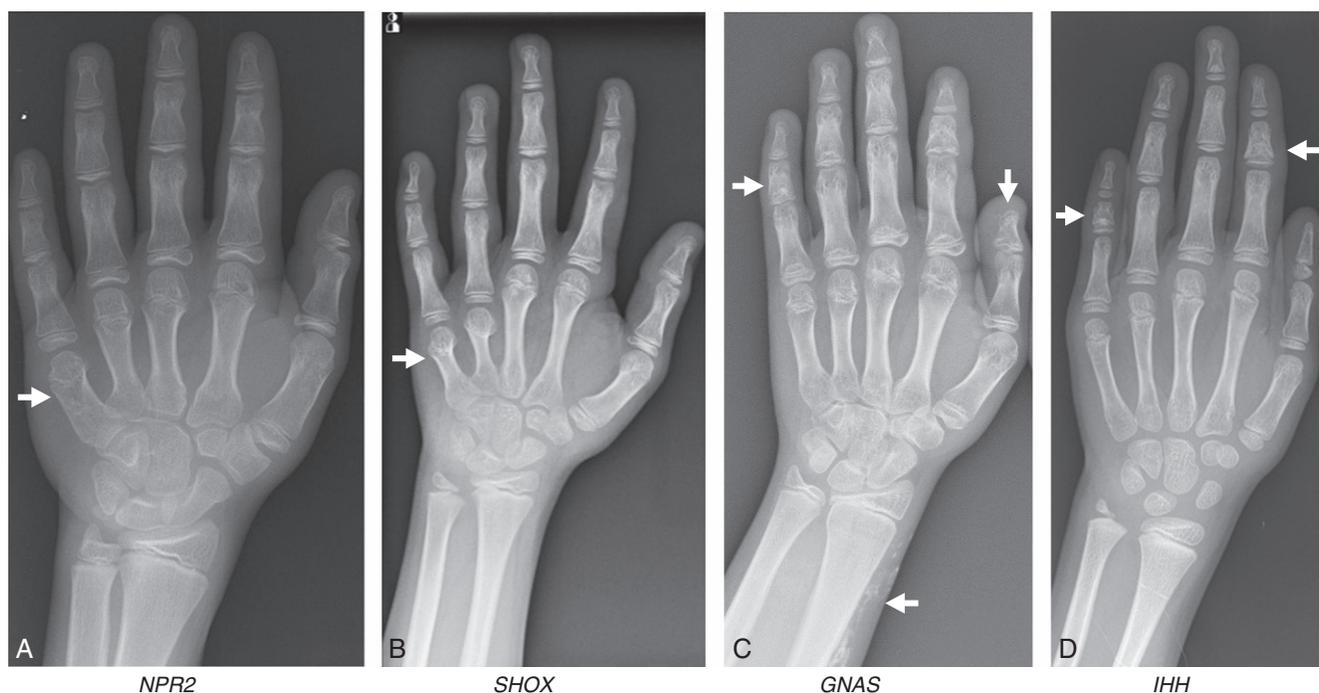


Fig. 11.10 Hand radiographs of children with short stature with nonspecific skeletal abnormalities associated with heterozygous variants in genes involved in growth plate development. A, Short metacarpals observed in some patients with natriuretic peptide receptor 2 (NPR2) variants; B, short fourth and fifth metacarpals in a patient with short stature homeobox (SHOX) haploinsufficiency; C, shortening of middle phalanges and distal phalanx of the thumb with the presence of subcutaneous ossifications in a patient with pseudopseudohypoparathyroidism because of guanine nucleotide binding protein(s) (GNAS) defect inherited from the father; and D, shortening of the middle phalanx of the second and fifth fingers with cone-shaped epiphyses associated with Indian hedgehog (IHH) gene defects.

additional causative genes and additional causative variants within known genes will be discovered and because the cost, coverage, and accuracy of the sequencing will improve.

Treatment of Short Stature

When a treatable cause of the short stature is uncovered, for example, celiac disease or hypothyroidism, there is usually little difficulty deciding on a plan to treat the underlying problem. Often, however, either the etiology remains unclear (idiopathic short stature) or the etiology is known but a specific treatment that specifically corrects the underlying etiology is not known. In these cases, the clinician and family may consider a nonspecific treatment to attempt to compensate for the underlying cause of diminished growth. Although multiple possible treatment approaches could potentially be used, the most common consideration is GH treatment.

Growth Hormone. hGH treatment of a patient with GH deficiency was first reported by Dr. Maurice Raben in 1958.³²⁷ He purified hGH from pituitary extracts from donor cadavers using hot glacial acetic acid, which retained GH but destroyed TSH, LH, and FSH. Soon thereafter, Wilhelmi developed another extraction method; he collected pituitaries in distilled water, froze them, and performed column extraction that yielded hGH, as well as other anterior pituitary hormones.³²⁸ Soon, national pituitary agencies were established in various countries to collect, extract, and dispense pituitary hGH for clinical use. About 400 pituitaries per year were needed to treat one child with hypopituitarism, so treatment was limited to the severest cases and dosing regimens were dictated by the limited hGH availability.³²⁹ The pituitary programs were terminated beginning in 1985 when Creutzfeldt-Jacob disease, a prion-mediated, progressive, and fatal spongiform encephalopathy, was diagnosed in patients who had previously received contaminated hGH extracted by the Wilhelmi method.³²⁹

Serendipitously, 1985 also saw the advent of GH production by recombinant DNA technology for clinical use; rhGH replaced pituitary hGH and has remained the sole source of GH treatment. Because the supply of rhGH was suddenly limitless—pharmaceutical companies could produce and sell as much as the market was willing to bear—the advent of rhGH not only increased the safety of GH treatment but opened the possibility to treating more patients with different indications at higher doses and for longer duration. rhGH use expanded from replacing GH deficiency (in patients with GH deficiency and PWS) to augmenting growth in patients who were GH replete but had other defects impairing their growth (Box 11.4). For example, GH treatment for ISS was approved by the FDA in 2003 for individuals with height below -2.25 SD (1.2 percentile), whose growth velocity would not be expected to allow attainment of an adult height in the normal range and whose diagnostic evaluation excluded other causes of growth failure that should be observed or treated by other means.³³⁰

Current rhGH treatment consists of nightly subcutaneous injections—subcutaneous because the polypeptide nature of rhGH precludes oral administration, and nightly, because most endogenous GH secretion occurs at night. Most rhGH manufacturers also created pen devices to facilitate administration by families. However, adherence with years of nightly injections remains challenging, and many companies have sought to develop long-acting rhGH preparations that may support long-term adherence via reduction in injection frequency; such preparations remain investigational.³³¹

Benefits. For children with GH deficiency, rhGH treatment provides both height and health benefits. Acceleration of statural growth is evident within the first few months of starting

BOX 11.4 Indications for Growth Hormone Treatment Approved by the US Food and Drug Administration

US FOOD AND DRUG ADMINISTRATION INDICATIONS WITH YEARS OF THEIR APPROVAL

Pediatric Indications

GH deficiency (1985)
Chronic renal insufficiency (1993)
Turner syndrome (1996)
Prader-Willi syndrome (2000)
Small-for-gestational age without catch-up growth (2001)
Idiopathic short stature (2003)
SHOX gene haploinsufficiency (2007)
Noonan syndrome (2008)

Adult Indications

HIV/AIDS-associated wasting (1996)
GH deficiency (1997)
Short bowel syndrome, dependent on parenteral nutrition (4-week course of GH) (2003)

AIDS, Acquired immunodeficiency syndrome; *GH*, growth hormone; *HIV*, human immunodeficiency virus.

rhGH treatment, with the greatest height velocities seen in the first year of treatment, slightly less in year 2, and slightly less still but persistent years 3 to the end of treatment. The increase in height velocity translates into an increase in height Z-score. Although historically individuals with untreated isolated GH deficiency had mean adult height Z-scores of -4.7 (range, -3.9 to -6),³³² those treated with rhGH commonly achieve adult heights in the normal range (i.e., less than 2 SD below the population mean and their midparental height). Patients with taller parents tend to have greater response to treatment than those with shorter parents, as do patients who are still prepubertal at initiation of treatment versus those already in puberty.³³³

In addition to height benefits, patients with GH deficiency derive health benefits from rhGH treatment owing to the effects of GH on lipid, protein, and glucose metabolism. Multiple studies have shown that rhGH treatment improves the decreased bone mineral density, decreased lean muscle mass, and increased fat mass seen in untreated children with GH deficiency. Although short-term studies showed a positive effect of rhGH treatment on left ventricular mass, data were inconsistent on cardiac performance measured by echocardiography (fractional shortening and left ventricular ejection fraction), vascular function (intimal media thickness at common carotid arteries), lipid profiles (although rhGH treatment lowers total and low-density lipoprotein cholesterol levels, data were inconsistent whether children with untreated GH deficiency had normal or abnormal profiles), and atherogenic indices.³¹⁹

For children receiving rhGH treatment who are not GH deficient (all indications except GH deficiency and Prader-Willi syndrome), benefits are limited to height only and even there, are often less dramatic than for children with GH deficiency.³³⁴ In a randomized controlled trial of children with ISS, approximately 6 years of GH treatment increased adult height by approximately 6 cm.³³⁵ Treatment responses vary widely, perhaps because ISS is such a heterogeneous categorization; some patients may experience no measurable increase in height Z-score. Furthermore, it is unclear whether the increase in height attributable to GH treatment has important psychological benefits.³³⁶ Thus it is important to counsel patients and their families accordingly before starting rhGH treatment, to

allow them to make an informed decision and to manage expectations.³¹⁹

Potential Adverse Effects. Potential adverse effects of rhGH treatment have been extensively reviewed recently by the Pediatric Endocrine Society^{319,337} and Growth Hormone Research Society,³³⁸ and results summarized herewith. The majority of safety data derive from postmarketing surveillance registries of rhGH, which were initially mandated by the FDA for all rhGH manufacturers for their respective products and then continued by the manufacturers themselves. Although these registries were well powered by their size (international scope and decades of data collection) to identify catastrophic or frequent adverse effects, there are some limitations. These include: (1) reliance on voluntary ascertainment for both occurrence and relatedness to rhGH treatment may lead to incomplete data capture, (2) on-treatment surveillance may miss any adverse effects that become manifest only after treatment has ended, (3) changes over time in rhGH dose and/or patient characteristics may alter the risk for adverse effects, and (4) lack of a valid control population precludes comparison to determine actual rhGH treatment effects on risk.

Adverse effects on rhGH treatment occur at low frequency (<3% of treated children) but are potentially serious and should be reviewed with patient-families before they decide to embark on treatment. Intracranial hypertension occurs at an estimated overall incidence of 28 per 100,000 treatment-years, generally during rhGH treatment initiation or dosage increases; it resolves with discontinuation of rhGH, after which rhGH may be restarted at half-dose and increased more gingerly. Slipped capital femoral epiphysis (SCFE) occurs at an estimated overall incidence of 73 per 100,000 treatment-years, with a median onset of 0.4 to 2.5 years after initiation of rhGH treatment, and requires surgical pinning of the capital femoral epiphysis to correct its malposition. Scoliosis progression can occur, especially in patient populations with higher baseline risk (PWS and Turner syndrome), and seems to result from rapid growth rather than rhGH per se.

Some apparent “adverse effects” of rhGH treatment actually reflect physiological GH actions. Physiological effects of GH on glucocorticoid metabolism (i.e., reducing hepatic 11 β -hydroxysteroid dehydrogenase 1-mediated conversion of inactive cortisone to active cortisol and increasing cytochrome P450 3A4-mediated cortisol catabolism) can unmask underlying adrenal insufficiency in those whose GH deficiency may be associated with MPHD. Likewise, by increasing peripheral deiodination of T4 to T3, rhGH can lower serum free T4 concentrations, which are often used to diagnose central hypothyroidism. GH physiologically decreases insulin sensitivity, and therefore rhGH treatment may induce glucose intolerance and manifest hyperglycemia in children with compromised insulin secretion or sensitivity, and lead to increased insulin requirement in children with diabetes mellitus already on insulin replacement. However, rhGH effects on insulin sensitivity occur only during treatment, with a return to baseline upon termination of rhGH treatment.

Risk of neoplasia with rhGH treatment has been a longstanding concern, based partly on indirect evidence. For example, IGF-1 promotes growth and metastases of various malignant cells in experimental models, and higher circulating IGF-1 concentrations are epidemiologically associated with increased risk of premenopausal breast cancer, colon cancer, and possibly prostate cancer.³³⁷ Epidemiological studies have suggested an increased risk of certain malignancies in patients with acromegaly,³³⁷ whereas individuals with Laron syndrome (GH insensitivity) seem protected against developing cancer.³³⁹

Surveillance studies generally have not found an increased risk for new malignancies in otherwise healthy children with

no prior history of cancer and no known predisposition to cancer. For patients with known tumor predisposition because of genetic or other medical conditions, data are insufficient to determine whether rhGH treatment further increases that risk. Published guidelines are generally based on retrospective data because randomized controlled trials have not been conducted. Per recent guidelines, rhGH treatment is contraindicated in patients with active malignancy, but pediatric cancer survivors who have completed cancer therapy and are without signs of active neoplastic disease may be candidates for rhGH treatment. rhGH treatment has not been found to raise their intrinsic risk of recurrence. Although rhGH treatment has been associated with a small early increase in the overall risk for subsequent primary neoplasms, subsequent studies found the risk related to irradiation rather than rhGH treatment.^{340,341} These studies of cancer risk are uncontrolled and retrospective, and thus the risks remain uncertain. Although few data are available to guide the appropriate interval between completion of cancer therapy and initiation of rhGH treatment, a standard waiting period of 12 months to establish “successful therapy” of the primary lesion has been recommended but can be altered depending on individual patient circumstances. Despite being histologically nonmalignant, craniopharyngiomas have a high rate of recurrence or progression after resection and are sometimes treated with irradiation, thereby increasing the patient’s risk to develop subsequent primary neoplasms. Craniopharyngiomas are commonly associated with GH deficiency, and there are no data to suggest treating them differently than malignant tumors with regard to observation periods before initiating rhGH treatment.

Long-term posttreatment risks are still poorly known because: (1) rhGH has only been available since 1985, and therefore the oldest treated patients are now in their 40s or early 50s; (2) the postmarketing surveillance registries lose track of patients once they stop treatment with that particular brand of rhGH; (3) studies designed to answer such questions require sufficient size and duration of follow-up; and (4) well-matched control groups are lacking. The ongoing multicenter “Safety and Appropriateness of Growth hormone treatments in Europe” (SAGhE) study was created to assess mortality and morbidity in adults in eight European countries (Belgium, France, Germany, Italy, the Netherlands, Sweden, Switzerland and the United Kingdom [England, Wales, and Scotland]) who had been treated in childhood with rhGH (not pituitary hGH) for approved indications. This industry-independent, cohort study compared 24,232 previously rhGH-treated patients, after an average 17.1 years of follow-up, with expected rates from national and local population data.³⁴² Early conflicting data from preliminary subgroup analyses were reviewed in the aforementioned society papers.^{319,337,338} Two additional SAGhE analyses were reported subsequently. For patients in the entire consortium who had received rhGH treatment for “isolated growth failure” (i.e., isolated GH deficiency, ISS, or SGA), cancer risk was not raised overall nor site specifically. However, for patients who did not have “isolated growth failure,” analyses found increased incidence of bone and bladder cancers and, with longer duration of follow-up, Hodgkin lymphoma in those without previous cancer, and increased cancer mortality risk associated with increasing rhGH daily dose for patients treated after previous cancer.³⁴³ Analysis of a five-country SAGhE cohort of 10,403 patients found a raised risk of meningioma related to previous irradiation for an underlying malignancy, but not to rhGH treatment.³⁴⁰ These studies are subject to inherent limitations, including the lack of a well-matched control group and the insufficient length of follow-up to fully assess the risk of the common malignancies (e.g., breast and colon cancer) that are statistically associated with IGF-1

concentrations and occur primarily in later adulthood. Further study is required.

Beyond the aforementioned considerations, which apply to all pediatric patients receiving rhGH treatment, there are additional risks pertaining to special populations. Patients with PWS have an underlying risk for both central and obstructive sleep apnea (OSA), sometimes leading to sudden death. Both GH and IGF-1 can stimulate adenotonsillar growth and might thereby exacerbate OSA. Thus polysomnography before initiating rhGH treatment and monitoring during rhGH treatment is recommended for patients with PWS. For those who develop OSA, rhGH treatment should be interrupted until their respiratory status can be stabilized by surgery (adenoidectomy and/or tonsillectomy, when appropriate) or continuous positive airway pressure. Another special population consists of critically ill patients in the intensive care setting. Increased mortality was observed with rhGH treatment in two placebo-controlled clinical trials in non-GH-deficient adult patients with acute critical illness because of complications following open-heart surgery, abdominal surgery or multiple accidental trauma, or those with acute respiratory failure. The safety of continuing rhGH treatment in pediatric patients receiving replacement doses for approved indications who concurrently develop these illnesses has not been established, and so potential benefits of rhGH treatment continuation should be weighed against potential risks.

On-Treatment Monitoring. During treatment, patients must be monitored at least every 6 months with auxological measurements, Tanner staging, and measurement of IGF-1 concentrations. IGF-1 concentrations provide information regarding treatment adherence and individual responsiveness to rhGH dose changes. Despite a lack of direct evidence, maintaining IGF-1 concentrations within age- and gender-specific normal ranges seems prudent.^{319,338} Soliciting a history of new or worsening headache, visual disturbance, or the presence of papilledema on funduscopic examination should prompt further evaluation for intracranial hypertension, and a history of hip pain or gait disturbance should elicit an evaluation for SCFE. Serial spinal examinations should be performed to screen for scoliosis. Assessment of cortisol and thyroid hormone sufficiency in patients whose GH deficiency may be related to MPHD and screening hemoglobin A1c in patients at risk for disturbed carbohydrate metabolism are warranted.^{319,338}

Treatment with rhGH at pediatric doses should not continue beyond epiphyseal fusion, lest acromegalic changes (e.g., disproportionate enlargement of the hands, feet, jaw, and tongue) be induced. When growth velocity drops to 2 to 2.5 cm/year, a bone age radiograph should be obtained to confirm epiphyseal fusion.³¹⁹ However, after a brief interruption in treatment, reassessment for adult GH deficiency can result in continued treatment for adult GH deficiency (with lower doses designed for the metabolic and body composition benefits outlined earlier). However, upon retesting, the majority of patients with isolated, idiopathic pediatric GH deficiency do not meet criteria for adult GH deficiency and do not require further treatment.

Shared Decision-Making Process. For children with non-GH-deficient short stature, the decision to treat with GH is difficult, in part because so many gaps remain in our understanding of the benefits and risks. The treatment of ISS with GH has been particularly controversial. As recommended by the latest guidelines for GH treatment by the Pediatric Endocrine Society, "In the USA, for children who meet FDA criteria, we suggest a shared decision-making approach to pursuing GH treatment for a child with ISS. The decision can be made on a case-by-case basis after assessment of physical and psychological burdens, and discussion of risks and benefits. We recommend against the routine use of GH in every child with height SDS (HtSDS) ≤ -2.25 ."³¹⁹

Several factors underlie this approach.³³⁰ Many patient-families seek evaluation and treatment of short stature out of concerns for psychosocial adaptation, both currently in childhood and projected into adulthood.³⁴⁴ However, evidence is limited to support either notion, that height is associated with psychological wellbeing and quality of life or that GH treatment improves this.³⁴⁵ Further, although GH treatment has been shown to increase the mean height of treated cohorts of patients with ISS, individual responses are highly variable, including some who do not increase their height SDS. With the same height meaning different things to different people, the variable responsiveness to treatment, and different perceptions of treatment burden, the risk-benefit consideration of GH treatment is a highly personal one.

Several markers of social success, including professional and mating, have been associated with tallness in males.^{115,345} Thus social pressures for tallness seem to affect males more than females and this leads to bias, conscious or subconscious, among parents seeking medical care for short children, among primary care clinicians who decide whether to pursue diagnostic testing and/or subspecialist referral, and among endocrinologists who prescribe GH treatment. The end result is that boys outnumbered girls in US pediatric GH registries 2:1 for all indications and 3:1 for ISS (the most socially driven indication) even though the prevalence of height below -2.25 SD (the FDA approved cut-off for GH treatment of ISS) did not differ by gender in a large, regional pediatric primary care population.³⁴⁶ Likewise, racial/ethnic disparities also have been observed in US pediatric GH registries.³⁴⁷ It behooves clinicians to be mindful of these tendencies, so that abnormal growth patterns are recognized and evaluated equally for all children as a potential sign of an underlying health problem.

Insulin-like Growth Factor-1. rhIGF-1 is available for treating patients with growth failure because of decreased IGF-1 concentrations that would not respond to GH treatment. It is used to treat severe primary IGF-1 deficiency because of GH insensitivity syndrome (Laron syndrome, caused by mutation in the GHR), *STAT5b* gene mutation, and *IGF1* gene mutations. In addition, rhIGF-1 is used to treat *GHI* gene deletion when patients have developed neutralizing antibodies that render further rhGH treatment ineffective. For unexplained severe PIGFD, where the degree of GH responsiveness is unclear, a trial of rhGH is recommended before turning to rhIGF-1 treatment.³¹⁹ rhGH treatment of patients with GH deficiency induces a greater growth response than rhIGF-1 treatment of patients with PIGFD, perhaps because of IGF-1-independent actions of GH at the growth plate, which the latter cannot replace. Further, rhGH treatment induces IGFBP-3, as well as IGF-1, making it more physiological than rhIGF-1 treatment, requires only once daily injections (versus twice daily injections of rhIGF-1), and does not cause hypoglycemia as does the latter (see later).

The most common cause of low IGF-1 concentrations in children and adolescents with growth stunting is undernutrition.³⁴⁸ A careful dietary history and inspection of the weight and BMI curves are important to screen for this possibility. For children with suspected undernutrition, nutritional intervention is the appropriate treatment rather than hormonal therapy. rhIGF-1 treatment is also not a standard treatment for children with a low IGF-1 concentration because of chronic systemic illness or children with mildly low IGF-1 concentrations associated with ISS or constitutional delay.

When indicated, rhIGF-1 treatment is administered as twice daily subcutaneous injections of 80 to 120 mcg/kg (also given outside the United States as 150–180 mcg/kg once daily injections). Hypoglycemia occurs in 42% of patients during their course of rhIGF-1 treatment, and although most

episodes are mild or moderate, severe hypoglycemic reactions (loss of consciousness, seizure) can occur.³¹⁹ Thus for safety, patient-families need to be educated about this risk, rhIGF-1 should be administered 20 minutes after a carbohydrate-containing meal or snack and withheld if the patient is not going to eat, and vigilance increased during intercurrent illnesses, including home glucometer use. rhIGF-1 treatment can also lead to hypertrophy of the tonsils and adenoids, hypersensitivity and allergic reactions, and reactions to the benzyl alcohol component of the diluent. Other potential adverse effects of rhIGF-1 treatment are similar to those of rhGH treatment.³¹⁹

Sex Steroids. Treatment with estrogens accelerates linear growth, causing a transient growth spurt, but also accelerates growth plate senescence, causing an earlier termination of linear growth. In general, the latter effect predominates, so that adult height is decreased. Treatment with aromatizable androgens can have similar effects because the androgens are converted to estrogens by the enzyme aromatase. However, treatment with nonaromatizable androgens, which cannot be converted to estrogens, may accelerate linear growth with less acceleration of growth plate senescence. Of nonaromatizable androgens, the best studied is oxandrolone. Oxandrolone treatment has been shown to increase adult height modestly in girls with Turner syndrome (see Chapter 17 on Turner syndrome for details). Oxandrolone increases linear growth rate in boys with constitutional delay of growth; however, no adequately powered, long-term, randomized controlled trials have evaluated the effect on adult height.^{349,350}

Gonadotropin Releasing Hormone Agonists. GnRH agonist treatment produces a highly effective, reversible suppression of the pituitary-gonadal axis. In a pubertal child, sex steroid production is greatly diminished, slowing both linear growth and growth plate senescence. In some situations at least, the latter effect predominates, resulting in a greater adult height. Although no long-term well-controlled trials are available, it is generally believed that GnRH agonist treatment in children with central precocious puberty diminishes the impairment in adult height³⁴⁹; (see Chapters 16 and 18 on Puberty and Its Disorders in the Female and Male, respectively, for details). In short adolescents with a normally timed puberty, GnRH treatment for 3.5 years, in a randomized controlled trial, initially slowed growth but also slowed bone age progression and allowed linear growth to continue longer; the net effect was an increase in adult height by approximately 4 cm.^{65,349} Potential adverse effects include decreased bone mineral density, as well as the psychological consequences of pubertal delay and of decreased stature during adolescence. Delivery options include regular intramuscular injections or an implantable device that must be removed or replaced approximately annually. This treatment is not approved by the FDA for children with short stature and a normally timed puberty and is used less frequently than GH treatment.

Aromatase Inhibitors. As noted earlier, estrogens transiently accelerate linear growth, but also accelerate growth plate senescence, causing an earlier termination of linear growth, and the net effect is generally a decrease in adult height. In men with estrogen deficiency resulting from biallelic mutations in the aromatase gene, the normal deceleration and cessation of linear growth in mid- to late adolescence is delayed, allowing for slow but persistent linear growth well into young adulthood resulting in tall stature.³⁵¹ Similar effects have been reported in estrogen resistance because of biallelic mutation in the estrogen receptor-alpha gene.³⁵¹ These observations suggest that pharmacological blockade of estrogen production with aromatase inhibitors during male adolescence might delay skeletal maturation and prolong linear growth, resulting in a greater adult

height. In principle, this treatment might have an advantage over GnRH agonist treatment by allowing the normal effects of androgen during male adolescence.

Randomized controlled trials of the aromatase inhibitor anastrozole or the more potent letrozole have been conducted in boys with short stature and in boys with constitutional delay. However, these studies have not been powered or were not analyzed to definitively assess the effect on adult height.^{352,353} In addition, questions about safety remain, some of which are suggested by the phenotype of aromatase-deficient men, including possible adverse effects on spermatogenesis, bone mineral density, lipids, insulin sensitivity, cognitive functions, and prostate growth. In addition, testosterone concentrations can rise to supraphysiological levels and vertebral deformities have been reported. Because of unanswered questions regarding efficacy and safety, aromatase inhibitors are not approved by the FDA for treatment of short stature and current guidelines by the US Pediatric Endocrine Society urge caution in the use of these agents outside of controlled clinical trials.³⁵⁴

C-Type Natriuretic Peptide Agonists for Achondroplasia.

ACH is the most common human skeletal dysplasia and is caused by a gain-of-function mutation in *FGFR3* that leads to excessive signaling in the MAPK pathway. Overactive MAPK signaling leads to impaired proliferation and terminal differentiation of chondrocytes and impaired synthesis of the extracellular matrix in the growth plate.⁵⁴ The binding of CNP to its receptor, NPR-B, generates cyclic guanosine monophosphate that activates cGKII, which inhibits the activation of MAPK pathway. Animal models of ACH have demonstrated that CNP overexpression rescues the impaired bone growth phenotype caused by *FGFR3* activation. This evidence suggests that CNP could be a potential therapy for ACH. Several clinical trials are in progress to evaluate the efficacy and safety of CNP analogues in children with ACH (www.clinicaltrials.gov).

Surgical Treatment. Based on distraction osteogenesis (i.e., osteotomy followed by a gradual rhythmic interruption of bone callus formation using an external fixation device, such as the Ilizarov frame), surgical leg lengthening was originally developed to correct length discrepancies or deformities. Bilateral leg lengthening surgery has been proposed as a treatment for some severe forms of short stature, especially those with shortening of limbs, for example, ACH.³⁵⁵ This technique can be applied to tibia and femoral lengthening alone or combined.^{355,356} The procedure is capable of promoting a significant total height gain (20 cm), but has a high frequency of complications and sequelae.³⁵⁵⁻³⁵⁷ Surgery can be performed in early childhood, but it is not recommended until the child is old enough to participate in the therapeutic decision. Although leg lengthening has the potential to bring better adaptability to patients with severe short stature, it is invasive and associated with a high risk of short- and long-term complications and is controversial among individuals with skeletal dysplasias (<https://www.lpaonline.org/>). For these reasons, the procedure should be performed in specialized centers and only on selected patients with severe short stature who have received adequate information about the procedure, risks, and consequences.

Tall Stature

A child may have a height above the normal range for many reasons. Often, the parents are also tall and the child's stature appears to be inherited as an oligogenic or polygenic trait. In these cases, the tall stature is considered a normal variation. Obesity is another common cause for stature above the normal range. However, there are also a large number of uncommon causes of tall stature, most of them genetic. Some

of these uncommon causes, such as Klinefelter syndrome, Beckwith-Wiedemann syndrome (BWS), Marfan syndrome, and GH excess have important health implications. Therefore in the child presenting with tall stature, a careful clinical evaluation is warranted.

Tall stature results from excessive growth plate chondrogenesis. This increased chondrogenesis can either arise from a primary cause within the growth plate chondrocytes themselves or can arise from a secondary cause, occurring elsewhere in the body and affecting the growth plate through a circulating, usually endocrine, factor.

Causes of Tall Stature

Secondary Growth Excess.

Endocrine Causes of Tall Stature

Growth Hormone/Insulin-Like Growth Factor-1 Excess. In children with increased growth rate but absence of pubertal signs, GH excess or hyperthyroidism should be considered. When the former occurs before epiphyseal fusion the result is rapid growth, metabolic changes similar to those observed in acromegalic patients, and elevated serum IGF-1 concentrations. GH concentrations may be elevated on an overnight GH profile, with no troughs detectable. Furthermore, serum GH concentrations are not suppressed by oral glucose administration during an oral glucose tolerance test. MRI may reveal a pituitary tumor in the form of a micro- or macroadenoma. Recent advances have revealed a genetic cause in a significant proportion of these patients. Somatic *GNAS1*, *PIK3CA*, and *USP8* mutations may be identified, or the tumor may occur as a component of syndromes, such as the MAS, the Carney complex, MEN 1 and Type 4, and *DICER* and *succinate dehydrogenase (SDH)*-related syndromes. Mutations in arylhydrocarbon-interacting protein (AIP) have been associated with familial pituitary adenomas (FIPA), and more recently, microduplications involving chromosome Xq26.3 have been associated with pituitary gigantism because of pituitary tumors as part of the X-linked acrogigantism (X-LAG) syndrome.

Familial Pituitary Adenomas. This condition is characterized by two or more cases of pituitary adenomas in a family in the absence of other associated tumors. Acromegaly/gigantism is associated with 54% of tumors, prolactinoma with 27%, nonfunctioning pituitary adenomas in 17%; prolactinoma and gigantism/acromegaly may occur in the same pedigree. *AIP* (a tumor suppressor gene) mutations are present in 15% to 30% of FIPA families, and in 20% of sporadic pediatric pituitary adenomas (PAs). The penetrance of PAs among *AIP* mutation-positive carriers is around 12% to 30%, suggesting the role of other disease modifiers. The mean age at diagnosis is 18 to 24 years; almost all *AIP* mutation positive cases were diagnosed by 40 years of age. *AIP* mutation-positive PAs are more likely to be macroadenomas (90%) and aggressive, needing multimodal therapy.

X-LAG Syndrome. This is associated with microduplications of Xq26.3 including the orphan G-protein-coupled receptor *GPR101*, which is significantly overexpressed in the pituitary of these patients. Mutations may be sporadic de novo, as well as familial germline Xq26.3 microduplications, and mutations may be mosaic. *GPR101* is also highly expressed in the hypothalamus and nucleus accumbens. Affected patients usually have gigantism of early onset, usually before the age of 5 years, and may have an increased appetite, acanthosis, coarse facial features, and excess sweating. There is a female preponderance, and the condition is associated with macroadenomas and somatotrope hyperplasia. Hyperprolactinaemia has been described in 85% of cases. *AIP* mutations account for 30% of pituitary gigantism, whereas X-LAG accounts for 10% cases.³⁵⁸

Sex Steroid Excess.

Early exposure to sex steroids causes growth acceleration in children. This early exposure can result from central precocious puberty; virilizing forms of congenital adrenal hyperplasia; ovarian cysts; or adrenal, testicular, or ovarian tumors; or exogenous sex steroids and other causes. Elevated estrogen concentrations accelerate skeletal maturation, including growth plate senescence, resulting in an advanced bone age, early cessation of growth, and early epiphyseal fusion. Because of this accelerated growth plate senescence and early growth cessation, the patient typically shows increased stature in childhood but decreased stature in adulthood. One of the goals of treating precocious puberty, whether central or peripheral, is to preserve adult height. In central precocious puberty treated with a GnRH analogue, the achieved adult height tends to be greater than the predicted adult height before treatment, modestly less than the predicted height at the end of treatment, and often modestly less than the midparental height, particularly if the treatment is not initiated soon after onset of puberty.³⁵⁹

Prolonged Growth Caused by Delayed Skeletal Maturation.

This comprises a heterogeneous group of diseases, including hypogonadism, aromatase deficiency, and estrogen resistance. In direct contrast to precocious puberty, these patients do not have a growth spurt because of the absence of, or resistance to, sex steroids. Growth rate is slow but, the deficiency in estrogen action also slows growth plate senescence, allowing these patients to keep growing into adulthood, developing tall stature with eunuchoid proportions (increased arm span and upper-to-lower segment ratio) only later in life. Consequently, untreated patients with hypogonadism may show normal stature in childhood, mildly short stature in early adolescence, and tall stature in adulthood.

Aromatase Deficiency. Aromatase deficiency is a rare disease with autosomal recessive inheritance. In males, the diagnosis is made later in adulthood, when there is tall stature, incomplete epiphyseal closure, eunuchoid proportions of the skeleton, osteoporosis, and obesity. Estrogen concentrations are low, whereas FSH, LH, and testosterone are slightly increased. The most intriguing features are the presence of steatohepatitis, insulin resistance with acanthosis nigricans, and high concentrations of triglycerides. The administration of low-dose estrogen allows completion of bone maturation after the complete closure of the epiphyses and thereafter leads to an increase in bone density. More than 30 mutations (point mutations, deletions and insertions) have been found in the gene *CYP19A1*, which encodes the P450 aromatase enzyme expressed in several tissues, such as gonad, brain, placental syncytiotrophoblast, breast, and adipose tissue, and catalyzes biosynthesis of estrogens from androgens.³⁶⁰

Estrogen Receptor α Deficiency. Rare cases of estrogen receptor α (*ER α*) deficiency have been reported with considerable phenotypic similarity to that of aromatase deficiency with tall stature and eunuchoid body proportions. The patients show continued linear growth into adulthood because of delayed skeletal maturation, osteoporosis, absent breast development with markedly elevated serum estrogen concentrations, and multicystic ovaries and amenorrhea in females. To date, the rare estrogen-resistant patients have had homozygous missense mutations in the *ER α* gene.^{361,362}

Thyrotoxicosis.

In hyperthyroidism, an increase in growth rate is associated with an advanced bone age, although stature and bone age are often still within the normal range.^{216,363} The diagnosis is made by measuring free T4 and TSH concentrations. The most common cause is Graves disease.

McCune-Albright Syndrome.

MAS is a rare cause of gonadotropin-independent precocious puberty, GH excess, and hyperthyroidism. It is a genetic

disorder caused by an activating mutation in the *GNAS* gene, which encodes the alpha subunit of the stimulatory G protein. The causative somatic mutation arises during embryogenesis and is distributed in a mosaic pattern. It is characterized as the triad of polyostotic fibrous dysplasia of bone, precocious puberty, and café-au-lait skin pigmentation. Moreover, other associated endocrinopathies have been recognized, including hyperthyroidism, GH excess, FGF23-mediated phosphate wasting, and hypercortisolism. Skin manifestations are common and are usually present at or shortly after birth. The café-au-lait spots typically have irregular margins giving them a “coast of Maine” appearance, and usually show an association with the midline of the body. In MAS, fibrous dysplasia of bone typically occurs at several sites (polyostotic), and commonly presents with fracture, deformity, and/or bone pain. Radiographs show characteristic expansive lesions with a “ground glass” appearance.

Other Endocrine Causes.

Familial glucocorticoid deficiency (FGD) is a condition characterized by resistance to ACTH. In addition to mutations in melanocortin receptor 2, several other causes have been identified. FGD is also associated with tall stature.³⁶⁴

Babies with congenital hyperinsulinemic hypoglycemia or those born to mothers with diabetes mellitus tend to be large for dates although they do not tend to be tall in the long term.

Nutritional Obesity.

Obesity has become the most common cause of tall stature. The growth chart is the most important diagnostic “test” in evaluating obesity; the endocrine causes of excessive weight gain and adiposity (glucocorticoid excess—both iatrogenic and endogenous Cushing syndrome, hypothyroidism, and GH deficiency) all tend to diminish statural growth, whereas nutritional (i.e., “exogenous”) obesity is associated with robust statural growth. Obesity also advances skeletal maturation, as evidenced by the bone age, and is associated with increased incidence of premature adrenarche and, in girls, early puberty. Thus although the height centiles of obese children typically exceed their midparental height centiles, these children typically stop growing younger such that adult height ends up at a centile lower than that followed in childhood, and thus obesity appears to have little effect on adult height.

Obesity has multiple effects on the GH/IGF-1 axis. Spontaneous GH secretion is reduced in both pulse frequency and amplitude, and lower amplitude secretion also can be evident on provocative GH testing.³⁴⁸ Despite the reduction in GH, circulating IGF-1 concentrations are similar to or, in some studies, higher in obese than in nonobese individuals. Some studies suggest that the amount of bioavailable IGF-1 is increased because of reductions in circulating IGFBP-1 and IGFBP-2 (IGFBP-3 remains unchanged). Circulating concentrations of GHBP also are increased.³⁴⁸

Perhaps the most extreme example is a phenomenon called *growth without GH*, whereby obese children can maintain normal growth velocity despite a clear absence of GH, such as from surgical resection of craniopharyngioma.³⁶⁵ Mechanisms implicated for maintaining their statural growth include insulin,^{365,366} leptin,³⁶⁶ sex hormones,³⁶⁶ and a growth-promoting adipokine, such as growth and differentiation factor 5.³⁶⁷

Primary Growth Excess.

In some children, the excessive growth plate chondrogenesis responsible for tall stature arises from a primary abnormality within the growth plate chondrocytes themselves. These primary causes include single gene defects that affect autocrine/paracrine signaling within the growth plate, cartilage extracellular matrix, or intracellular regulatory pathways. In other children, the underlying cause is not a single gene defect but rather another genetic abnormality, such as a chromosomal anomaly, a genetic CNV (deletion or duplication), or imprinting disorder.

Disorders Involving Autocrine/Paracrine Factors.

Fibroblast growth factor-3 (*FGFR3*) negatively regulates growth plate chondrogenesis. Consequently, as discussed earlier in the chapter, gain-of-function mutations in *FGFR3* impair growth plate chondrogenesis and can present clinically as a chondrodysplasia with short stature, such as hypochondroplasia or ACH. Other mutations in *FGFR3*, likely involving a loss of function, appear to cause excessive growth plate chondrogenesis, presenting clinically as tall stature with skeletal abnormalities.^{56,368}

CNP acts through NPR2 to stimulate growth plate chondrogenesis (see discussion earlier). As a result, loss-of-function mutations in NPR2 slow chondrogenesis to cause short stature. Conversely, gain-of-function mutations in NPR2 cause tall stature, associated with scoliosis and macrodactyly of the great toe.³⁶⁹

Simpson-Golabi-Behmel syndrome type 1 (SGBS1) is characterized by pre- and postnatal overgrowth, distinctive facies, intellectual disability, and a variety of congenital anomalies. It is X-linked and can be caused by mutations in *GPC3* or 4. The glypicans encoded by these genes are cell surface heparan sulfate proteoglycans. There is evidence that glypicans are involved in BMP, FGF, hedgehog (HH), and wingless-integrated (WNT) signaling.

Disorders Involving Cartilage Extracellular Matrix/Fluid.

Fibrillin-1 (*FBN1*) is a major component of microfibrils, which are found extracellularly in connective tissues. Some heterozygous mutations in *FBN1* cause Marfan syndrome, which is inherited in an autosomal dominant fashion and has multiple skeletal, ocular, and cardiovascular manifestations. Skeletal abnormalities include tall stature with disproportionately long limbs and digits, as well as pectus excavatum or carinatum and scoliosis. Eye findings include myopia and ectopia lentis. Cardiovascular manifestations include aortic root enlargement with a high risk of aortic dissection.³⁷⁰ Other mutations in *FBN1* can cause acromelic dysplasias, which are characterized by short stature with disproportionate shortening of the distal limbs.

Homocystinuria caused by cystathionine β -synthase deficiency shows some similarities to Marfan syndrome. It can affect the skeleton, causing tall stature with disproportionately long extremities, scoliosis, and pectus excavatum.³⁷¹ Other manifestations include myopia and ectopia lentis, thromboembolism, and developmental delay.³⁷¹ There is evidence that the excessive linear growth is caused by high extracellular concentrations of homocysteine.³⁷²

Disorders Involving Intracellular Factors.

Klinefelter syndrome is a relatively common chromosomal disorder that is usually associated with a 47,XXY karyotype, sometimes with mosaicism. The classical features include a male with tall stature with relatively long lower extremities, small testes usually with azoospermia, and gynecomastia (see Chapter 18 on Puberty and Its Disorders in the Male for details).³⁷³ The tall stature and increased lower leg length is thought to be caused by increased copy number of *SHOX*, a transcription factor that appears to be important for growth plate function. *SHOX* is located in a pseudoautosomal region on the X and Y chromosomes and escapes X inactivation. Therefore normal males and females have two active copies of *SHOX*, whereas girls with Turner syndrome have only one copy, causing short stature, and boys with Klinefelter syndrome have three active copies, causing tall stature.³⁷³

Mutations in genes that affect epigenetic modifications can cause overgrowth. Sotos syndrome is characterized by overgrowth, which is typically of prenatal onset. The overgrowth affects the limbs more than the spine and is accompanied by macrocephaly and advanced bone age. In addition, there are characteristic facial features and variable developmental delay. It can be caused by heterozygous mutations in *NSD1*, which encodes a histone methyltransferase and therefore affects epigenetic control of gene expression. Malan syndrome, which is

also termed *Sotos syndrome 2*, is a similar disorder that is characterized by overgrowth, macrocephaly, characteristic facies, and developmental delay and is caused by mutations in gene nuclear factor I X (*NFIX*).³⁷⁴ Mutations in *NFIX* can also cause Marshall-Smith syndrome, which can include tall stature with advanced bone age, intellectual disability, and distinctive facies.

Weaver syndrome has features similar to Sotos syndrome, including tall stature, advanced bone age, characteristic facies, and variable intellectual disability. It can be caused by mutations in *EZH2*, *EED*, or *SUZ12*, which participate in the PRC2 protein complex and are involved in histone methylation, an epigenetic modification. A similar overgrowth syndrome is caused by heterozygous mutations in *DNMT3a*, which encodes a DNA methyltransferase that affects DNA methylation.

BWS manifests as overgrowth, typically both pre- and post-natal, which can disproportionately affect different tissues resulting in macroglossia and hemihypertrophy. Patients have an increased risk of abdominal wall defects at birth, hyperinsulinism, and embryonal tumors. BWS can be caused by genetic or epigenetic abnormalities in a cluster of imprinted genes in the 11p15.5–11p15.4 region. Often, there is abnormal DNA methylation in this region and abnormal expression of the imprinted genes, *CDKN1C* (a cell cycle inhibitor) and *IGF2* (a paracrine growth factor).³⁷⁵

Perlman syndrome is an overgrowth syndrome similar to BWS but inherited in an autosomal recessive fashion. It is caused by mutations in *DIS3L2*, which is thought to be involved in messenger RNA degradation.

Idiopathic Growth Excess. The majority of healthy tall children with no apparent cause of growth excess will be classified as idiopathic tall stature, including familial tall stature and constitutional advance in growth and adolescence.³⁷⁶ Both are diagnoses of exclusion and the most common diagnoses among people with tall stature. Children with familial tall stature usually maintain growth at approximately the same high percentile and reach an adult height within their target range. Constitutional advance in growth is characterized by a developmental pattern opposite to constitutional delay of growth. It can be associated, although not exclusively, with obesity. These patients show accelerated growth with advanced bone age and relatively early puberty, usually resulting in normal adult height. In this group of children with isolated tall stature, it is very unusual to identify a monogenic cause for overgrowth. The growth pattern and height of these children is probably explained by the combination of several common variants in a typical polygenic effect. However, the ability to predict tall stature using polymorphisms identified in GWAS height is limited.³⁷⁷

Diagnostic Approach to the Patient With Tall Stature

Although we can define tall stature as a height SDS over 2, in the absence of other symptoms and signs, the large majority of tall children and adolescents are healthy and have familial tall stature or constitutional advance in growth.³⁷⁶ In addition, tall stature has a wider social acceptance than short stature and secondary conditions resulting in overgrowth are infrequent. For these reasons, more restricted referral criteria are usually used to improve the identification of pathological overgrowth conditions and thereby reduce unnecessary testing. Patients with more severe tall stature, those with height significantly above the mean parental height or who cross upward through percentiles during childhood and/or puberty are significantly more likely to have tall stature caused by a specific pathological condition and require clinical investigation. Furthermore, presence of intellectual disability, macrocephaly, or dysmorphic features can indicate a syndromic tall stature condition (in general with a genetic basis). In children with tall stature and these additional features, a more detailed evaluation for genetic syndromes should be considered.

The main differential diagnoses of children with tall stature are precocious exposure to sex steroids, supernumerary sex chromosome aneuploidies (e.g., Klinefelter syndrome), growth hormone excess (i.e., pituitary gigantism), BWS, Marfan syndrome, Sotos syndrome, Weaver syndrome, and other rare genetic conditions (see section on Causes of Tall Stature earlier). Despite the complexity and multiplicity of potential causes for tall stature, several diagnoses can be obtained from a carefully documented medical history and a comprehensive physical examination (Table 11.7). Depending on specific clinical findings from the medical history and physical examination, special investigations are often required to confirm the initial clinical impression (Table 11.8).

Determination of growth patterns are a critical part of the medical history (Fig. 11.11). Tall children who cross percentiles upward should be investigated for precocious exposure to sex steroids, GH excess, and hyperthyroidism. On the other hand,

TABLE 11.7 Key Findings in the Medical History and Physical Examination of Children With Tall Stature

Key Findings	Disorders
Family history suggesting an autosomal recessive inheritance and/or presence of consanguinity	Homocystinuria, CATSHL syndrome
Family history suggesting an autosomal dominant inheritance of tall stature	Familial tall stature, Marfan syndrome, epiphyseal chondrodysplasia, Miura type (increase of signal by <i>CNP/NPR-B</i>)
Developmental delay, intellectual disability and behavior problems	Sotos, Weaver syndrome, homocystinuria, fragile X-chromosome, <i>DNMT3A</i> mutations, and others
Increased birth weight and length SDS for gestational age	Beckwith-Wiedemann, Sotos, Weaver syndromes, <i>IGF1R</i> duplications, and others
Head circumference SDS	Macrocephaly is observed in Sotos, Weaver, <i>DNMT3A</i> mutations, and fragile-X syndromes Microcephaly is observed in CATSHL syndrome
Presence of precocious secondary sexual characteristics	Precocious sex steroids exposure (precocious puberty, virilizing disorders)
Signs of hypogonadism (small testes, amenorrhea, underdevelopment of breast and body hair)	Several different causes; in males, mainly Klinefelter syndrome
Marfanoid habitus (arm span that exceeds the height, arachnodactyly and hyperlaxity)	Marfan, Sotos, homocystinuria, epiphyseal chondrodysplasia, Miura type
Macroductyly of the great toes	Epiphyseal chondrodysplasia, Miura type (increase of signal by <i>CNP/NPR-B</i>)
Body disproportion (arm span that exceeds the height and/or sitting height : total height SDS < -2)	Klinefelter syndrome
Dysmorphic facial features	Several syndromic forms of overgrowth conditions, each one with particular features
Severe kyphoscoliosis	CATSHL syndrome, epiphyseal chondrodysplasia, Miura type

The listed signs and symptoms are diagnostically helpful when present but may be absent in some children with the associated disorders. *CATSHL*, Camptodactyly, tall stature, and hearing loss syndrome (recessive *FGFR3* mutation); *CNP*, C-type natriuretic peptide; *NPR-B*, natriuretic peptide receptor B; *SDS*, standard deviation score.

TABLE 11.8 Complementary Diagnostic Tests That Can be Useful During Investigation of a Patient With Tall Stature

Examination	Objective
Wrist and hand x-ray for bone age determination	Advanced bone age is often observed in patients with Sotos syndrome, Weaver syndrome, Beckwith-Wiedemann syndrome, precocious sex steroids exposure, constitutional advance of growth, and obesity. Delayed bone age is observed in hypogonadism, aromatase deficiency, and estrogen resistance
Serum IGF-1, random GH and OGTT for GH suppression	Screening for GH excess
Serum TSH, free-T4	Screening for hyperthyroidism
LH, FSH, Testosterone/estradiol levels	For evaluation of precocious sex steroids exposure in presence of physical signs or hypogonadism
Serum homocysteine	Screening for homocystinuria mainly in patients with Marfanoid habitus, consanguineous parents and/or intellectual disability
Karyotype	Assessment of chromosome aberrations (i.e., Klinefelter syndrome, 47 XYY, 47 XXX) or CAIS
Echocardiogram	Assessment of aortic root dilation (i.e., Marfan syndrome)
Ophthalmological evaluation	Assessment of <i>ectopia lentis</i> (i.e., Marfan syndrome and homocystinuria)
<i>FBN1</i> sequence	To confirm or exclude the diagnosis of Marfan syndrome in patients with suggestive clinical findings
Molecular karyotype (SNP or CGH array)	To identify submicroscopic copy number variation (deletion or duplication), mainly in patients with unexplained developmental delay/intellectual disability, autism spectrum disorders, and/or multiple congenital anomalies
High-throughput sequence (whole exome or gene panel sequence)	To simultaneously investigate the presence of pathogenic variants in several genes associated with tall stature, especially in patients with high suspicion for genetic causes

CAIS, Complete androgen insensitivity; CGH, comparative genomic hybridization; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; T4, thyroxine, TSH, thyroid-stimulating hormone.

children who are above 2 SD in height and remain in the same percentile and are compatible with the target height of their percentile suggest familial tall stature, in the absence of other positive findings. Sotos and Weaver syndromes should be considered particularly in children born large for gestational age that remain with height SDS above 2 and have associated macrocephaly and/or cognitive impairment.³⁷⁸ BWS should be investigated in children with pre- and postnatal overgrowth associated with neonatal hypoglycemia, macroglossia, omphalocele, umbilical hernia, and/or a history of an embryonal tumor (e.g., Wilms tumor, hepatoblastoma, neuroblastoma, rhabdomyosarcoma).³⁷⁹ Disproportionately tall stature (long

legs, arm span that exceeds the height, and/or sitting height: total height SDS < -2), small testes, gynecomastia, learning disability, and/or increased gonadotropin concentrations suggest Klinefelter syndrome.³⁸⁰ Tall children with personal or family histories of ectopia lentis (subluxation of the lenses) or aortic root dilation should be investigated for Marfan syndrome.³⁸¹ Finally, there are children whose height is within the reference range during childhood, but who have prolonged growth beyond adolescence resulting in a height SDS above 2 in adulthood,³⁷⁶ a pattern which can result from impaired estrogen production or action.

Genetic tests are important to confirm diagnostic hypotheses and also to investigate patients with syndromic overgrowth of unknown cause. Because of the heterogeneity of genes that can cause pathological tall stature, the large size of some genes and the rarity of these conditions, a multigene strategy using next-generation sequencing (exome sequencing or targeted panel sequencing) may be more efficient than targeted sequencing of a single candidate gene.³⁷⁶ Moreover, molecular karyotyping (CGH or SNP microarrays) is also a useful tool to investigate patients with overgrowth associated with developmental delay, intellectual disability, or autism spectrum disorders.

Treatment of Tall Stature

Treatment for Growth Hormone Excess. The treatment for pituitary gigantism can be complex, and includes medical therapy using somatostatin analogues, which result in biochemical cure in 70% with tumor shrinkage in 75%. Cabergoline can also be tried and is particularly effective in patients with tumors secreting both GH and prolactin. The GH antagonist, Pegvisomant, can also be used although it does not cause tumor shrinkage. Transsphenoidal resection of the tumor is associated with biochemical cure in 75% to 95%. Radiotherapy is used in resistant cases, but may be associated with complications including hypopituitarism, visual impairment, and possibly secondary tumors.

Treatment for Other Endocrine Causes of Tall Stature. When tall stature is caused by excess sex steroids or thyroid hormone, the underlying endocrine abnormality is usually treatable. The treatments for precocious puberty, congenital adrenal hyperplasia, and hyperthyroidism are discussed elsewhere.

Treatment for Nonendocrine Tall Stature. In general, tall stature that does not have a specific pathological etiology does not require treatment. However, tall stature in a child may cause anxiety to parents, who may then request treatment to cease growth progression. No evidence-based guideline has been created with respect to the selection of potential candidates for adult height reduction. Treatment decisions are often influenced by the predicted adult height, based on a radiograph of the nondominant hand and wrist. Typically, treatment is only considered for adolescents whose predicted adult height is more than 2.5 SD above the population mean. Unfortunately, the available methods to predict adult height, including the Bayley-Pinneau method and TW Mark 1 and 2 methods, have limited accuracy and, depending on the bone age, a tendency to overestimate adult height.³⁸²

Various types of treatment, either hormonal or surgical, have been used to reduce growth in children with tall stature.

One approach for an excessive height prediction that has been studied is to induce puberty early, thereby leading to early cessation of growth and early epiphyseal fusion, using testosterone in males and estrogens in females. However, this use of sex steroids has declined over the last 20 years, in part because of adverse treatment effects.³⁸³ In the short term, boys treated

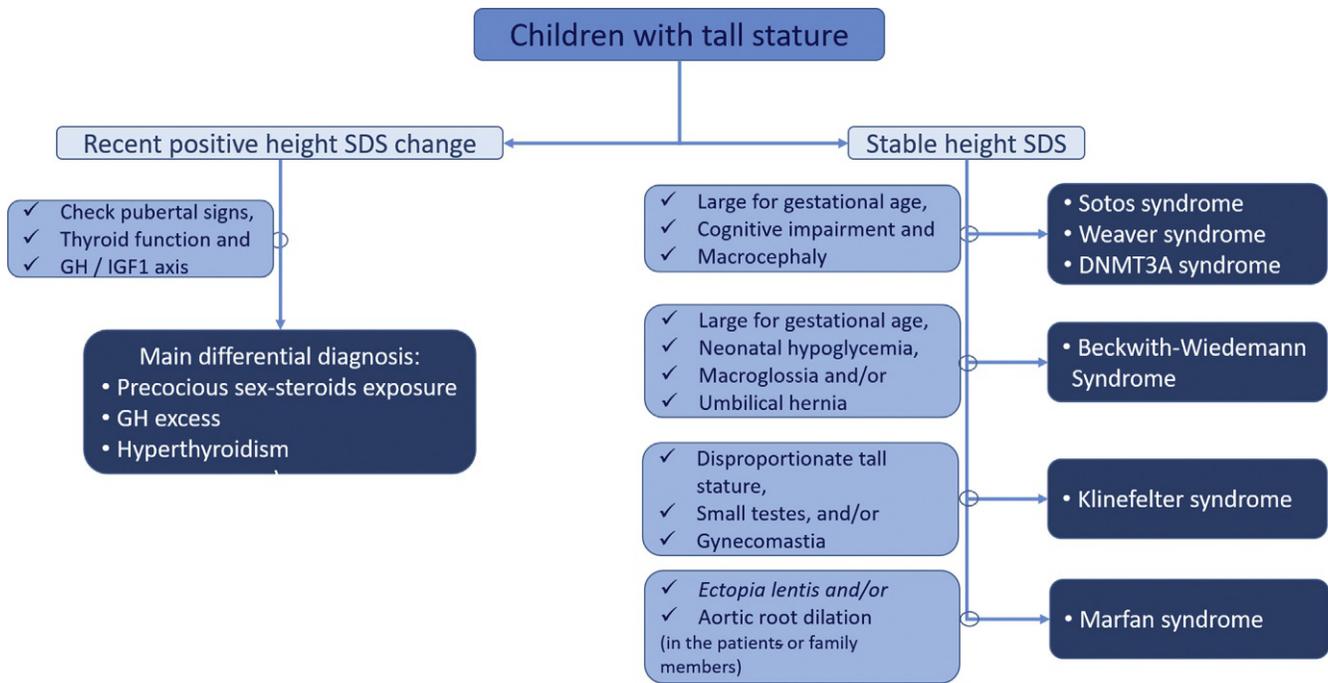


Fig. 11.11 Overview of investigation of children with tall stature.

with androgens may present with myalgia, acne, gynecomastia, and weight gain, whereas girls treated with high doses of estrogen may have weight gain, night cramps, galactorrhea, ovarian cysts, and predisposition to thrombosis. There is also evidence that tall women who were treated with high-dose estrogen are at risk of decreased fertility.³⁸⁴ Some have advocated inducing puberty by the use of more physiological doses of sex steroids before the tall child progresses into puberty as a means of reducing final adult height. There remains a significant amount of uncertainty regarding the efficacy of exogenous sex steroids in limiting final height, whether used as part of a high or lower dose strategy.

Nocturnal infusion of octreotide, a somatostatin analogue, has been reported to reduce GH secretion and height prediction in tall children. However, a more recent study showed that long-term treatment with a somatostatin analogue does not reduce final height in a manner sufficient to justify treatment in tall stature.³⁸⁵

The most common surgical procedure for reducing growth is bilateral percutaneous epiphysiodesis of the distal femur and proximal tibia and fibula. Epiphysiodesis is a surgical intervention for reducing final height by approximately 5 cm, which is typically performed at a bone age exceeding 12.5 years in girls with a stature of 170 cm and 14 years in boys with a stature more than 185 cm.³⁸³

REFERENCES

1. Kelberman D, Rizzoti K, Lovell-Badge R, Robinson IC, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev.* 2009;30:790–829.
2. Davis SW, Castinetti F, Carvalho LR, Ellsworth BS, Potok MA, Lyons RH, et al. Molecular mechanisms of pituitary organogenesis: in search of novel regulatory genes. *Mol Cellular Endocrinol.* 2010;323:4–19.
3. Davis SW, Mortensen AH, Camper SA. Birthdating studies reshape models for pituitary gland cell specification. *Dev Biol.* 2011;352:215–227.
4. Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, et al. Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors

- in mice and humans. *Proc Natl Acad Sci U S A.* 2011;108:11482–11487.
5. Andoniadou CL, Gaston-Massuet C, Reddy R, Schneider RP, Blasco MA, Le Tissier P, et al. Identification of novel pathways involved in the pathogenesis of human adamantinomatous craniopharyngioma. *Acta Neuropathol.* 2012;124:259–271.
6. Fang Q, George AS, Brinkmeier ML, Mortensen AH, Gergics P, Cheung LY, Daly AZ, et al. Genetics of combined pituitary hormone deficiency: roadmap into the genome era. *Endocr Rev.* 2016;37:636–675.
7. Andoniadou CL, Matsushima D, Mousavy Gharavy SN, Signore M, Mackintosh AI, Schaeffer M, et al. Sox2(+) stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential. *Cell Stem Cell.* 2013;13:433–445.
8. Fauquier T, Rizzoti K, Dattani M, Lovell-Badge R, Robinson IC. SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland. *Proc Natl Acad Sci U S A.* 2008;105:2907–2912.
9. Castinetti F, Davis SW, Brue T, Camper SA. Pituitary stem cell update and potential implications for treating hypopituitarism. *Endocr Rev.* 2011;32:453–471.
10. Suga H, Kadoshima T, Minaguchi M, Ohgushi M, Soen M, Nakano T, et al. Self-formation of functional adenohypophysis in three-dimensional culture. *Nature.* 2011;480:57–62.
11. Baumann G. Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. *Endocr Rev.* 1991;12:424–449.
12. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature.* 1999;402:656–660.
13. Pantel J, Legendre M, Cabrol S, Hilal L, Hajaji Y, Morisset S, et al. Loss of constitutive activity of the growth hormone secretagogue receptor in familial short stature. *J Clin Invest.* 2006;116:760–768.
14. Pantel J, Legendre M, Nivot S, Morisset S, Vie-Luton MP, le Bouc Y, et al. Recessive isolated growth hormone deficiency and mutations in the ghrelin receptor. *J Clin Endocrinol Metab.* 2009;94:4334–4341.
15. Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol.* 2003;23:7973–7981.
16. Nass R, Toogood AA, Hellmann P, Bissonette E, Gaylinn B, Clark R, Thormer MO. Intracerebroventricular administration of the rat growth hormone (GH) receptor antagonist G118R stimulates GH secretion: evidence for the existence of short loop negative feedback of GH. *J Neuroendocrinol.* 2000;12:1194–1199.

17. Raz B, Janner M, Petkovic V, Lochmatter D, Eble A, Dattani MT, et al. Influence of growth hormone (GH) receptor deletion of exon 3 and full-length isoforms on GH response and final height in patients with severe GH deficiency. *J Clin Endocrinol Metab.* 2008;93:974–980.
18. Brown RJ, Adams JJ, Pelekanos RA, Wan Y, McKinstry WJ, Palethorpe K, et al. Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nat Struct Mol Biol.* 2005;12:814–821.
19. Waters MJ. The growth hormone receptor. *Growth Horm IGF Res.* 2016;28:6–10.
20. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, et al. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. *Lancet (London, England).* 2001;358:1754–1759.
21. Hwa V, Nadeau K, Wit JM, Rosenfeld RG. STAT5b deficiency: lessons from STAT5b gene mutations. *Best Pract Res Clin Endocrinol Metab.* 2011;25:61–75.
22. Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. The somatomedin hypothesis: 2001. *Endocr Rev.* 2001;22:53–74.
23. Blum WF, Wudy SA. In: Ranke, ME, Mullis, PE, eds. *Insulin-like growth factors and their binding proteins.* In: *Diagnostics of Endocrine Function in Children and Adolescents.* 4th ed. Basel, Switzerland: Karger; 2011:157–182.
24. Yang J, Anzo M, Cohen P. Control of aging and longevity by IGF-I signaling. *Exp Gerontol.* 2005;40:867–872.
25. Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, et al. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci U S A.* 2008;105:3438–3442.
26. Brown J, Jones EY, Forbes BE. Keeping IGF-II under control: lessons from the IGF-II-IGF2R crystal structure. *Trends Biochem Sci.* 2009;34:612–619.
27. Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr Rev.* 1999;20:761–787.
28. Dauber A, Munoz-Calvo MT, Barrios V, Domene HM, Kloverpris S, Serra-Juhe C, et al. Mutations in pregnancy-associated plasma protein A2 cause short stature due to low IGF-I availability. *EMBO Mol Med.* 2016;8:363–374.
29. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell.* 1993;75:73–82.
30. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med.* 1996;335:1363–1367.
31. Begemann M, Zirn B, Santen G, Wirthgen E, Soellner L, Buttler HM, et al. Paternally Inherited IGF2 Mutation and Growth Restriction. *N Engl J Med.* 2015;373:349–356.
32. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med.* 2003;349:2211–2222.
33. Nebesio TD, Wise MD, Perkins SM, Eugster EA. Does clinical management impact height potential in children with severe acquired hypothyroidism? *J Pediatr Endocrinol Metab.* 2011;24:893–896.
34. van Gucht ALM, Moran C, Meima ME, Visser WE, Chatterjee K, Visser TJ, Peeters RP. Resistance to thyroid hormone due to heterozygous mutations in thyroid hormone receptor alpha. *Curr Top Dev Biol.* 2017;125:337–355.
35. Dumitrescu AM, Refetoff S. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, et al., eds. *Impaired sensitivity to thyroid hormone: defects of transport, metabolism and action.* South Dartmouth (MA): Endotext; 2000.
36. Nilsson O, Marino R, De Luca F, Phillip M, Baron J. Endocrine regulation of the growth plate. *Horm Res.* 2005;64:157–165.
37. Rivkees SA, Danon M, Herrin J. Prednisone dose limitation of growth hormone treatment of steroid-induced growth failure. *J Pediatr.* 1994;125:322–325.
38. Oz OK, Millsaps R, Welch R, Birch J, Zerwekh JE. Expression of aromatase in the human growth plate. *J Mol Endocrinol.* 2001;27:249–253.
39. Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER. Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. *J Clin Endocrinol Metab.* 1993;76:996–1001.
40. De Leon DD, Stanley CA. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Permanent neonatal diabetes mellitus.* Seattle (WA): GeneReviews(R); 1993.
41. Kosztolanyi G. Leprechaunism/Donohue syndrome/insulin receptor gene mutations: a syndrome delineation story from clinicopathological description to molecular understanding. *Eur J Pediatr.* 1997;156:253–255.
42. McFarland MB, Trylovich CG, Langer O. Anthropometric differences in macrosomic infants of diabetic and nondiabetic mothers. *J Matern Fetal Med.* 1998;7:292–295.
43. Misra M, Klibanski A. Endocrine consequences of anorexia nervosa. *Lancet Diabetes Endocrinol.* 2014;2:581–592.
44. Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: mechanisms and management. *Nat Rev Endocrinol.* 2017;13:174–186.
45. Gat-Yablonski G, De Luca F. Effect of nutrition on statural growth. *Horm Res Paediatr.* 2017;88:46–62.
46. DeGroot LJ. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, et al., eds. *The non-thyroidal illness syndrome.* South Dartmouth: Endotext; 2000.
47. de Groot CJ, van den Berg A, Ballieux B, et al. Determinants of advanced bone age in childhood obesity. *Horm Res Paediatr.* 2017;87:254–263.
48. Shalitin S, Kiess W. Putative effects of obesity on linear growth and puberty. *Horm Res Paediatr.* 2017;88:101–110.
49. Fennoy I. Effect of obesity on linear growth. *Curr Opin Endocrinol Diabetes Obes.* 2013;20:44–49.
50. Sederquist B, Fernandez-Vojvodich P, Zaman F, Savendahl L. Recent research on the growth plate: impact of inflammatory cytokines on longitudinal bone growth. *J Mol Endocrinol.* 2014;53:T35–T44.
51. Wong SC, Dobie R, Altowati MA, Werther GA, Farquharson C, Ahmed SF. Growth and the growth hormone-insulin like growth factor 1 axis in children with chronic inflammation: current evidence, gaps in knowledge, and future directions. *Endocr Rev.* 2016;37:62–110.
52. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol.* 2015;225:R67–R81.
53. Baron J, Savendahl L, De Luca F, Dauber A, Phillip M, Wit JM, Nilsson O. Short and tall stature: a new paradigm emerges. *Nat Rev Endocrinol.* 2015;11:735–746.
54. Vasques GA, Amhold JJ, Jorge AA. Role of the natriuretic peptide system in normal growth and growth disorders. *Horm Res Paediatr.* 2014;82:222–229.
55. Kant SG, Cervenkova I, Balek L, Trantirek L, Santen GW, de Vries MC, et al. A novel variant of FGFR3 causes proportionate short stature. *Eur J Endocrinol.* 2015;172:763–770.
56. Escobar LF, Tucker M, Bamshad M. A second family with CATSHL syndrome: Confirmatory report of another unique FGFR3 syndrome. *Am J Med Genet A.* 2016;170:1908–1911.
57. Yakar S, Werner H, Rosen CJ. Insulin-like growth factors: actions on the skeleton. *J Mol Endocrinol.* 2018;61:T115–T137.
58. Walenkamp MJ, Losekoot M, Wit JM. Molecular IGF-1 and IGF-1 receptor defects: from genetics to clinical management. *Endocr Dev.* 2013;24:128–137.
59. Kronenberg HM. PTHrP and skeletal development. *Ann N Y Acad Sci.* 2006;1068:1–13.
60. Juppner H. Role of parathyroid hormone-related peptide and Indian hedgehog in skeletal development. *Pediatr Nephrol.* 2000;14:606–611.
61. Vasques GA, Funari MFA, Ferreira FM, Aza-Carmona M, Sentchordi-Montane L, Barraza-Garcia J, et al. IHH gene mutations causing short stature with nonspecific skeletal abnormalities and response to growth hormone therapy. *J Clin Endocrinol Metab.* 2018;103:604–614.
62. Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev.* 2009;19:230–236.
63. Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. *Proc Natl Acad Sci U S A.* 2001;98:6871–6876.
64. Smith EP, Specker B, Korach KS. Recent experimental and clinical findings in the skeleton associated with loss of estrogen hormone

- or estrogen receptor activity. *J Steroid Biochem Mol Biol*. 2010;118:264–272.
65. Yanovski JA, Rose SR, Municchi G, et al. Treatment with a luteinizing hormone-releasing hormone agonist in adolescents with short stature. *N Engl J Med*. 2003;348:908–917.
 66. Hero M. Aromatase Inhibitors in the Treatment of Short Stature. *Endocr Dev*. 2016;30:130–140.
 67. Prader A, Tanner JM, von HG. Catch-up growth following illness or starvation. *An example of developmental canalization in man J Pediatr*. 1963;62:646–659.
 68. Tanner JM. Regulation of growth in size in mammals. *Nature*. 1963;199:845–850.
 69. Lui JC, Nilsson O, Baron J. Growth plate senescence and catch-up growth. *Endocr Dev*. 2011;21:23–29.
 70. Emons JA, Boersma B, Baron J, Wit JM. Catch-up growth: testing the hypothesis of delayed growth plate senescence in humans. *J Pediatr*. 2005;147:843–846.
 71. de Wit CC, Sas TC, Wit JM, Cutfield WS. Patterns of catch-up growth. *J Pediatr*. 2013;162:415–420.
 72. Jelenkovic A, Sund R, Hur YM, et al. Genetic and environmental influences on height from infancy to early adulthood: an individual-based pooled analysis of 45 twin cohorts. *Sci Rep*. 2016;6:28496.
 73. Guo MH, Hirschhorn JN, Dauber A. Insights and implications of genome wide association studies of height. *J Clin Endocrinol Metab*. 2018;103(9):3155–3168.
 74. Wood AR, Esko T, Yang J, et al. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet*. 2014;46:1173–1186.
 75. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet*. 2018;27:3641–3649.
 76. Marouli E, Graff M, Medina-Gomez C, et al. Rare and low-frequency coding variants alter human adult height. *Nature*. 2017;542:186–190.
 77. Voss LD, Wilkin TJ, Bailey BJ, Betts PR. The reliability of height and height velocity in the assessment of growth (the Wessex Growth Study). *Arch Dis Child*. 1991;66:833–837.
 78. Lipman TH, Hench K, Logan JD, DiFazio DA, Hale PM, Singer-Granick C. Assessment of growth by primary health care providers. *J Pediatr Health Care*. 2000;14:166–171.
 79. Lipman TH, Hench KD, Benyi T, Delaune J, Gilluly KA, Johnson L, et al. A multicentre randomised controlled trial of an intervention to improve the accuracy of linear growth measurement. *Arch Dis Child*. 2004;89:342–346.
 80. Van den Broeck J, Hokken-Koelega A, Wit J. Validity of height velocity as a diagnostic criterion for idiopathic growth hormone deficiency and Turner syndrome. *Horm Res*. 1999;51:68–73.
 81. Grummer-Strawn LM, Reinold C, Krebs NF, Centers for Disease C, Prevention. Use of World Health Organization and CDC growth charts for children aged 0–59 months in the United States. *MMWR Recomm Rep*. 2010;59:1–15.
 82. Tanner JM, Davies PS. Clinical longitudinal standards for height and height velocity for North American children. *J Pediatr*. 1985;107:317–329.
 83. De Sanctis V. *Manual of growth charts and body standard measurements*. 2nd ed. Pisa, Italy: Pacini Editore S.p.A; 2016.
 84. Albanese A, Stanhope R. Does constitutional delayed puberty cause segmental disproportion and short stature? *Eur J Pediatr*. 1993;152:293–296.
 85. Turan S, Bereket A, Omar A, Berber M, Ozen A, Bekiroglu N. Upper segment/lower segment ratio and armspan-height difference in healthy Turkish children. *Acta Paediatr*. 2005;94:407–413.
 86. Malaquias AC, Scalco RC, Fontenele EG, Costalonga EF, Baldin AD, Braz AF, et al. The sitting height/height ratio for age in healthy and short individuals and its potential role in selecting short children for SHOX analysis. *Horm Res Paediatr*. 2013;80:449–456.
 87. National Health and Nutrition Examination Survey Data. *Hyattsville, MD: Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS)*. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 1988.
 88. Frisancho AR. *Anthropometric Standards: An Interactive Nutritional Reference of Body Size and Body Composition for Children and Adults*. Ann Arbor, MI: The University of Michigan Press; 2008.
 89. Geister KA, Camper SA. Advances in Skeletal Dysplasia Genetics. *Annu Rev Genomics Hum Genet*. 2015;16:199–227.
 90. Greulich WW, Pyle SI. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. Stanford. Stanford, CA: Stanford University Press; 1959.
 91. Tanner JM, Healy MJR, Cameron N, et al. *Assessment of Skeletal Maturity and Prediction of Adult Height (TW2 Method)*. London: Academic Press; 2001.
 92. Unrath M, Thodberg HH, Schweizer R, Ranke MB, Binder G, Martin DD. Automation of bone age reading and a new prediction model improve adult height prediction in children with short stature. *Horm Res Paediatr*. 2012;78:312–319.
 93. Flor-Cisneros A, Leschek EW, Merke DP, Barnes KM, Coco M, Cutler Jr GB, Baron J. In boys with abnormal developmental tempo, maturation of the skeleton and the hypothalamic-pituitary-gonadal axis remains synchronous. *J Clin Endocrinol Metab*. 2004;89:236–241.
 94. Marshall WA. Interrelationships of skeletal maturation, sexual development and somatic growth in man. *Ann Hum Biol*. 1974;1:29–40.
 95. Flor-Cisneros A, Roemmich JN, Rogol AD, Baron J. Bone age and onset of puberty in normal boys. *Mol Cell Endocrinol*. 2006;254–255:202–206.
 96. Filipsson R, Hall K. Prediction of adult height of girls from height and dental maturity at ages 6–10 years. *Ann Hum Biol*. 1975;2:355–363.
 97. Luo ZC, Albertsson-Wikland K, Karlberg J. Target height as predicted by parental heights in a population-based study. *Pediatr Res*. 1998;44:563–571.
 98. Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. *J Pediatr*. 1952;40:423–441.
 99. Roche AF, Wainer H, Thissen D. The RWT method for the prediction of adult stature. *Pediatrics*. 1975;56:1027–1033.
 100. Zachmann M, Sobradillo B, Frank M, Frisch H, Prader A. Bayley-Pinneau, Roche-Wainer-Thissen, and Tanner height predictions in normal children and in patients with various pathologic conditions. *J Pediatr*. 1978;93:749–755.
 101. Khamis HJ, Roche AF. Predicting adult stature without using skeletal age: the Khamis-Roche method. *Pediatrics*. 1994;94:504–507.
 102. Topor LS, Feldman HA, Bauchner H, Cohen LE. Variation in methods of predicting adult height for children with idiopathic short stature. *Pediatrics*. 2010;126:938–944.
 103. Dauber A, Rosenfeld RG, Hirschhorn JN. Genetic evaluation of short stature. *J Clin Endocrinol Metab*. 2014;99:3080–3092.
 104. Wit JM. International classification of pediatric endocrine diagnoses. *Horm Res Paediatr*. 2016;86:212–214.
 105. Bhutta ZA, Berkley JA, Bandsma RHJ, Kerac M, Trehan I, Briend A. Severe childhood malnutrition. *Nat Rev Dis Primers*. 2017;3:17067.
 106. Williams AM, Suchdev PS. Assessing and improving childhood nutrition and growth globally. *Pediatr Clin North Am*. 2017;64:755–768.
 107. Lifshitz F. Nutrition and growth. *J Clin Res Pediatr Endocrinol*. 2009;1:157–163.
 108. Mairs R, Nicholls D. Assessment and treatment of eating disorders in children and adolescents. *Arch Dis Child*. 2016;101:1168–1175.
 109. Bonamico M, Scire G, Mariani P, Pasquino AM, Triglionone P, Scaccia S, et al. Short stature as the primary manifestation of monosymptomatic celiac disease. *J Pediatr Gastroenterol Nutr*. 1992;14:12–16.
 110. Kanof ME, Lake AM, Bayless TM. Decreased height velocity in children and adolescents before the diagnosis of Crohn's disease. *Gastroenterology*. 1988;95:1523–1527.
 111. Giglio L, Candusso M, D'Orazio C, Mastella G, Faraguna D. Failure to thrive: the earliest feature of cystic fibrosis in infants diagnosed by neonatal screening. *Acta Paediatr*. 1977;86:1162–1165.
 112. Yakoob MY, Lo CW. Nutrition (micronutrients) in child growth and development: a systematic review on current evidence, recommendations and opportunities for further research. *J Dev Behav Pediatr*. 2017;38:665–679.

113. Prasad AS, Miale Jr A, Farid Z, Sandstead HH, Schuler AR. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. *J Lab Clin Med.* 1963;61:537–549.
114. Prasad AS. Clinical and biochemical manifestations of zinc deficiency in human subjects. *J Am Coll Nutr.* 1985;4:65–72.
115. Grimberg A, Lifschitz F. Worrisome growth. In: *Pediatric Endocrinology*. 5th ed. New York: Informa Healthcare; 2006. Vol 2.
116. Alatzoglou KS, Webb EA, Le Tissier P, Dattani MT. Isolated growth hormone deficiency (GHD) in childhood and adolescence: recent advances. *Endocr Rev.* 2014;35:376–432.
117. Growth Hormone Research S. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab.* 2000;85:3990–3993.
118. Patel L, McNally RJ, Harrison E, Lloyd IC, Clayton PE. Geographical distribution of optic nerve hypoplasia and septo-optic dysplasia in Northwest England. *J Pediatr.* 2006;148:85–88.
119. Arslanian SA, Rothfus WE, Foley Jr TP, Becker DJ. Hormonal, metabolic, and neuroendocrine abnormalities associated with septo-optic dysplasia. *Acta Endocrinol.* 1984;107:282–288.
120. GH Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab.* 2000;85:3990–3993.
121. Wales JK, Quarrell OW. Evidence for possible Mendelian inheritance of septo-optic dysplasia. *Acta Paediatr (Oslo, Norway: 1992).* 1996;85:391–392.
122. Murray PG, Paterson WF, Donaldson MD. Maternal age in patients with septo-optic dysplasia. *J Pediatr Endocrinol Metab.* 2005;18:471–476.
123. Alatzoglou KS, Dattani MT. Genetic forms of hypopituitarism and their manifestation in the neonatal period. *Early Hum Dev.* 2009;85:705–712.
124. Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, et al. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat Genet.* 1996;14:357–360.
125. Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, et al. Loss-of-function mutations in the human GLI2 gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proc Natl Acad Sci U S A.* 2003;100:13424–13429.
126. Franca MM, Jorge AA, Carvalho LR, Costalonga EF, Vasques GA, et al. Novel heterozygous nonsense GLI2 mutations in patients with hypopituitarism and ectopic posterior pituitary lobe without holoprosencephaly. *J Clin Endocrinol Metab.* 2010;95:E384–E391.
127. Kelberman D, Rizzotti K, Avilion A, Bitner-Glindzicz M, Cianfarani S, Collins J, et al. Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans. *J Clin Invest.* 2006;116:2442–2455.
128. Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, Gregory JW, et al. SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development. *J Clin Endocrinol Metab.* 2008;93:1865–1873.
129. Alatzoglou, K.S., Andoniadou, C.L., Kelberman, D., Buchanan, C. R., Crolla, J., Arriazu, M.C., et al. SOX2 haploinsufficiency is associated with slow progressing hypothalamo-pituitary tumours. *Hum Mut.* 32, 1376–1380.
130. Woods KS, Cundall M, Turton J, Rizotti K, Mehta A, Palmer R, et al. Over- and underdosage of SOX3 is associated with infundibular hypoplasia and hypopituitarism. *Am J Hum Genet.* 2005;76:833–849.
131. Acerini CL, Tasker RC. Traumatic brain injury induced hypothalamic-pituitary dysfunction: a paediatric perspective. *Pituitary.* 2007;10:373–380.
132. Agha A, Thompson CJ. Anterior pituitary dysfunction following traumatic brain injury (TBI). *Clin Endocrinol.* 2006;64:481–488.
133. Benvenega S, Campenni A, Ruggeri RM, Trimarchi F. Clinical review 113: Hypopituitarism secondary to head trauma. *J Clin Endocrinol Metab.* 2000;85:1353–1361.
134. Lieberman SA, Oberoi AL, Gilkison CR, Masel BE, Urban RJ. Prevalence of neuroendocrine dysfunction in patients recovering from traumatic brain injury. *J Clin Endocrinol Metab.* 2001;86:2752–2756.
135. Agha A, Sherlock M, Phillips J, Tormey W, Thompson CJ. The natural history of post-traumatic neurohypophysial dysfunction. *Eur J Endocrinol.* 2005;152:371–377.
136. Leal-Cerro A, Flores JM, Rincon M, Murillo F, Pujol M, Garcia-Pesquera F, et al. Prevalence of hypopituitarism and growth hormone deficiency in adults long-term after severe traumatic brain injury. *Clin Endocrinol.* 2005;62:525–532.
137. Aimaretti G, Ambrosio MR, Di Somma C, Gasperi M, Cannavo S, Scaroni C, et al. Residual pituitary function after brain injury-induced hypopituitarism: a prospective 12-month study. *J Clin Endocrinol Metab.* 2005;90:6085–6092.
138. Einaudi S, Matarazzo P, Peretta P, Grossetti R, Giordano F, Altare F, et al. Hypothalamo-hypophysial dysfunction after traumatic brain injury in children and adolescents: a preliminary retrospective and prospective study. *J Pediatr Endocrinol Metab.* 2006;19:691–703.
139. Niederland T, Makovi H, Gal V, Andreka B, Abraham CS, Kovacs J. Abnormalities of pituitary function after traumatic brain injury in children. *J Neurotrauma.* 2007;24:119–127.
140. Keenan HT, Hooper SR, Wetherington CE, Nocera M, Runyan DK. Neurodevelopmental consequences of early traumatic brain injury in 3-year-old children. *Pediatrics.* 2007;119:e616–e623.
141. Chigo E, Masel B, Aimaretti G, Leon-Carrion J, Casanueva F.F., Dominguez-Morales M.R., et al. Consensus guidelines on screening for hypopituitarism following traumatic brain injury. *Brain Inj.* 19, 711–724.
142. Schaefer S, Boegershausen N, Meyer S, Ivan D, Schepelmann K, Kann PH. Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system. *Eur J Endocrinol.* 2008;158:3–9.
143. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med.* 2007;357:2153–2165.
144. Takano K. Sarcoidosis of the hypothalamus and pituitary. *Int Med (Tokyo, Japan).* 2004;43:894–895.
145. Murialdo G, Tamagno G. Endocrine aspects of neurosarcoidosis. *J Endocrinol Invest.* 2002;25:650–662.
146. Caturegli P, Newschaffer C, Olivi A, Pomper M.G., Burger P.C., Rose N.R. Autoimmune hypophysitis. *Endocr Rev.* 26, 599–614.
147. de Graaf JH, Egeler RM. New insights into the pathogenesis of Langerhans cell histiocytosis. *Curr Opin Pediatr.* 1997;9:46–50.
148. Nanduri VR, Bareille P, Pritchard J, Stanhope R. Growth and endocrine disorders in multisystem Langerhans' cell histiocytosis. *Clin Endocrinol.* 2000;53:509–515.
149. Donadieu J, Rolon MA, Thomas C, Brugieres L, Plantaz D, Emile JF, et al. Endocrine involvement in pediatric-onset Langerhans' cell histiocytosis: a population-based study. *J Pediatr.* 2004;144:344–350.
150. Maghnie M, Cosi G, Genovese E, Manca-Bitti ML, Cohen A, Zecca S, et al. Central diabetes insipidus in children and young adults. *N Engl J Med.* 2000;343:998–1007.
151. Donadieu J, Rolon MA, Pion I, Thomas C, Doz F, Barkaoui M, et al. Incidence of growth hormone deficiency in pediatric-onset Langerhans cell histiocytosis: efficacy and safety of growth hormone treatment. *J Clin Endocrinol Metab.* 2004;89:604–609.
152. Maghnie M, Arico M, Villa A, Genovese E, Beluffi G, Severi F. MR of the hypothalamic-pituitary axis in Langerhans cell histiocytosis. *AJNR.* 1992;13:1365–1371.
153. Kaltsas GA, Powles TB, Evanson J, Plowman PN, Drinkwater JE, Jenkins PJ, et al. Hypothalamo-pituitary abnormalities in adult patients with langerhans cell histiocytosis: clinical, endocrinological, and radiological features and response to treatment. *J Clin Endocrinol Metab.* 2000;85:1370–1376.
154. Lafferty AR, Chrousos GP. Pituitary tumors in children and adolescents. *J Clin Endocrinol Metab.* 1999;84:4317–4323.
155. Ezzat S, Asa SL. Mechanisms of disease: the pathogenesis of pituitary tumors. *Nat Clin Pract Endocrinol Metabol.* 2006;2:220–230.
156. Adan L, Trivin C, Sainte-Rose C, Zucker JM, Hartmann O, Brauner R. GH deficiency caused by cranial irradiation during childhood: factors and markers in young adults. *J Clin Endocrinol Metab.* 2001;86:5245–5251.
157. Josefson J, Listernick R, Fanguarso JR, Charrow J, Habiby R. Growth hormone excess in children with neurofibromatosis type

- 1-associated and sporadic optic pathway tumors. *J Pediatr*. 2011;158:433–436.
158. el-Mahdy W, Powell M. Transsphenoidal management of 28 symptomatic Rathke's cleft cysts, with special reference to visual and hormonal recovery. *Neurosurgery*. 1998;42:7–16. discussion 16–17.
 159. Rajab A, Kelberman D, de Castro SC, Biebermann H, Shaikh H, Pearce K, et al. Novel mutations in LHX3 are associated with hypopituitarism and sensorineural hearing loss. *Hum Mol Genet*. 2008;17:2150–2159.
 160. Netchine I, Sobrier ML, Krude H, Schnabel D, Maghnie M, Marcos E, et al. Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency. *Nat Genet*. 2000;25:182–186.
 161. Bhangoo AP, Hunter CS, Savage JJ, Anhalt H, Pavlakis S, Walvoord EC, et al. Clinical case seminar: a novel LHX3 mutation presenting as combined pituitary hormonal deficiency. *J Clin Endocrinol Metab*. 2006;91:747–753.
 162. Machinis K, Pantel J, Netchine I, Leger J, Camand OJ, Sobrier ML, et al. Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4. *Am J Hum Genet*. 2001;69:961–968.
 163. Wu W, Cogan JD, Pfaffle RW, Dasen JS, Frisch H, O'Connell SM, et al. Mutations in PROP1 cause familial combined pituitary hormone deficiency. *Nat Genet*. 1998;18:147–149.
 164. Turton JP, Mehta A, Raza J, Woods KS, Tiulpakov A, Cassar J, et al. Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clin Endocrinol*. 2005;63:10–18.
 165. Kelberman D, Dattani MT. Role of transcription factors in midline central nervous system and pituitary defects. *Endocr Dev*. 2009;14:67–82.
 166. Deladoey J, Stocker P, Mullis PE. Autosomal dominant GH deficiency due to an Arg183His GH-1 gene mutation: clinical and molecular evidence of impaired regulated GH secretion. *J Clin Endocrinol Metab*. 2001;86:3941–3947.
 167. Binder G, Nagel BH, Ranke MB, Mullis PE. Isolated GH deficiency (IGHD) type II: imaging of the pituitary gland by magnetic resonance reveals characteristic differences in comparison with severe IGHD of unknown origin. *Eur J Endocrinol*. 2002;147:755–760.
 168. Hess O, Hujeirat Y, Wajnrajch MP, et al. Variable phenotypes in familial isolated growth hormone deficiency caused by a G6664A mutation in the GH-1 gene. *J Clin Endocrinol Metab*. 2007;92:4387–4393.
 169. Binder G, Keller E, Mix M, et al. Isolated GH deficiency with dominant inheritance: new mutations, new insights. *J Clin Endocrinol Metab*. 2001;86:3877–3881.
 170. Mullis PE, Robinson IC, Salemi S, Eble A, Besson A, Vuissoz JM, et al. Isolated autosomal dominant growth hormone deficiency: an evolving pituitary deficit? A multicenter follow-up study. *J Clin Endocrinol Metab*. 2005;90:2089–2096.
 171. Salvatori R, Fan X, Phillips 3rd JA, Espigares-Martin R, Martin De Lara I, Freeman KL, et al. Three new mutations in the gene for the growth hormone (gh)-releasing hormone receptor in familial isolated gh deficiency type ib. *J Clin Endocrinol Metab*. 2001;86:273–279.
 172. Alatzoglou KS, Turton JP, Kelberman D, Clayton PE, Mehta A, Buchanan C, et al. Expanding the spectrum of mutations in GH1 and GHRHR: genetic screening in a large cohort of patients with congenital isolated growth hormone deficiency. *J Clin Endocrinol Metab*. 2009;94:3191–3199.
 173. Blum WF, Klammt J, Amselem S, Pfaffle HM, Legendre M, Sobrier ML, et al. Screening a large pediatric cohort with GH deficiency for mutations in genes regulating pituitary development and GH secretion: frequencies, phenotypes and growth outcomes. *EBioMedicine*. 2018;36:390–400.
 174. Abrahams JJ, Trefelner E, Boulware SD. Idiopathic growth hormone deficiency: MR findings in 35 patients. *AJNR*. 1991;12:155–160.
 175. Hamilton J, Blaser S, Daneman D. MR imaging in idiopathic growth hormone deficiency. *AJNR*. 1998;19:1609–1615.
 176. Kornreich L, Horev G, Lazar L, Schwarz M, Sulkes J, Pertzalan A. MR findings in growth hormone deficiency: correlation with severity of hypopituitarism. *AJNR*. 1998;19:1495–1499.
 177. Leger J, Danner S, Simon D, Garel C, Czernichow P. Do all patients with childhood-onset growth hormone deficiency (GHD) and ectopic neurohypophysis have persistent GHD in adulthood? *J Clin Endocrinol Metab*. 2005;90:650–656.
 178. Gelwane G, Garel C, Chevenne D, Armoogum P, Simon D, Czernichow P, Leger J. Subnormal serum insulin-like growth factor-I levels in young adults with childhood-onset nonacquired growth hormone (GH) deficiency who recover normal gh secretion may indicate less severe but persistent pituitary failure. *J Clin Endocrinol Metab*. 2007;92:3788–3795.
 179. Karavitaki N, Cudlip S, Adams CB, Wass JA. Craniopharyngiomas. *Endocr Rev*. 2006;27:371–397.
 180. Muller HL, Merchant TE, Puget S, Martinez-Barbera JP. New outlook on the diagnosis, treatment and follow-up of childhood-onset craniopharyngioma. *Nat Rev Endocrinol*. 2017;13:299–312.
 181. Bogusz A, Muller HL. Childhood-onset craniopharyngioma: latest insights into pathology, diagnostics, treatment, and follow-up. *Expert Rev Neurotherap*. 2018;18:793–806.
 182. Muller HL. Craniopharyngioma. *Endocr Rev*. 2014;35:513–543.
 183. Fahlbusch R, Honegger J, Paulus W, Huk W, Buchfelder M. Surgical treatment of craniopharyngiomas: experience with 168 patients. *J Neurosurg*. 1999;90:237–250.
 184. Sekine S, Shibata T, Kokubu A, Morishita Y, Noguchi M, Nakanishi Y, et al. Craniopharyngiomas of adamantinomatous type harbor beta-catenin gene mutations. *Am J Pathol*. 2002;161:1997–2001.
 185. Muller HL, Gebhardt U, Etavard-Gorris N, Korenke E, Warmuth-Metz M, Kolb R, et al. Prognosis and sequela in patients with childhood craniopharyngioma – results of HIT-ENDO and update on KRANIOPHARYNGEOM 2000. *Klin Padiatr*. 2004;216:343–348.
 186. Muller HL, Albanese A, Calaminus G, Hargrave D, Garre ML, Gebhardt U, et al. Consensus and perspectives on treatment strategies in childhood craniopharyngioma: results of a meeting of the Craniopharyngioma Study Group (SIOP), Genova, 2004. *J Pediatr Endocrinol Metab*. 2006;19(Suppl 1):453–454.
 187. Spoudeas HA, Saran F, Pizer B. A multimodality approach to the treatment of craniopharyngiomas avoiding hypothalamic morbidity: a UK perspective. *J Pediatr Endocrinol Metab*. 2006;19(Suppl 1):447–451.
 188. Ajithkumar T, Mazhari AL, Stickan-Verfurth M, Kramer PH, Fuentes CS, Lambert J, et al. Proton therapy for craniopharyngioma - an early report from a Single European Centre. *Clin Oncol (Royal College of Radiologists (Great Britain))*. 2018;30:307–316.
 189. Loche S, Bizzarri C, Maghnie M, Faedda A, Tziaila C, Autelli M, et al. Results of early reevaluation of growth hormone secretion in short children with apparent growth hormone deficiency. *J Pediatr*. 2002;140:445–449.
 190. Thomas M, Massa G, Maes M, Beckers D, Craen M, Francois I, et al. Growth hormone (GH) secretion in patients with childhood-onset GH deficiency: retesting after one year of therapy and at final height. *Horm Res*. 2003;59:7–15.
 191. Marin G, Domene HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler Jr GB. The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. *J Clin Endocrinol Metab*. 1994;79:537–541.
 192. Martinez AS, Domene HM, Ropelato MC, Jasper HG, Pennisi PA, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. *J Clin Endocrinol Metab*. 2000;85:4168–4172.
 193. Stanley TL, Levitsky LL, Grinspoon SK, Misra M. Effect of body mass index on peak growth hormone response to provocative testing in children with short stature. *J Clin Endocrinol Metab*. 2009;94:4875–4881.
 194. Takahashi Y, Chihara K. Clinical significance and molecular mechanisms of bioinactive growth hormone (review). *Int J Mol Med*. 1998;2:287–291.
 195. Besson A, Salemi S, Deladoey J, Vuissoz JM, Eble A, Bidlingmaier M, et al. Short stature caused by a biologically inactive mutant growth hormone (GH-C53S). *J Clin Endocrinol Metab*. 2005;90:2493–2499.
 196. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958-2003. *J Clin Endocrinol Metab*. 2004;89:1031–1044.

197. David A, Hwa V, Metherell LA, Netchine J, Camacho-Hubner C, Clark AJ, et al. Evidence for a continuum of genetic, phenotypic, and biochemical abnormalities in children with growth hormone insensitivity. *Endocr Rev.* 2011;32:472–497.
198. Schilbach K, Bidlingmaier M. Growth hormone binding protein - physiological and analytical aspects. *Best Pract Res Clin Endocrinol Metab.* 2015;29:671–683.
199. Storr HL, Chatterjee S, Metherell LA, Foley C, Rosenfeld RG, Backeljauw PF, et al. Non-classical growth hormone insensitivity (GHI): characterization of mild abnormalities of GH action. *Endocr Rev.* 2019;40(2):476–505.
200. Shapiro L, Chatterjee S, Ramadan DG, et al. Whole-exome sequencing gives additional benefits compared to candidate gene sequencing in the molecular diagnosis of children with growth hormone or IGF-1 insensitivity. *Eur J Endocrinol.* 2017;177:485–501.
201. Jorge AA, Arnhold IJ. Growth hormone receptor exon 3 isoforms and their implication in growth disorders and treatment. *Horm Res.* 2009;71(Suppl 2):55–63.
202. Renehan AG, Solomon M, Zwahlen M, Morjaria R, Whatmore A, Audi L, et al. Growth hormone receptor polymorphism and growth hormone therapy response in children: a Bayesian meta-analysis. *Am J Epidemiol.* 2012;175:867–877.
203. Zoledziewska M, Sidore C, Chiang CWK, Sanna S, Mulas A, Steri M, et al. Understanding Society Scientific G. Height-reducing variants and selection for short stature in Sardinia. *Nat Genet.* 2015;47:1352–1356.
204. Cohen AC, Nadeau KC, Tu W, Hwa V, Dionis K, Bezrodnik L, et al. Cutting edge: Decreased accumulation and regulatory function of CD4+ CD25(high) T cells in human STAT5b deficiency. *J Immunol.* 2006;177:2770–2774.
205. Scalco RC, Hwa V, Domene HM, Jasper HG, Belgorosky A, Marino R, et al. STAT5B mutations in heterozygous state have negative impact on height: another clue in human stature heritability. *Eur J Endocrinol.* 2015;173:291–296.
206. Klammt J, Neumann D, Gevers EF, Andrew SF, Schwartz ID, Rockstroh D, et al. Dominant-negative STAT5B mutations cause growth hormone insensitivity with short stature and mild immune dysregulation. *Nat Commun.* 2018;9:2105.
207. Gutierrez M, Scaglia P, Keselman A, Martucci L, Karabatas L, Domene S, et al. Partial growth hormone insensitivity and dysregulatory immune disease associated with de novo germline activating STAT3 mutations. *Mol Cell Endocrinol.* 2018;473:166–177.
208. Domené HM, Hwa V, Argente J, Wit JM, Camacho-Hübner C, Jasper HG, et al. Group IAC. Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Horm Res.* 2009;72:129–141.
209. Heath KE, Argente J, Barrios V, Pozo J, Diaz-Gonzalez F, Martos-Moreno GA, et al. Primary acid-labile subunit deficiency due to recessive IGFALS mutations results in postnatal growth deficit associated with low circulating insulin growth factor (IGF)-I, IGF binding protein-3 levels, and hyperinsulinemia. *J Clin Endocrinol Metab.* 2008;93:1616–1624.
210. Batey L, Moon JE, Yu Y, Wu B, Hirschhorn JN, Shen Y, Dauber A. A novel deletion of IGF1 in a patient with idiopathic short stature provides insight into IGF1 haploinsufficiency. *J Clin Endocrinol Metab.* 2014;99:E153–E159.
211. Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, et al. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J Clin Endocrinol Metab.* 2005;90:2855–2864.
212. Klammt J, Kiess W, Pfaffle R. IGF1R mutations as cause of SGA. *Best Pract Res Clin Endocrinol Metab.* 2011;25:191–206.
213. Ester WA, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CA, Govaerts LC, et al. Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. *J Clin Endocrinol Metab.* 2009;94:4717–4727.
214. Caliebe J, Broekman S, Boogaard M, Bosch CA, Ruivenkamp CA, Oostdijk W, et al. IGF1, IGF1R and SHOX mutation analysis in short children born small for gestational age and short children with normal birth size (idiopathic short stature). *Horm Res Paediatr.* 2012;77:250–260.
215. Rivkees SA, Bode HH, Crawford JD. Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. *N Engl J Med.* 1988;318:599–602.
216. Crocker MK, Kaplowitz P. Treatment of paediatric hyperthyroidism but not hypothyroidism has a significant effect on weight. *Clin Endocrinol.* 2010;73:752–759.
217. Lomenick JP, El-Sayyid M, Smith WJ. Effect of levo-thyroxine treatment on weight and body mass index in children with acquired hypothyroidism. *J Pediatr.* 2008;152:96–100.
218. Nukatsuka M, Fujioka A, Nakagawa F, Ohshimo H, Kitazato K, Fukushima M. The antitumor activity of DPD inhibitory-fluoropyrimidine (DIF) and non-DIF alone or in combination with paclitaxel against orthotopically implanted human breast cancer. *Gan to kagaku ryoho.* 2002;29:803–806.
219. Moran C, Habeb AM, Kahaly GJ, Kampmann C, Hughes M, Marek J, et al. Homozygous resistance to thyroid hormone beta: Can combined antithyroid drug and triiodothyroacetic acid treatment prevent cardiac failure? *J Endocr Soc.* 2017;1:1203–1212.
220. Bochukova E, Schoenmakers N, Agostini M, Schoenmakers E, Rajanayagam O, Keogh JM, et al. A mutation in the thyroid hormone receptor alpha gene. *N Engl J Med.* 2012;366:243–249.
221. Tyłki-Szymanska A, Acuna-Hidalgo R, Krajewska-Walasek M, Lecka-Ambroziak A, Steehouwer M, Gilissen C, et al. Thyroid hormone resistance syndrome due to mutations in the thyroid hormone receptor alpha gene (THRA). *J Med Genet.* 2015;52:312–316.
222. van Mullem AA, Chrysis D, Eythimiadou A, Chroni E, Tsatsoulis A, de Rijke YB, et al. Clinical phenotype of a new type of thyroid hormone resistance caused by a mutation of the TRalpha1 receptor: consequences of LT4 treatment. *J Clin Endocrinol Metab.* 2013;98:3029–3038.
223. Moran C, Schoenmakers N, Agostini M, Schoenmakers E, Offiah A, Kydd A, et al. An adult female with resistance to thyroid hormone mediated by defective thyroid hormone receptor alpha. *J Clin Endocrinol Metab.* 2013;98:4254–4261.
224. Baron J, Huang Z, Oerter KE, Bacher JD, Cutler Jr GB. Dexamethasone acts locally to inhibit longitudinal bone growth in rabbits. *Am J Physiol.* 1992;263:E489–E492.
225. Mosier Jr HD, Smith Jr FG, Schultz MA. Failure of catch-up growth after Cushing's syndrome in childhood. *Am J Dis Child (1960).* 1972;124:251–253.
226. Weise M, Flor A, Barnes KM, Cutler Jr GB, Baron J. Determinants of growth during gonadotropin-releasing hormone analog therapy for precocious puberty. *J Clin Endocrinol Metab.* 2004;89:103–107.
227. Nilsson O, Weise M, Landman EB, Meyers JL, Barnes KM, Baron J. Evidence that estrogen hastens epiphyseal fusion and cessation of longitudinal bone growth by irreversibly depleting the number of resting zone progenitor cells in female rabbits. *Endocrinology.* 2014;155:2892–2899.
228. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for R. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol.* 2004;31:390–392.
229. McErlane F, Carrasco R, Kearsley-Fleet L, Baildam EM, Wedderburn LR, Foster HE, et al. Growth patterns in early juvenile idiopathic arthritis: Results from the Childhood Arthritis Prospective Study (CAPS). *Semin Arthritis Rheum.* 2018;48:53–60.
230. Bechtold S, Ripperger P, Dalla Pozza R, Bonfig W, Hafner R, Michels H, Schwarz HP. Growth hormone increases final height in patients with juvenile idiopathic arthritis: data from a randomized controlled study. *J Clin Endocrinol Metab.* 2007;92:3013–3018.
231. Burbige EJ, Huang SH, Bayless TM. Clinical manifestations of Crohn's disease in children and adolescents. *Pediatrics.* 1975;55:866–871.
232. Mack DR, Langton C, Markowitz J, LeLeiko N, Griffiths A, Bousvaros A, Evans J, et al. Pediatric Inflammatory Bowel Disease Collaborative Research Group. Laboratory values for children with newly diagnosed inflammatory bowel disease. *Pediatrics.* 2007;119:1113–1119.
233. Sawczenko A, Ballinger AB, Savage MO, Sanderson IR. Clinical features affecting final adult height in patients with pediatric-onset Crohn's disease. *Pediatrics.* 2006;118:124–129.
234. Thomas AG, Holly JM, Taylor F, Miller V. Insulin like growth factor-I, insulin like growth factor binding protein-1, and insulin in childhood Crohn's disease. *Gut.* 1993;34:944–947.

235. Wong SC, Kumar P, Galloway PJ, Blair JC, Didi M, Dalzell AM, et al. A preliminary trial of the effect of recombinant human growth hormone on short-term linear growth and glucose homeostasis in children with Crohn's disease. *Clin Endocrinol (Oxf)*. 2011;74:599–607.
236. Denson LA, Kim MO, Bezold R, Carey R, Osuntokun B, Nylund C, et al. A randomized controlled trial of growth hormone in active pediatric Crohn disease. *J Pediatr Gastroenterol Nutr*. 2010;51:130–139.
237. Rees L. Growth hormone therapy in children with CKD after more than two decades of practice. *Pediatr Nephrol*. 2016;31:1421–1435.
238. Mahan JD, Warady BA, Consensus C. Assessment and treatment of short stature in pediatric patients with chronic kidney disease: a consensus statement. *Pediatr Nephrol*. 2006;21:917–930.
239. Ingulli EG, Mak RH. Growth in children with chronic kidney disease: role of nutrition, growth hormone, dialysis, and steroids. *Curr Opin Pediatr*. 2014;26:187–192.
240. Roelfsema V, Clark RG. The growth hormone and insulin-like growth factor axis: its manipulation for the benefit of growth disorders in renal failure. *J Am Soc Nephrol*. 2001;12:1297–1306.
241. Troib A, Landau D, Kachko L, Rabkin R, Segev Y. Epiphyseal growth plate growth hormone receptor signaling is decreased in chronic kidney disease-related growth retardation. *Kidney Int*. 2013;84:940–949.
242. Sharma AP, Sharma RK, Kapoor R, Kornecki A, Sural S, Filler G. Incomplete distal renal tubular acidosis affects growth in children. *Nephrol Dial Transplant*. 2007;22:2879–2885.
243. Laing CM, Toye AM, Capasso G, Unwin RJ. Renal tubular acidosis: developments in our understanding of the molecular basis. *Int J Biochem Cell Biol*. 2005;37:1151–1161.
244. Fuente R, Gil-Pena H, Claramunt-Taberner D, Hernandez O, Fernandez-Iglesias A, Alonso-Duran L, et al. X-linked hypophosphatemia and growth. *Rev Endocr Metab Disord*. 2017;18:107–115.
245. Ichikawa S, Sorenson AH, Imel EA, Friedman NE, Gertner JM, Econs MJ. Intronic deletions in the SLC34A3 gene cause hereditary hypophosphatemic rickets with hypercalciuria. *J Clin Endocrinol Metab*. 2006;91:4022–4027.
246. Liu ES, Zalutskaya A, Chae BT, Zhu ED, Gori F, Demay MB. Phosphate interacts with PTHrP to regulate endochondral bone formation. *Endocrinology*. 2014;155:3750–3756.
247. Costello CL, Gellatly M, Daniel J, Justo RN, Weir K. Growth restriction in infants and young children with congenital heart disease. *Congenit Heart Dis*. 2015;10:447–456.
248. Mitchell IM, Logan RW, Pollock JC, Jamieson MP. Nutritional status of children with congenital heart disease. *Br Heart J*. 1995;73:277–283.
249. Blasquez A, Clouzeau H, Fayon M, Mouton JB, Thambo JB, Enaud R, Lamireau T. Evaluation of nutritional status and support in children with congenital heart disease. *Eur J Clin Nutr*. 2016;70:528–531.
250. Powell GF, Brasel JA, Blizzard RM. Emotional deprivation and growth retardation simulating idiopathic hypopituitarism. I. Clinical evaluation of the syndrome. *N Engl J Med*. 1967;276:1271–1278.
251. Widdowson EM. Mental contentment and physical growth. *Lancet*. 1951;1:1316–1318.
252. Powell GF, Brasel JA, Raiti S, Blizzard RM. Emotional deprivation and growth retardation simulating idiopathic hypopituitarism. II. Endocrinologic evaluation of the syndrome. *N Engl J Med*. 1967;276:1279–1283.
253. Powell GF, Hopwood NJ, Barratt ES. Growth hormone studies before and during catch-up growth in a child with emotional deprivation and short stature. *J Clin Endocrinol Metab*. 1973;37:674–679.
254. Albanese A, Hamill G, Jones J, Skuse D, Matthews DR, Stanhope R. Reversibility of physiological growth hormone secretion in children with psychosocial dwarfism. *Clin Endocrinol (Oxf)*. 1994;40:687–692.
255. Munoz-Hoyos A, Molina-Carballo A, Augustin-Morales M, et al. Psychosocial dwarfism: psychopathological aspects and putative neuroendocrine markers. *Psychiatry Res*. 2011;188:96–101.
256. Johnson DE, Guthrie D, Smyke AT, Koga SF, Fox NA, Zeanah CH, Nelson CA. Growth and associations between auxology, caregiving environment, and cognition in socially deprived Romanian children randomized to foster vs ongoing institutional care. *Arch Pediatr Adolesc Med*. 2010;164:507–516. 3rd.
257. Johnson DE, Gunnar IV MR. Growth failure in institutionalized children. *Monogr Soc Res Child Dev*. 2011;76:92–126.
258. Wakeling, E.L., Brioude, F., Lokulo-Sodipe, O., O'Connell, S.M., Salem, J., Blik, J., et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. *Nat Rev Endocrinol*, 13, 105–124.
259. Rockstroh D, Pfaffle H, Le Duc D, Rossler F, Schlensog-Schuster F, Heiker JT, et al. A new p.(Ile66Serfs*93) IGF2 variant is associated with pre- and postnatal growth retardations. *Eur J Endocrinol*. 2018;180(1):K1–K13.
260. Abi Habib W, Brioude F, Edouard T, Bennett JT, Lienhardt-Roussie A, Tixier F, et al. Genetic disruption of the oncogenic HMG2-PLAG1-IGF2 pathway causes fetal growth restriction. *Genet Med*. 2018;20:250–258.
261. Hisado-Oliva A, Garre-Vazquez AI, Santaolalla-Caballero F, Belinchon A, Barreda-Bonis AC, Vasques GA, et al. Heterozygous NPR2 mutations cause disproportionate short stature, similar to Leri-Weill dyschondrosteosis. *J Clin Endocrinol Metab*. 2015;100:E1133–E1142.
262. Hisado-Oliva A, Ruzafa-Martin A, Sentchordi L, Funari MFA, Bezanilla-Lopez C, Alonso-Bernaldez M, et al. Mutations in C-natriuretic peptide (NPPC): a novel cause of autosomal dominant short stature. *Genet Med*. 2018;20:91–97.
263. Bocciardi R, Giorda R, Buttgerit J, Gimelli S, Divizia MT, Beri S, et al. Overexpression of the C-type natriuretic peptide (CNP) is associated with overgrowth and bone anomalies in an individual with balanced t(2;7) translocation. *Hum Mutat*. 2007;28:724–731.
264. Horton WA, Hall JG, Hecht JT. Achondroplasia. *Lancet*. 2007;370:162–172.
265. Bober MB, Bellus GA, Nikkel SM, Tiller GE. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Hypochondroplasia*. Seattle (WA): GeneReviews(R); 1993.
266. Toydemir RM, Brassington AE, Bayrak-Toydemir P, Krakowiak PA, Jorde LB, Whitby FG, et al. A novel mutation in FGFR3 causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet*. 2006;79:935–941.
267. Mantovani G, Bastepe M, Monk D, de Sanctis L, Thiele S, Usardi A, et al. Diagnosis and management of pseudohypoparathyroidism and related disorders: first international Consensus Statement. *Nat Rev Endocrinol*. 2018;14:476–500.
268. Haldeman-Englert CR, Hurst ACE, Levine MA. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Disorders of GNAS Inactivation*. Seattle (WA): GeneReviews(R); 1993.
269. Gao B, Guo J, She C, Shu A, Yang M, Tan Z, Yang X, Guo S, Feng G, He L. Mutations in IHH, encoding Indian hedgehog, cause brachydactyly type A-1. *Nat Genet*. 2001;28:386–388.
270. Gkourogianni A, Andrew M, Tyzinski L, Crocker M, Douglas J, Dunbar N, et al. Clinical Characterization of Patients With Autosomal Dominant Short Stature due to AggreCan Mutations. *J Clin Endocrinol Metab*. 2017;102:460–469.
271. Wit JM, Oostdijk W, Losekoot M, van Duyvenvoorde HA, Ruivenkamp CA, Kant SG. Mechanisms in endocrinology: novel genetic causes of short stature. *Eur J Endocrinol*. 2015;174:R145–R173.
272. Marchini A, Ogata T, Rappold GA. A Track Record on SHOX: From Basic Research to Complex Models and Therapy. *Endocr Rev*. 2016;37:417–448.
273. Blum WF, Ross JL, Zimmermann AG, Quigley CA, Child CJ, Kalifa G, et al. GH treatment to final height produces similar height gains in patients with SHOX deficiency and Turner syndrome: results of a multicenter trial. *J Clin Endocrinol Metab*. 2013;98:E1383–E1392.
274. Roberts AE, Allanson JE, Tartaglia M, Gelb BD. Noonan syndrome. *Lancet*. 2013;381:333–342.
275. Villani A, Greer MC, Kalish JM, Nakagawara A, Nathanson KL, Pajtler KW, et al. Recommendations for cancer surveillance in individuals with RASopathies and other rare genetic conditions with increased cancer risk. *Clin Cancer Res*. 2017;23:e83–e90.

276. Tajan M, Paccoud R, Branka S, Edouard T, Yart A. The rasopathy family: consequences of germline activation of the RAS/MAPK Pathway. *Endocr Rev.* 2018;39:676–700.
277. Malaquias AC, Brasil AS, Pereira AC, Arnhold IJ, Mendonca BB, Bertola DR, Jorge AA. Growth standards of patients with Noonan and Noonan-like syndromes with mutations in the RAS/MAPK pathway. *Am J Med Genet A.* 2012;158A:2700–2706.
278. Allanson JE, Roberts AE. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Noonan Syndrome*. Seattle (WA): GeneReviews(R); 1993.
279. Wang SR, Carmichael H, Andrew SF, Miller TC, Moon JE, Derr MA, et al. Large-scale pooled next-generation sequencing of 1077 genes to identify genetic causes of short stature. *J Clin Endocrinol Metab.* 2013;98:E1428–E1437.
280. Levine MA. An update on the clinical and molecular characteristics of pseudohypoparathyroidism. *Curr Opin Endocrinol Diabetes Obes.* 2012;19:443–451.
281. Holder-Espinasse M. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *3-M Syndrome*. Seattle (WA): GeneReviews(R); 1993.
282. Huber C, Munnich A, Cormier-Daire V. The 3M syndrome. *Best Pract Res Clin Endocrinol Metab.* 2011;25:143–151.
283. Dauber A, Stoler J, Hechter E, Safer J, Hirschhorn JN. Whole exome sequencing reveals a novel mutation in CUL7 in a patient with an undiagnosed growth disorder. *J Pediatr.* 2013;162:202–204. e201.
284. Woods CG. DNA repair disorders. *Arch Dis Child.* 1998;78:178–184.
285. Arora H, Chacon AH, Choudhary S, McLeod MP, Meshkov L, Nouri K, Izakovic J. Bloom syndrome. *Int J Dermatol.* 2014;53:798–802.
286. Petryk A, Kanakatti Shankar R, Giri N, Hollenberg AN, Rutter MM, Nathan B, et al. Endocrine disorders in Fanconi anemia: recommendations for screening and treatment. *J Clin Endocrinol Metab.* 2015;100:803–811.
287. Mehta PA, Tolar J. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Fanconi Anemia*. Seattle (WA): GeneReviews(R); 1993.
288. Sanz MM, German J, Cunniff C. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Bloom's Syndrome*. Seattle (WA): GeneReviews(R); 1993.
289. Alkuraya FS. Primordial dwarfism: an update. *Curr Opin Endocrinol Diabetes Obes.* 2015;22:55–64.
290. Klingseisen A, Jackson AP. Mechanisms and pathways of growth failure in primordial dwarfism. *Genes Dev.* 2011;25:2011–2024.
291. Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tumer Z, Monk D, et al. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenet.* 2015;7:123.
292. Eggermann K, Blied J, Brioude F, Algar E, Buiting K, Russo S, et al. EMQN best practice guidelines for the molecular genetic testing and reporting of chromosome 11p15 imprinting disorders: Silver-Russell and Beckwith-Wiedemann syndrome. *Eur J Hum Genet.* 2016;24:1377–1387.
293. Wakeling EL, Amero SA, Alders M, Blied J, Forsythe E, Kumar S, et al. Epigenotype-phenotype correlations in Silver-Russell syndrome. *J Med Genet.* 2010;47:760–768.
294. Burman P, Ritzen EM, Lindgren AC. Endocrine dysfunction in Prader-Willi syndrome: a review with special reference to GH. *Endocr Rev.* 2001;22:787–799.
295. Irizarry KA, Miller M, Freemark M, Haqq AM. Prader-Willi syndrome: genetics, metabolomics, hormonal function, and new approaches to therapy. *Adv Pediatr.* 2016;63:47–77.
296. Driscoll DJ, Miller JL, Schwartz S, Cassidy SB. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Prader-Willi Syndrome*. Seattle (WA): GeneReviews(R); 1993.
297. Kagami M, Nagasaki K, Kosaki R, Horikawa R, Naiki Y, Saitoh S, et al. Temple syndrome: comprehensive molecular and clinical findings in 32 Japanese patients. *Genet Med.* 2017;19:1356–1366.
298. Gomes LG, Cunha-Silva M, Crespo RP, Ramos CO, Montenegro LR, Canton A, et al. DLK1 is a novel link between reproduction and metabolism. *J Clin Endocrinol Metab.* 2018;104(6):2112–2120.
299. Jacobs PA. An opportune life: 50 years in human cytogenetics. *Annu Rev Genomics Hum Genet.* 2014;15:29–46.
300. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, et al. International Turner Syndrome Consensus G. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol.* 2017;177:G1–G70.
301. Homma TK, Krepischi ACV, Furuya TK, Honjo RS, Malaquias AC, Bertola DR, et al. Recurrent copy number variants associated with syndromic short stature of unknown cause. *Horm Res Paediatr.* 2018;89:13–21.
302. Wudy SA, Hagemann S, Dempfle A, Ringler G, Blum WF, Berthold LD, et al. Children with idiopathic short stature are poor eaters and have decreased body mass index. *Pediatrics.* 2005;116:e52–e57.
303. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab.* 2007;92:804–810.
304. Wollmann HA. Intrauterine growth restriction: definition and etiology. *Horm Res.* 1998;49(Suppl 2):1–6.
305. Khetarpal P, Das S, Panigrahi I, Munshi A. Primordial dwarfism: overview of clinical and genetic aspects. *Mol Genet Genomics.* 2016;291:1–15.
306. Mericq V, Martinez-Aguayo A, Uauy R, Iniguez G, Van der Steen M, Hokken-Koelega A. Long-term metabolic risk among children born premature or small for gestational age. *Nat Rev Endocrinol.* 2017;13:50–62.
307. Verkauskienė R, Petraitiene I, Albertsson Wikland K. Puberty in children born small for gestational age. *Horm Res Paediatr.* 2013;80:69–77.
308. Wakeling EL, Brioude F, Lokulo-Sodipe O, O'Connell SM, Salem J, Blied J, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. *Nat Rev Endocrinol.* 2017;13:105–124.
309. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. participants ISSCW. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab.* 2008;93:4210–4217.
310. Cianfarani S, Liguori A, Boemi S, Maghnie M, Iughetti L, Wasniewska M, et al. Inaccuracy of insulin-like growth factor (IGF) binding protein (IGFBP)-3 assessment in the diagnosis of growth hormone (GH) deficiency from childhood to young adulthood: association to low GH dependency of IGF-II and presence of circulating IGFBP-3 18-kilodalton fragment. *J Clin Endocrinol Metab.* 2005;90:6028–6034.
311. Prakash SK, Crenshaw ML, Bäckeljauw PF, Silberbach M, Scurlock C, Culin DD, et al. 45,X mosaicism in a population-based biobank: implications for Turner syndrome. *Genet Med.* 2018;21:1882–1883.
312. Sisley S, Trujillo MV, Khoury J, Bäckeljauw P. Low incidence of pathology detection and high cost of screening in the evaluation of asymptomatic short children. *J Pediatr.* 2013;163:1045–1051.
313. Blum WF, Alherbish A, Alsagheir A, El Awwa A, Kaplan W, Koledova E, Savage MO. The growth hormone-insulin-like growth factor-I axis in the diagnosis and treatment of growth disorders. *Endocr Connect.* 2018;7:R212–R222.
314. Ranke MB, Schweizer R, Elmlinger MW, et al. Relevance of IGF-I, IGFBP-3, and IGFBP-2 measurements during GH treatment of GH-deficient and non-GH-deficient children and adolescents. *Horm Res.* 2001;55:115–124.
315. Nunez SB, Municchi G, Barnes KM, Rose SR. Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 concentrations compared to stimulated and night growth hormone in the evaluation of short children—a clinical research center study. *J Clin Endocrinol Metab.* 1996;81:1927–1932.
316. Rosenfeld RG, Wilson DM, Lee PD, Hintz RL. Insulin-like growth factors I and II in evaluation of growth retardation. *J Pediatr.* 1986;109:428–433.
317. Hawkes CP, Grimberg A. Measuring growth hormone and insulin-like growth factor-I in infants: what is normal? *Pediatr Endocrinol Rev.* 2013;11:126–146.
318. Juul A, Kastrup KW, Pedersen SA, Skakkebaek NE. Growth hormone (GH) provocative retesting of 108 young adults with

- childhood-onset GH deficiency and the diagnostic value of insulin-like growth factor I (IGF-I) and IGF-binding protein-3. *J Clin Endocrinol Metab.* 1997;82:1195–1201.
319. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, et al. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. *Horm Res Paediatr.* 2016;86:361–397.
 320. Tan TY, Dillon OJ, Stark Z, Schofield D, Alam K, Shrestha R, et al. Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing for Ambulant Children With Suspected Monogenic Conditions. *JAMA Pediatr.* 2017;171:855–862.
 321. Durand C, Rappold GA. Height matters—from monogenic disorders to normal variation. *Nat Rev Endocrinol.* 2013;9:171–177.
 322. Murray PG, Clayton PE, Chernausek SD. A genetic approach to evaluation of short stature of undetermined cause. *Lancet Diabetes Endocrinol.* 2018;6(7):564–574.
 323. Hauer NN, Popp B, Schoeller E, Schuhmann S, Heath KE, Hisado-Oliva A, et al. Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature. *Genet Med.* 2018;20:630–638.
 324. Wang SR, Jacobsen CM, Carmichael H, Edmund AB, Robinson JW, Olney RC, et al. Heterozygous mutations in natriuretic peptide receptor-B (NPR2) gene as a cause of short stature. *Hum Mutat.* 2015;36:474–481.
 325. Dillon OJ, Lunke S, Stark Z, Yeung A, Thorne N, Melbourne Genomics Health A, et al. Exome sequencing has higher diagnostic yield compared to simulated disease-specific panels in children with suspected monogenic disorders. *Eur J Hum Genet.* 2018;26:644–651.
 326. Freire BL, Homma TK, Funari MFA, Lerario AM, Vasques GA, Malaquias AC, et al. Multigene sequencing analysis of children born small for gestational age with isolated short stature. *J Clin Endocrinol Metab.* 2019;104(6):2023–2030.
 327. Raben MS. Treatment of a pituitary dwarf with human growth hormone. *J Clin Endocrinol Metab.* 1958;18:901–903.
 328. Wilhelmi AE. Fractionation of human pituitary glands. *Can J Biochem Physiol.* 1961;39:1659–1668.
 329. Laron Z. The era of cadaveric pituitary extracted human growth hormone (1958-1985): biological and clinical aspects. *Pediatr Endocrinol Rev.* 2018;16:11–16.
 330. Grimberg A, Allen DB. Growth hormone treatment for growth hormone deficiency and idiopathic short stature: new guidelines shaped by the presence and absence of evidence. *Curr Opin Pediatr.* 2017;29:466–471.
 331. Christiansen JS, Backeljauw PF, Bidlingmaier M, Biller BM, Boguszewski MC, Casanueva FF, et al. Growth Hormone Research Society perspective on the development of long-acting growth hormone preparations. *Eur J Endocrinol.* 2016;174:C1–C8.
 332. Wit JM, Kamp GA, Rikken B. Spontaneous growth and response to growth hormone treatment in children with growth hormone deficiency and idiopathic short stature. *Pediatr Res.* 1996;39:295–302.
 333. Grimberg A, Cohen P. Optimizing growth hormone therapy in children. *Horm Res.* 1997;48(Suppl 5):11–15.
 334. Loche S, Carta L, Ibba A, Guzzetti C. Growth hormone treatment in non-growth hormone-deficient children. *Ann Pediatr Endocrinol Metab.* 2014;19:1–7.
 335. Albertsson-Wikland K, Aronson AS, Gustafsson J, Hagenas L, Ivarsson SA, Jonsson B, et al. Dose-dependent effect of growth hormone on final height in children with short stature without growth hormone deficiency. *J Clin Endocrinol Metab.* 2008;93:4342–4350.
 336. Gardner M, Scerbak T, Sandberg DE. Psychosocial aspects of short stature and rhGH treatment: implicit trends over 60+ years. *Pediatr Endocrinol Rev.* 2018;16:129–141.
 337. Raman S, Grimberg A, Waguespack SG, et al. Risk of neoplasia in pediatric patients receiving growth hormone therapy—a report from the Pediatric Endocrine Society Drug and Therapeutics Committee. *J Clin Endocrinol Metab.* 2015;100:2192–2203.
 338. Allen DB, Backeljauw P, Bidlingmaier M, Biller BM, Boguszewski M, Burman P, et al. GH safety workshop position paper: a critical appraisal of recombinant human GH therapy in children and adults. *Eur J Endocrinol.* 2016;174: P1–9.
 339. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, et al. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Translat Med.* 2011;3: 70ra13.
 340. Swerdlow AJ, Cooke R, Beckers D, Butler G, Carel JC, Cianfarani S, et al. Risk of meningioma in European patients treated with growth hormone in childhood: results from the SAGhE cohort. *J Clin Endocrinol Metab.* 2019;104:658–664.
 341. Patterson BC, Chen Y, Sklar CA, Neglia J, Yasui Y, Mertens A, et al. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the childhood cancer survivor study. *J Clin Endocrinol Metab.* 2014;99:2030–2037.
 342. Swerdlow AJ, Cooke R, Albertsson-Wikland K, Borgstrom B, Butler G, Cianfarani S, et al. Description of the SAGhE cohort: a large European study of mortality and cancer incidence risks after childhood treatment with recombinant growth hormone. *Horm Res Paediatr.* 2015;84:172–183.
 343. Swerdlow AJ, Cooke R, Beckers D, Borgstrom B, Butler G, Carel JC, et al. Cancer risks in patients treated with growth hormone in childhood: the SAGhE European cohort study. *J Clin Endocrinol Metab.* 2017;102:1661–1672.
 344. Grimberg A, Cousounis P, Cucchiara AJ, Lipman TH, Ginsburg KR. Parental concerns influencing decisions to seek medical care for a child's short stature. *Horm Res Paediatr.* 2015;84:338–348.
 345. Cutfield WS, Albert BB. Growth hormone treatment for idiopathic short stature. *Pediatr Endocrinol Rev.* 2018;16:113–122.
 346. Grimberg A, Huerta-Saenz L, Grundmeier R, et al. Gender bias in U.S. pediatric growth hormone treatment. *Sci Rep.* 2015;5:11099.
 347. Grimberg A, Lindberg A, Wajnrach M, Cucchiara AJ, Camacho-Hubner C. Racial/ethnic disparities in US pediatric growth hormone treatment. *Horm Res Paediatr.* 2018;90:102–108.
 348. Hawkes CP, Grimberg A. Insulin-like growth factor-I is a marker for the nutritional state. *Pediatr Endocrinol Rev.* 2015;13:499–511.
 349. Wit JM, Oostdijk W. Novel approaches to short stature therapy. *Best Pract Res Clin Endocrinol Metab.* 2015;29:353–366.
 350. Schroor EJ, van Weissenbruch MM, Knibbe P, Delemarre-van de Waal HA. The effect of prolonged administration of an anabolic steroid (oxandrolone) on growth in boys with constitutionally delayed growth and puberty. *Eur J Pediatr.* 1995;154:953–957.
 351. MacGillivray MH, Morishima A, Conte F, Grumbach M, Smith EP. Pediatric endocrinology update: an overview. The essential roles of estrogens in pubertal growth, epiphyseal fusion and bone turnover: lessons from mutations in the genes for aromatase and the estrogen receptor. *Horm Res.* 1998;49(Suppl 1):2–8.
 352. Hero M, Toiviainen-Salo S, Wickman S, Makitie O, Dunkel L. Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J Bone Miner Res.* 2010;25:1536–1543.
 353. Mauras N, Ross JL, Gagliardi P, Yu YM, Hossain J, Permuy J, et al. Randomized trial of aromatase inhibitors, growth hormone, or combination in pubertal boys with idiopathic, short stature. *J Clin Endocrinol Metab.* 2016;101:4984–4993.
 354. Shulman DI, Francis GL, Palmert MR, Eugster EA, Lawson Wilkins Pediatric Endocrine Society D, Therapeutics C. Use of aromatase inhibitors in children and adolescents with disorders of growth and adolescent development. *Pediatrics.* 2008;121: e975–e983.
 355. Kim SJ, Pierce W, Sabharwal S. The etiology of short stature affects the clinical outcome of lower limb lengthening using external fixation. A systematic review of 18 trials involving 547 patients. *Acta Orthop.* 2014;85:181–186.
 356. Schiedel F, Rodl R. Lower limb lengthening in patients with disproportionate short stature with achondroplasia: a systematic review of the last 20 years. *Disabil Rehabil.* 2012;34:982–987.
 357. Donaldson J, Aftab S, Bradish C. Achondroplasia and limb lengthening: Results in a UK cohort and review of the literature. *J Orthop.* 2015;12:31–34.
 358. Marques P, Korbonits M. Genetic aspects of pituitary adenomas. *Endocrinol Metab Clin North Am.* 2017;46:335–374.
 359. Klein KO, Barnes KM, Jones JV, Feuillan PP, Cutler Jr GB. Increased final height in precocious puberty after long-term treatment with LHRH agonists: the National Institutes of Health experience. *J Clin Endocrinol Metab.* 2001;86:4711–4716.

360. Marino R, Perez Garrido N, Costanzo M, Guercio G, Juanes M, Rocco C, et al. Five new cases of 46,XX aromatase deficiency: clinical follow-up from birth to puberty, a novel mutation, and a founder effect. *J Clin Endocrinol Metab.* 2015;100:E301–E307.
361. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med.* 1994;331:1056–1061.
362. Quaynor SD, Stradtman Jr EW, Kim HG, et al. Delayed puberty and estrogen resistance in a woman with estrogen receptor alpha variant. *N Engl J Med.* 2013;369:164–171.
363. Wong GW, Lai J, Cheng PS. Growth in childhood thyrotoxicosis. *Eur J Pediatr.* 1999;158:776–779.
364. Elias LL, Huebner A, Metherell LA, Canas A, Warne GL, Bitti ML, et al. Tall stature in familial glucocorticoid deficiency. *Clin Endocrinol.* 2000;53:423–430.
365. Geffner ME. The growth without growth hormone syndrome. *Endocrinol Metab Clin North Am.* 1996;25:649–663.
366. Phillip M, Moran O, Lazar L. Growth without growth hormone. *J Pediatr Endocrinol Metab.* 2002;15(Suppl 5):1267–1272.
367. Shtaf B, Dror N, Bar-Maisels M, Phillip M, Gat-Yablonski G. Growth without growth hormone: can growth and differentiation factor 5 be the mediator? *Growth Factors.* 2015;33:309–318.
368. Makrythanasis P, Temtamy S, Aglan MS, Otaify GA, Hamamy H, Antonarakis SE. A novel homozygous mutation in FGFR3 causes tall stature, severe lateral tibial deviation, scoliosis, hearing impairment, camptodactyly, and arachnodactyly. *Hum Mutat.* 2014;35:959–963.
369. Miura K, Kim OH, Lee HR, Namba N, Michigami T, Yoo WJ, Choi IH, Ozono K, Cho TJ. Overgrowth syndrome associated with a gain-of-function mutation of the natriuretic peptide receptor 2 (NPR2) gene. *Am J Med Genet A.* 2014;164A:156–163.
370. Dietz H. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Marfan syndrome.* Seattle (WA): GeneReviews(R); 1993.
371. Sacharow SJ, Picker JD, Levy HL. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency.* Seattle (WA): GeneReviews(R); 1993.
372. Fritzer-Szekeres M, Blom HJ, Boers GH, Szekeres T, Lubec B. Growth promotion by homocysteine but not by homocystic acid: a role for excessive growth in homocystinuria or proliferation in hyperhomocysteinemia? *Biochim Biophys Acta.* 1998;1407:1–6.
373. Kanakis GA, Nieschlag E. Klinefelter syndrome: more than hypogonadism. *Metabolism.* 2018;86:135–144.
374. Priolo M, Schanze D, Tatton-Brown K, Mulder PA, Tenorio J, Kooblall K, et al. Further delineation of Malan syndrome. *Hum Mutat.* 2018;39:1226–1237.
375. Brioude F, Kalish JM, Mussa A, Foster AC, Blik J, Ferrero GB, et al. Expert consensus document: clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol.* 2018;14:229–249.
376. Albuquerque EV, Scalco RC, Jorge AA. Management of endocrine disease: diagnostic and therapeutic approach of tall stature. *Eur J Endocrinol.* 2017;176:R339–R353.
377. Liu F, Hendriks AE, Ralf A, Boot AM, Benyi E, Sävendahl L, et al. Common DNA variants predict tall stature in Europeans. *Hum Genet.* 2014;133:587–597.
378. Tatton-Brown K, Cole TRP, Rahman N. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Sotos Syndrome.* Seattle (WA): GeneReviews(R); 1993.
379. Shuman C, Beckwith JB, Weksberg R. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Beckwith-Wiedemann Syndrome.* Seattle (WA): GeneReviews(R); 1993.
380. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter Syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev.* 2018;39:389–423.
381. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De Backer J, Devereux RB, et al. The revised Ghent nosology for the Marfan syndrome. *J Med Genet.* 2010;47:476–485.
382. Joss EE, Temperli R, Mullis PE. Adult height in constitutionally tall stature: accuracy of five different height prediction methods. *Arch Dis Child.* 1992;67:1357–1362.
383. Hannema SE, Savendahl L. The evaluation and management of tall stature. *Horm Res Paediatr.* 2016;85:347–352.
384. Hendriks AE, Laven JS, Valkenburg O, Fong SL, Fauser BC, de Ridder MA, et al. Fertility and ovarian function in high-dose estrogen-treated tall women. *J Clin Endocrinol Metab.* 2011;96:1098–1105.
385. Noordam C, van Daalen S, Otten BJ. Treatment of tall stature in boys with somatostatin analogue 201-995: effect on final height. *Eur J Endocrinol.* 2006;154:253–257.
386. Millar DS, Lewis MD, Horan M, Newsway V, Easter TE, Gregory JW, et al. Novel mutations of the growth hormone 1 (GH1) gene disclosed by modulation of the clinical selection criteria for individuals with short stature. *Hum Mutat.* 2003;21:424–440.
387. Goddard AD, Covello R, Luoh SM, Clackson T, Attie KM, Gesundheit N, et al. Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group. *N Engl J Med.* 1995;333:1093–1098.
388. Domene HM, Bengolea SV, Martinez AS, Ropelato MG, Pennisi P, Scaglia P, et al. Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. *N Engl J Med.* 2004;350:570–577.
389. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. Intrauterine Growth Retardation Study G. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med.* 2003;349:2211–2222.
390. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet.* 1997;16:54–63.
391. Nilsson O, Guo MH, Dunbar N, Popovic J, Flynn D, Jacobsen C, et al. Short stature, accelerated bone maturation, and early growth cessation due to heterozygous aggrecan mutations. *J Clin Endocrinol Metab.* 2014;99:E1510–E1518.
392. Vasques GA, Amano N, Docko AJ, Funari MF, Quedas EP, Nishi MY, et al. Heterozygous mutations in natriuretic peptide receptor-B (NPR2) gene as a cause of short stature in patients initially classified as idiopathic short stature. *J Clin Endocrinol Metab.* 2013;98:E1636–E1644.
393. Strande NT, Riggs ER, Buchanan AH, Ceyhan-Birsoy O, DiStefano M, Dwight SS, et al. Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the Clinical Genome Resource. *Am J Hum Genet.* 2017;100:895–906.
394. Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, et al. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. *J Clin Endocrinol Metab.* 1987;64(1):51–58.
395. Gregory LC, Dattani MT. The molecular basis of congenital hypopituitarism and related disorders. *J Clin Endocrinol Metab.* 2020;1105(6):dgz184. <https://doi.org/10.1210/clinem/dgz184>. PMID: 31702014.
396. Green H, Morikawa M, Nixon T. A dual effector theory of growth-hormone action. *Differentiation.* 1985;29(3):195–198. <https://doi.org/10.1111/j.1432-0436.1985.tb00316.x>.

12

Disorders of the Posterior Pituitary

Abhinash Srivatsa, Joseph A. Majzoub

CHAPTER OUTLINE

INTRODUCTION	357
PHYSIOLOGY OF OSMOTIC AND VOLUME REGULATION	357
Osmotic Sensor and Effector Pathways	357
Volume Sensor and Effector Pathways	364
APPROACH TO THE PATIENT: DIFFERENTIAL DIAGNOSIS OF DISORDERS OF WATER METABOLISM	368
Hyponatremia	368
Polyuria, Polydipsia, and Hypernatremia	368
SPECIFIC DISORDERS OF WATER METABOLISM	371
Hyponatremia With Normal Regulation of Vasopressin	371
Hyponatremia With Abnormal Regulation of Vasopressin	374
Other Causes of True and Factitious Hyponatremia	376
Hypernatremia With Inappropriate Decreased Vasopressin Secretion or Action	376
CONCLUDING REMARKS	383
References	383

INTRODUCTION

Maintenance of the tonicity of extracellular fluids within a very narrow range is crucial for proper cell function.¹ Extracellular osmolality regulates cell shape, as well as intracellular concentrations of ions and other osmolytes. Furthermore, proper extracellular ionic concentrations are necessary for the correct function of ion channels, action potentials, and other modes of intercellular communication. Extracellular fluid tonicity is regulated almost exclusively by the amount of water intake and excretion, whereas extracellular volume is regulated by the level of sodium chloride intake and excretion. In children and adults, normal blood tonicity is maintained over a 10-fold variation in water intake by a coordinated interaction among thirst, vasopressin, and renal systems. Dysfunction in any of these systems can result in abnormal regulation of blood osmolality, which if not properly recognized and treated may cause life-threatening dysfunction in neuronal and other cellular activities.

The posterior pituitary, or the neurohypophysis, secretes the nonapeptide hormones vasopressin (also termed *antidiuretic hormone [ADH]*) and oxytocin. Vasopressin controls water homeostasis, and oxytocin regulates smooth muscle contraction during parturition and lactation. Disorders of vasopressin secretion and action lead to clinically important derangements in water metabolism. In this chapter, the physiology of water and volume regulation is summarized, a symptom-based approach to the differential diagnosis of the diseases of water homeostasis is presented, and a review of the pathology and treatment of disorders involving these systems is provided.

PHYSIOLOGY OF OSMOTIC AND VOLUME REGULATION

The control of plasma tonicity and intravascular volume involves a complex integration of endocrine, neural, and paracrine pathways. Osmotic sensor and effector pathways control the regulation of vasopressin release and signal transduction, whereas volume homeostasis is determined largely through the action of the renin-angiotensin-aldosterone system, with contributions from both vasopressin and the natriuretic peptide family. An improved understanding of the anatomic structures and molecules involved has developed through recent molecular biologic and physiologic studies.

Osmotic Sensor and Effector Pathways

Vasopressin and Oxytocin Biochemistry

Vasopressin and oxytocin are evolutionarily-related peptides (paralogs), having arisen from gene duplication of a phylogenetically common molecule approximately 450 million years ago.² Both peptides consist of a 6-amino-acid disulfide ring, plus a 3-amino-acid tail, with amidation of the carboxy terminus.³ As early as 1895, a potent biologic principle—consisting of vascular pressor activity, “birth quickening,” and milk secretory effects—was recognized in neurohypophyseal extracts.⁴ The sequences of the individual peptides with pressor and antidiuretic capacity (vasopressin) and oxytocic capacity were determined by du Vigneaud and colleagues during the mid-1950s,³ culminating in the synthesis of each hormone in its biologically active form.^{5,6} In most mammals, vasopressin and oxytocin differ in only two amino acids—one substitution within the ring and one within the tail structure (Fig. 12.1). Exploration of the structure-function relationship of specific amino acids, within both vasopressin and oxytocin, has allowed characterization of molecules with important clinical use. Although the vasopressor activity was markedly diminished by the replacement of l-arginine with d-arginine at position 8 of the vasopressin molecule, the duration of action was prolonged, and the antidiuretic action enhanced by the amino-terminal deamidation, creating the analogue desmopressin (desamino-d-arginine vasopressin [dDAVP]) (see Fig. 12.1).⁷ Whereas the antidiuretic:pressor ratio for vasopressin is 1:1, this ratio for dDAVP is between 2000:1 and 3000:1. This difference is almost entirely caused by the differential binding of dDAVP to the V2 and V1 receptors (2054:1). dDAVP, with an antidiuretic potency between 2 times and 3 times that of its parent vasopressin,⁸ is now routinely used in clinical practice.

The association of vasopressin and oxytocin with specific proteins, the neurophysins, while stored in the neurohypophysis was apparent as early as 1900.⁹ Subsequent isolation and characterization of the neurophysins revealed two distinct forms, one type exclusively associated with vasopressin and the other exclusively associated with oxytocin.^{10,11} Both are single-polypeptide chains of molecular weight 10,000 daltons. Despite extensive biophysical characterization, including

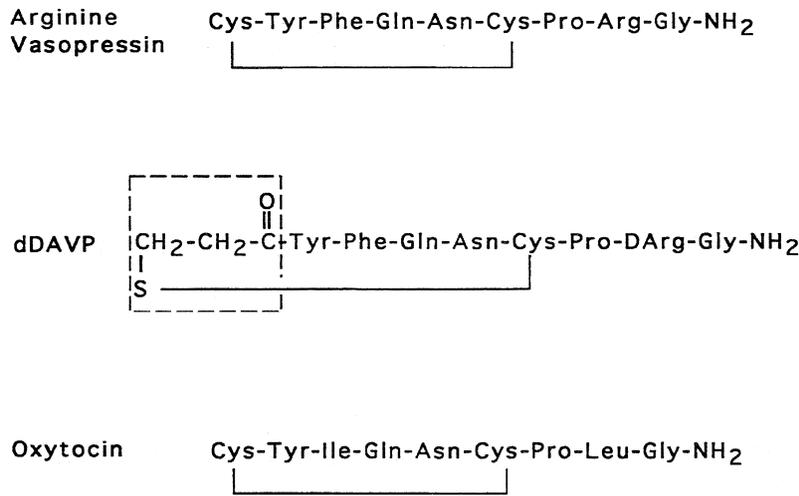


Fig. 12.1 Structures of vasopressin, desamino-d-arginine vasopressin (dDAVP), and oxytocin. In dDAVP, the deamidated cysteine is enclosed in the box.

crystallography of the oxytocin-neurophysin complex,^{12,13} the biologic function of the neurophysins remains unknown. Possible roles for the neurophysins include hormone stabilization against degradation during intracellular storage, more efficient packaging within secretory granules, enhancement of posttranslational processing by the proenzyme convertases, and hormone transport in blood. Recently, copeptin, a segment of vasopressin-associated neurophysin, has been developed as an indicator of vasopressin secretion.¹⁴

The common origin of vasopressin and its neurophysin from a single larger precursor was first proposed by Sachs and colleagues,¹⁵ who showed increased incorporation of 35S cysteine, infused into canine third ventricle, into vasopressin isolated from the hypothalamus compared with vasopressin isolated from the posterior pituitary. Isolation of the larger precursor from the hypothalamus, followed by trypsin

digestion, produced fragments of size similar to that of vasopressin and its neurophysin, with vasopressin immunoreactivity in the 1000-dalton component.¹⁷

Since 1990, molecular genetic analyses have enhanced the understanding of the synthesis, the processing, and the evolution of the vasopressin and oxytocin preprohormones. All mammalian vasopressin and oxytocin genes each consist of three exons (Fig. 12.2).¹⁸ The first exon encodes the 19-amino-acid signal peptide, followed by vasopressin or oxytocin nonapeptides. This is followed by a 3-amino-acid protease cleavage site, leading into the first nine amino acids of neurophysin II (for vasopressin) or neurophysin I (for oxytocin). After interruption of the coding region by an intron, exon 2 continues with neurophysin coding sequences. The third exon completes the sequence of the neurophysin and, for vasopressin only, is followed by coding information for an

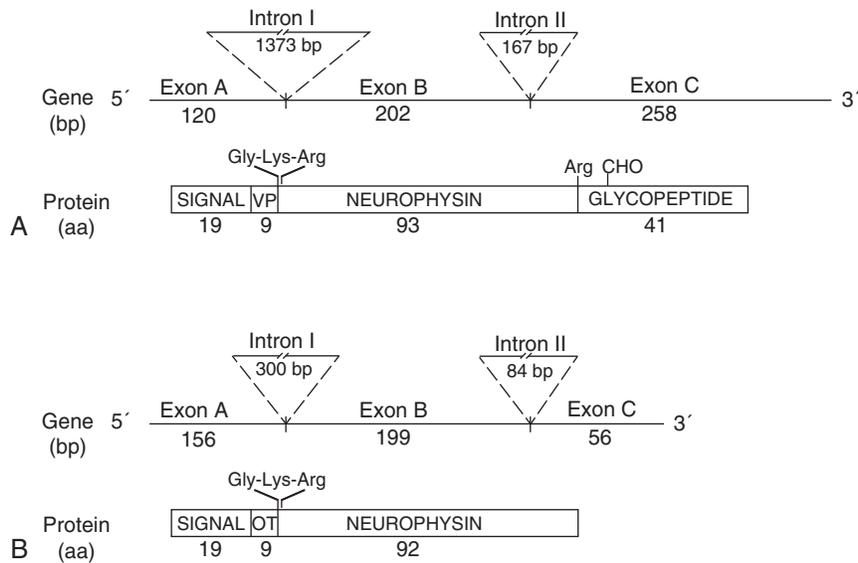


Fig. 12.2 Structure of the human genes and peptide products of vasopressin (VP) (A) and oxytocin (OT) (B). Shown are the sizes of exons and intron, in nucleotide base pairs (bp) and peptide products in amino acids (aa). Depicted are the amidation-dibasic cleavage signal (Gly-Lys-Arg) at the carboxy terminus of vasopressin and oxytocin, and the monobasic cleavage signal at the end of neurophysin. CHO, Carbohydrate; signal, signal peptide.

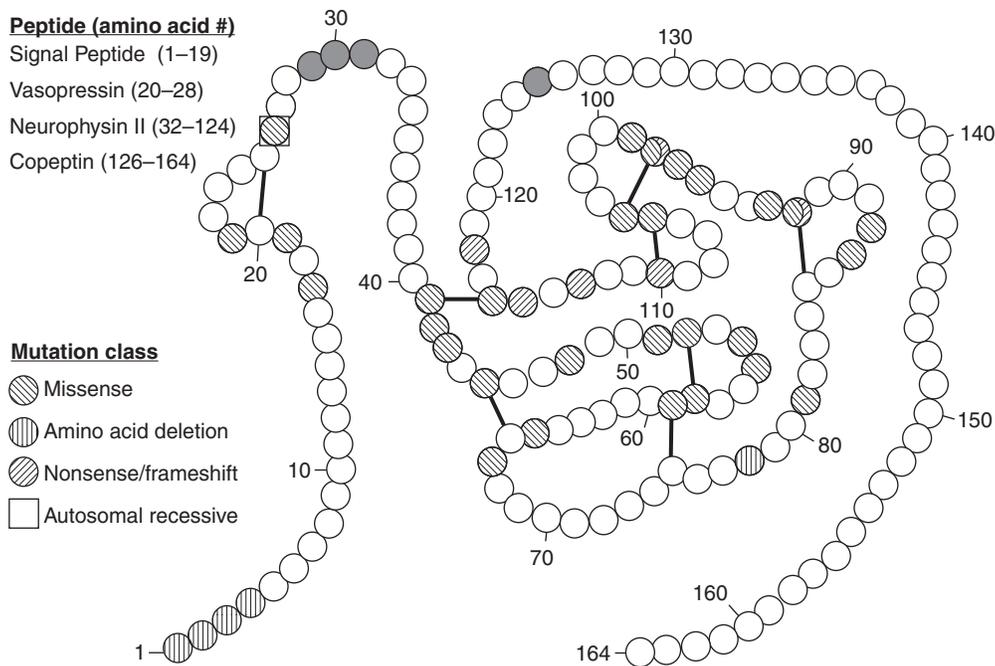


Fig. 12.3 Structure of preprovasopressin peptide and most frequent AVP mutations in central diabetes insipidus. The 164-amino-acid preprovasopressin peptide consists of signal peptide, vasopressin, neurophysin II, and copeptin. Vasopressin and neurophysin II are separated by three basic residues (gray), which serve as peptide cleavage and amidation sites. The 16 cysteines in preprovasopressin are connected by eight putative disulfide bridges, many of which are mutated in autosomal dominant diabetes insipidus. Amino acid mutations are classified as missense, deletion, or nonsense/frameshift. Most mutations are inherited with an autosomal dominant pattern, except at amino acid 26, which has an autosomal recessive pattern.

additional 39-amino-acid glycopeptide (copeptin) whose function is unclear. Preprovasopressin contains 16 cysteines, which likely participate in eight disulfide bridges that determine the tertiary structure of the protein (Fig. 12.3). One cysteine pair is present in vasopressin peptide, whereas the rest are in neurophysin.

In all mammalian species analyzed thus far, oxytocin and vasopressin genes are adjacent in chromosomal location (chromosome 20 in the human 19) and linked tail to tail, in opposite transcriptional orientation. In the human, they are separated by only 12 kb.¹⁹ This likely explains their origin from the ancient duplication of a common ancestral gene.²⁰ Whether this adjacent linkage is of regulatory significance is unclear.

Expression of vasopressin and oxytocin genes occurs in the hypothalamic paraventricular and supraoptic nuclei.¹ The magnocellular components of each of these nuclei are the primary neuronal populations involved in water balance, with vasopressin synthesized in these areas carried by means of axonal transport to the posterior pituitary, its primary site of storage and release into the systemic circulation (Fig. 12.4). The bilaterally paired hypothalamic paraventricular and supraoptic nuclei are separated from one another by relatively large distances (approximately 1 cm). Their axons course caudally, converge at the infundibulum, and terminate at different levels within the pituitary stalk and the posterior pituitary gland (see Fig. 12.4). Vasopressin is also synthesized in distinct parvocellular neurons of the paraventricular nucleus, where it has a role in modulation of hypothalamic-pituitary-adrenal axis activity. In this site, vasopressin is colocalized in cells that synthesize corticotropin-releasing hormone,^{21,22} and both are

secreted at the median eminence and carried through the hypothalamic-hypophyseal portal capillary system to the anterior pituitary, where together they act as the major regulators of adrenocorticotrophic hormone synthesis and release.²³ Vasopressin is also present in the hypothalamic suprachiasmatic nucleus, the circadian pacemaker of the body, where its function is unknown.

Regulation of Vasopressin Secretion and Thirst

Osmotic Regulation. The rate of secretion of vasopressin from the paraventricular and supraoptic nuclei is influenced by several physiologic variables, including plasma osmolality and intravascular volume, as well as nausea and a number of pharmacologic agents. The major osmotically active constituents of blood are sodium, chloride, and glucose (with insulin deficiency). Normal blood osmolality ranges between 280 and 290 mOsm/kg H₂O.

The work of Verney²⁴ first demonstrated the relationship of increased vasopressin release in response to increasing plasma osmolality, as altered by infusion of sodium chloride or sucrose. At that time, it was postulated that there existed intracranial sensors sensitive to changes in plasma osmolality. Multiple researchers have subsequently confirmed that plasma vasopressin concentration increases in response to increasing plasma tonicity, with the location of the osmosensor, likely to be within the circumventricular organ, neuronal nuclei surrounding the third ventricle, which lacks a blood-brain barrier.²⁵ The organ vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO), areas of the preoptic hypothalamus outside the blood-brain barrier, are likely sites

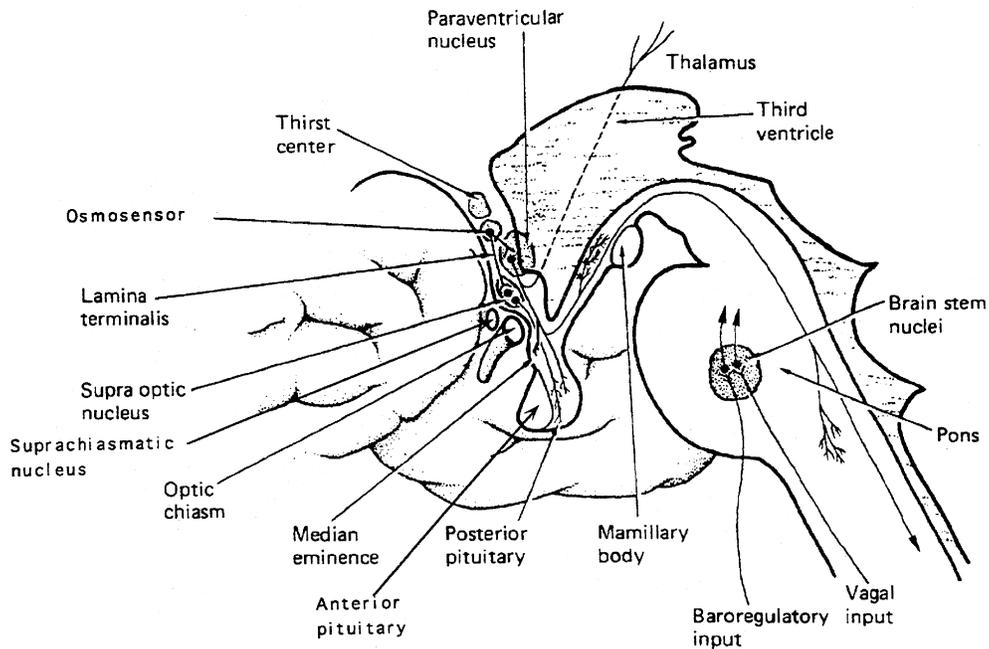


Fig. 12.4 Vasopressin cells in the hypothalamus. Diagram of vasopressin cell bodies in the supraoptic, paraventricular, and suprachiasmatic hypothalamic nuclei, and axonal termination in the posterior pituitary and median eminence. Because vasopressin axons terminate at different levels in the pituitary stalk and posterior pituitary, the amount of permanent cell loss, following neurosurgical insult, is determined by the highest level of damage, which will dictate the degree of vasopressin axon transection and retrograde neuronal degeneration. (Modified with permission from Baylis, P.H. (1989). Vasopressin and its neurophysin. In: Degroot, L.D. (ed). Endocrinology, 2nd ed. WB Saunders, Philadelphia, p. 213.)

of both osmosensing, because lesions of the OVLT result in impaired vasopressin secretion and hypernatremia.^{26,27} Also the site of action of angiotensin II, infused intracerebrally or peripherally, to produce vasopressin secretion and antidiuresis resides within the OVLT.^{28–30}

The pattern of secretion of vasopressin into blood has been characterized extensively in normal individuals and in those with abnormalities in water homeostasis. Normally, at a serum osmolality of less than 280 mOsm/kg, plasma vasopressin concentration is at or below 1 pg/mL, the lower limit of detection of most radioimmunoassays.³¹ Above 283 mOsm/kg—the normal threshold for vasopressin release—plasma vasopressin concentration increases in proportion to plasma osmolality, up to a maximum concentration of about 20 pg/mL at a blood osmolality of approximately 320 mOsm/kg (Fig. 12.5). The osmosensor can detect as little as a 1% change in blood osmolality. Plasma concentrations in excess of 5 pg/mL are also found with nausea, hypotension, hypovolemia, and insulin-induced hypoglycemia, but further increments in urine concentration do not occur, because peak antidiuretic effect is achieved at 5 pg/mL. The rate of increase of plasma vasopressin concentration, and thus the sensitivity of the osmosensor, exhibits substantial (as much as 10-fold) interindividual variation as plasma osmolality increases.³² The set-point for vasopressin secretion varies in a single individual, in relation to changes in volume status and hormonal environment (e.g., pregnancy³³) or glucocorticoid status.^{34,35} After the seventh week of gestation, osmotic thresholds for both vasopressin release and thirst are reduced by approximately 10 mOsm/kg (see Fig. 12.5), such that normal blood osmolality, during pregnancy, is approximately 273 mOsm/kg (serum sodium 135 mEq/L).^{33,35} Similarly, thresholds for vasopressin release and thirst during the luteal phase of the menstrual cycle are approximately 5 mOsm/kg lower than those in the follicular phase.^{36,37} Human chorionic gonadotropin, during pregnancy,³⁸ and luteinizing hormone, during the second half

of the menstrual cycle, may contribute to these changes in osmotic thresholds.

The sensation of thirst, a more integrated cortical activity, is determined by other anatomically distinct hypothalamic neurons, with afferents involving the ventromedial nucleus,³⁹ and subfornical organ.⁴⁰ The activation of the thirst mechanism is probably mediated by angiotensin II.⁴¹ Whether the osmosensor for thirst and vasopressin release are the same is not certain, although this is suggested by lesions in the anteroventral

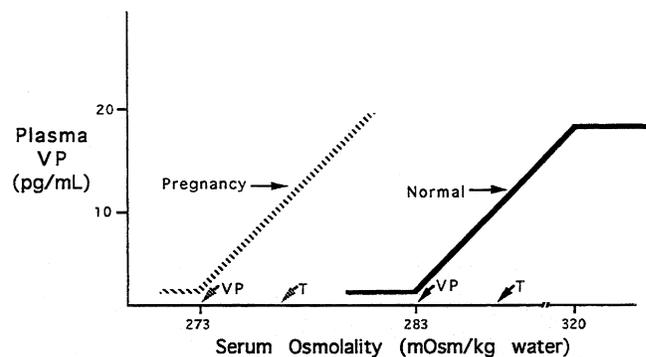


Fig. 12.5 Osmotic thresholds for vasopressin and thirst. The threshold for vasopressin release is below that for thirst. In nonpregnant persons there is linear increase in vasopressin (VP) release up to a serum osmolality of 320 mOsm/kg, after which no further increase occurs. In pregnancy, there is a decreased threshold for vasopressin release and thirst sensation, with no change in the sensitivity (slope) of the vasopressin-osmolality relationship. Vasopressin secretion in pregnancy presumably also plateaus at some level of hyperosmolality, although this has not been studied. Normal nonpregnant persons, solid line and arrows; pregnant women, dashed line and arrows. T, Threshold for thirst; VP, threshold for vasopressin secretion.

region of the third ventricle that abolish both thirst sensation and vasopressin release.⁴² It makes physiologic sense that the threshold for thirst (293 mOsm/kg) is approximately 10 mOsm/kg higher than that for vasopressin release⁴³ (see Fig. 12.5). Otherwise, during the development of hyperosmolality, the initial activation of thirst and water ingestion would result in polyuria, without activation of vasopressin release, causing a persistent diuretic state. Immediately after water ingestion, before a change in blood osmolality or volume, vasopressin concentration falls and thirst ceases.⁴⁴ The degree of suppression is directly related to the coldness⁴⁵ and volume⁴⁶ of the ingested fluid. Water that bypasses the pharynx does not have this effect.⁴⁷ The effect is probably mediated by chemoreceptors present in the oropharynx that project to the subfornical organ, which results in decreased thirst and vasopressin release.^{40,48,49} This "presystemic" regulation of thirst and vasopressin secretion, before osmolality changes, guards against both the rapid overdrinking of fluids after prior intense thirst, and reversal of anti-diuresis with the onset of water ingestion.

As noted earlier, water balance is regulated in two ways: (1) vasopressin secretion stimulates water reabsorption by the kidney, thereby reducing future water loss, and (2) thirst stimulates water ingestion, thereby restoring previous water loss. Ideally, these two systems work in parallel to efficiently regulate extracellular fluid tonicity (Fig. 12.6); however, each system by itself can maintain plasma osmolality in the near-normal range. For example, in the absence of vasopressin secretion but with free access to water, thirst drives water ingestion up to the 5 to 10 L/m² of urine output seen with vasopressin deficiency. Conversely, an intact vasopressin secretory system can compensate for some degree of disordered thirst regulation. When both vasopressin secretion and thirst are compromised, however, by either disease or iatrogenic means, there is great risk of the occurrence of life-threatening abnormalities in plasma osmolality.⁵⁰

Nonosmotic Regulation. Separate from osmotic regulation, vasopressin has been shown to be secreted in response to alterations in intravascular volume. Afferent baroreceptor pathways arising from the right and left atria and the aortic arch (carotid sinus) are stimulated by increasing intravascular

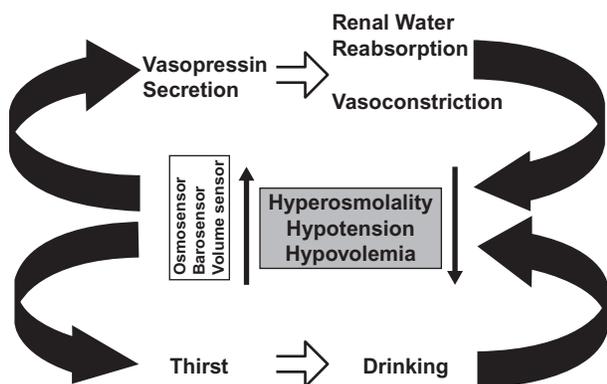


Fig. 12.6 Regulation of vasopressin secretion and serum osmolality. Hyperosmolality, hypovolemia, or hypotension are sensed by osmosensors, volume sensors, or barosensors, respectively. These stimulate both vasopressin secretion and thirst. Vasopressin, acting on the kidney causes increased reabsorption of water (antidiuresis) and acting on the vasculature, causes vasoconstriction. Thirst causes increased water ingestion. The results of these dual negative feedback loops cause a reduction in hyperosmolality or hypotension/hypovolemia. Additional stimuli for vasopressin secretion include nausea, hypoglycemia, and pain.

volume and stretch of vessel walls, and they send signals through the vagus and glossopharyngeal nerves, respectively, to the brain stem nucleus tractus solitarius. Noradrenergic fibers from the nucleus tractus solitarius synapse on the hypothalamic paraventricular nucleus and the supraoptic nucleus and, on stimulation, inhibit vasopressin secretion.⁵¹

The pattern of vasopressin secretion in response to volume, as opposed to osmotic stimuli, is markedly different (Fig. 12.7). Although minor changes in plasma osmolality

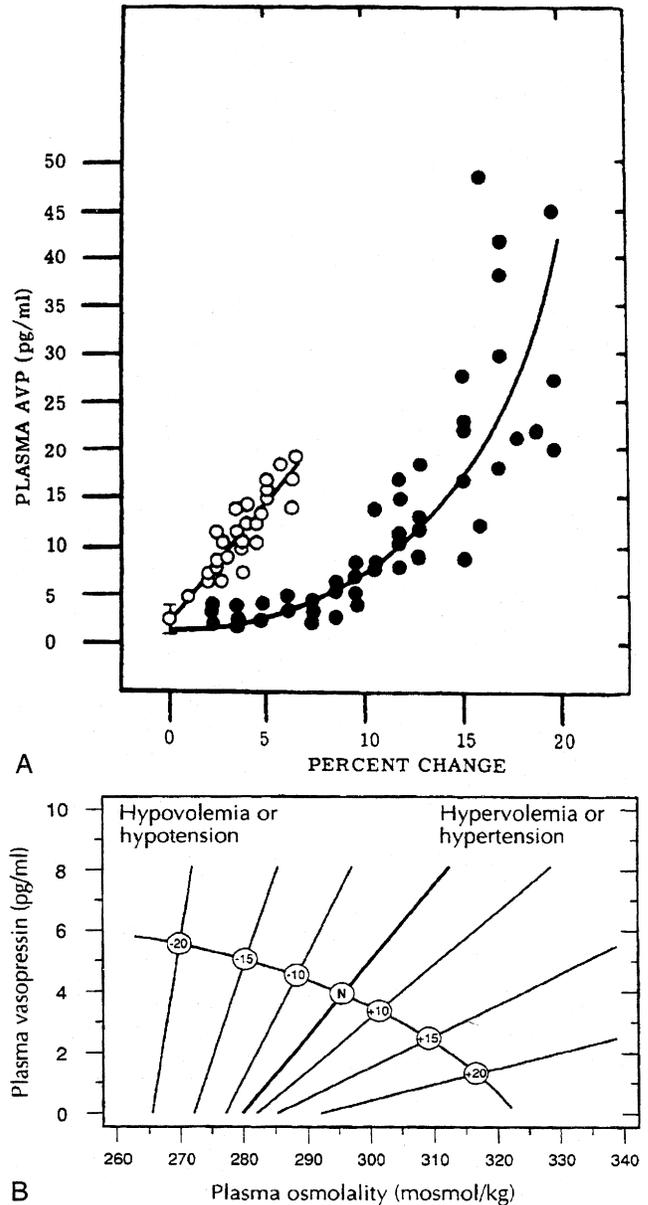


Fig. 12.7 Relationships between osmotic and nonosmotic stimuli for vasopressin release. A, Relationship of plasma vasopressin (AVP) concentration to the percent increase in blood osmolality (open circles) or decrease in blood volume (closed circles). B, Alteration of sensitivity of osmotic stimulation of vasopressin secretion by volume or pressure stimuli. (From Shaikh, Z.H.A., Taylor, H.C., Maroo, P.V., Llerena, L.A. (2000). Syndrome of inappropriate antidiuretic hormone secretion associated with lisinopril. *Ann Pharmacother*, 34, 176–179; van Laar, T., Lammers, G.-J., Roos, R.A.C., Gerritsen, J.J., Meinders, A.E. (1998). Antiparkinsonian drugs causing inappropriate antidiuretic hormone secretion. *Mov Disord*, 13, 176–178. With permission.)

above 280 mOsm/kg evoke linear increases in plasma vasopressin, substantial alteration in intravascular volume is required for alteration in vasopressin output.⁵² No change in vasopressin secretion is seen until blood volume decreases by approximately 8%. With intravascular volume deficits exceeding 8%, vasopressin concentration increases exponentially. Furthermore, osmotic and hemodynamic stimuli can interact in a mutually synergistic fashion, so that the response to either stimulus may be enhanced by the concomitant presence of the other (see Fig. 12.7). When blood volume or blood pressure decreases by approximately 25%, vasopressin concentrations are evident of 20- to 30-fold above normal, and vastly exceeding those required for maximal antidiuresis. Mice with deletion of the V1a vasopressin receptor gene, *Avpr1*, indicate that vasopressin acting through this receptor is required to maintain normal blood pressure, even at low concentrations of blood vasopressin.⁵³

Nausea, as evoked by apomorphine, motion sickness, or vasovagal reactions, is a very potent stimulus for vasopressin secretion. This effect is likely mediated by afferents from the area postrema of the brain stem and may result in vasopressin concentrations two to three orders of magnitude above basal levels. Nicotine is also a strong stimulus for vasopressin release.⁵⁴ These pathways probably do not involve osmotic or hemodynamic sensor systems, because blockade of the emetic stimulus, with dopamine or opioid antagonists, does not alter the vasopressin response to hypernatremia or hypovolemia.

Vasopressin secretion is inhibited by glucocorticoids; because of this, loss of negative regulation of vasopressin secretion occurs in the setting of primary or secondary glucocorticoid insufficiency.⁵⁵ The insertion of aquaporin-2 (AQP2) in the renal luminal membrane is stimulated by nitric oxide.⁵⁶ Glucocorticoids inhibit nitric oxide synthase,⁵⁷ thereby providing a mechanism by which they may facilitate free water clearance, by inhibiting nitric oxide-induced insertion of AQP2 into the luminal membrane. The effects of cortisol loss of both enhancing hypothalamic vasopressin production and directly impairing free water excretion are important considerations in the evaluation of the patient with hyponatremia, as is subsequently discussed.

Vasopressin Metabolism

Once in the circulation, vasopressin has a half-life of only 5 to 10 minutes, owing to its rapid degradation by a cysteine aminoterminal peptidase called *vasopressinase*. A synthetic analogue of vasopressin, desmopressin, is insensitive to aminoterminal degradation and thus has a much longer half-life of 8 to 24 hours. During pregnancy, the placenta secretes increased amounts of this vasopressinase,^{58,59} resulting in a 4-fold increase in the metabolic clearance rate of vasopressin.⁶⁰ Normal women compensate with an increase in vasopressin secretion, but women with preexisting deficits in vasopressin secretion or action,⁶¹ or those with increased concentrations of placental vasopressinase, associated with liver dysfunction⁶² or multiple gestations,⁶³ may develop diabetes insipidus in the last trimester, which resolves in the immediate postpartum period.⁶⁴ As expected, this form of diabetes insipidus responds to treatment with desmopressin but not with vasopressin.^{59,65,66}

Sites of Vasopressin Action

Vasopressin Receptors. Vasopressin, released from the posterior pituitary and the median eminence, affects the function of several tissue types by binding to members of a family of G protein-coupled cell surface receptors, which subsequently transduce ligand binding into alterations of intracellular

second messenger pathways. Biochemical and cell biologic studies have defined three receptor types, designated V1, V2, and V3 (or V1b). The major sites of V1 receptor expression are on vascular smooth muscle^{53,67} and hepatocytes,⁶⁸⁻⁷¹ where receptor activation results in vasoconstriction^{72,73} and glycogenolysis,⁷⁴ respectively. The latter activity may be augmented by stimulation of glucagon secretion from the pancreas.⁷⁴ The V1 receptor on platelets also stimulates platelet aggregation.⁷⁵ V1 receptor activation mobilizes intracellular calcium stores through phosphatidylinositol hydrolysis.^{73,76} Despite its initial characterization as a powerful pressor agent, the concentration of vasopressin needed to significantly increase blood pressure is several-fold higher than that required for maximal antidiuresis,⁷⁷ although substantial vasoconstriction in renal and splanchnic vasculature can occur at lower concentrations.⁷⁸ The cloning of the V1 receptor^{67,68,70} has greatly elucidated the relationship of the vasopressin (and oxytocin^{79,80}) receptors and, through sensitive in situ hybridization analysis, has further localized V1 expression to the liver and the vasculature of the renal medulla, as well as to many sites within the brain, including the hippocampus, the amygdala, the hypothalamus, and the brain stem.^{69,71} Compared with their normal counterparts, mice genetically modified to be deficient in the V1 receptor (V1a KO) have been found to have insulin resistance, increased hepatic glucose production, decreased hepatic glycogen content, and decreased aldosterone secretion, despite a lower plasma volume, lower basal blood pressure, greater degree of lipolysis, and impaired nuclear transport of the renal tubular mineralocorticoid receptor.⁸¹ The V3 (or V1b) receptor is present on corticotrophs in the anterior pituitary⁸² and acts through the phosphatidylinositol pathway⁸³ to increase adrenocorticotropic hormone secretion.⁸⁴ Its binding profile for vasopressin analogues resembles more closely that of the V1 than the V2 receptor. The structure of this receptor has been determined in humans by cloning of its complementary deoxyribonucleic acid (DNA).^{83,85} Its structure is similar to that of the V1 and oxytocin receptors, and it is expressed in the kidney, as well as in the pituitary. Mice with deletion of the V1b (V3) receptor gene (*V1bKO*) have been created and studied.^{86,87} As expected, they have defective activation of the pituitary-adrenal axis, following some acute and chronic stressors. Male *V1bKO* mice were also found to have decreased aggression and social motivation.⁸⁸

Modulation of water balance occurs through the action of vasopressin on V2 receptors, located primarily in the renal collecting tubule, along with other sites in the kidney, including the thick ascending limb of the loop of Henle and periglomerular tubules.⁸⁹ It is also present on vascular endothelial cells in some systemic vascular beds, where vasopressin stimulates vasodilation,⁹⁰ possibly through activation of nitric oxide synthase.⁹¹ Vasopressin also stimulates von Willebrand factor, factor VIIIa, and tissue plasminogen activator through V2-mediated actions. Because of this, desmopressin is used to improve the prolonged bleeding times characteristic of uremia, type I von Willebrand disease, and hemophilia.⁹² The V2 receptor consists of 370 amino acids encoding seven transmembrane domains characteristic of the G protein-coupled receptors.^{89,93} These transmembrane domains share approximately 60% sequence identity with the V1 receptor, but substantially less with other members of this family (Fig. 12.8). Unlike the V1 and V3 receptors, the V2 receptor acts through adenylate cyclase to increase intracellular cyclic adenosine monophosphate (AMP) concentration. The human V2 receptor gene is located on the long arm of the X chromosome (Xq28),⁹⁴ at the locus associated with congenital, X-linked vasopressin-resistant diabetes insipidus. Mice in which *V2R* has been deleted have a similar nephrogenic diabetes insipidus phenotype.⁹⁵

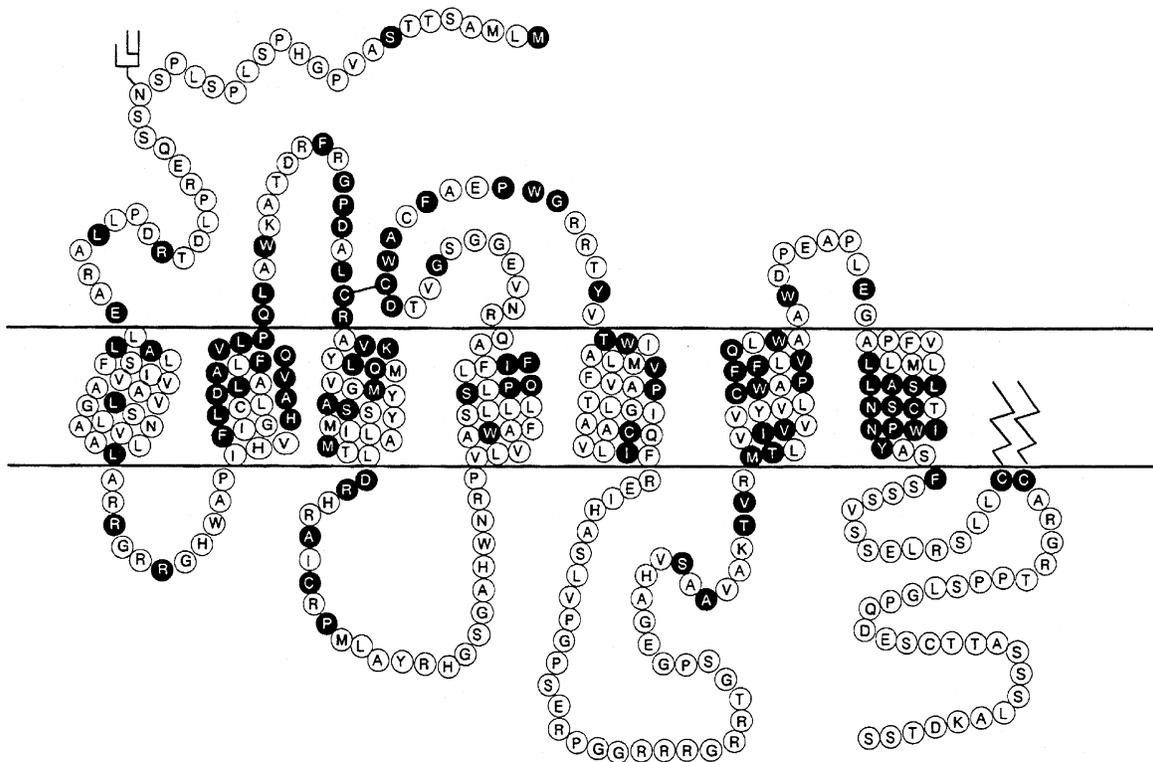


Fig. 12.8 Structure of the V1 and V2 vasopressin receptors, and the oxytocin receptor. Depicted, are predicted membrane topology, with the extracellular domain at the top of the figure and amino acids in the one-letter code. Amino acids in *open circles* encode the V1 receptor, whereas those in *black circles* are common to all three receptors. (Baylis, P.H. (1989). Vasopressin and its neurophysin. In: Degroot, L.J., ed. Endocrinology. 2nd ed. WB Saunders, Philadelphia, p. 213. With permission.)

Renal Cascade of Vasopressin Function. Vasopressin-induced increases in intracellular cyclic AMP, as mediated by the V2 receptor, triggers a complex pathway of events resulting in increased permeability of the collecting duct to water and efficient water transit across an otherwise minimally permeable epithelium (Fig. 12.9).¹ Activation of a cyclic AMP-dependent protein kinase imparts remodeling of cytoskeletal microtubules and microfilaments that culminate in the insertion of aggregates of water channels into the apical membrane.^{96,97} These mechanisms may involve a vesicle-associated membrane protein-2–like protein (VAMP-2), which also regulates synaptic vesicle activity in neuronal terminals,⁹⁸ and its associated receptor syntaxin-4.⁹⁹

Insertion of the water channels causes an up to 100-fold increase in water permeability of the apical membrane, allowing water movement along its osmotic gradient into the hypertonic inner medullary interstitium from the tubule lumen and excretion of a concentrated urine (see Fig. 12.9). The molecular analysis of the water channels has revealed a family of related proteins, designated aquaporins, that differ in their sites of expression and pattern of regulation.^{97,100} Each protein consists of a single polypeptide chain with six membrane-spanning domains (Fig. 12.10). Although functional as monomers, they form homotetramers in the plasma membrane.

AQP2 is expressed mostly within the kidney,¹⁰¹ primarily within the collecting duct.¹⁰² It is also expressed in the vas deferens, at least in the rat, although it is not regulated by vasopressin in this location.¹⁰³ Studies with immunoelectron microscopy have demonstrated large amounts of AQP2 in the apical plasma membrane and subapical vesicles of the collecting duct, consistent with the “membrane shuttling” model

of water channel aggregate insertion into the apical membrane after vasopressin stimulation.¹⁰³ Studies analyzing the mechanism by which AQP2 traffics to the apical plasma membrane have demonstrated that vasopressin-induced, protein kinase A–mediated serine phosphorylation at amino acid 256 is required for its exocytosis,¹⁰⁴ a process also requiring a heterotrimeric G protein of the G_i family.¹⁰⁵ In response to water restriction or desmopressin infusion in humans, the content of urinary AQP2 in both soluble and membrane-bound forms has been found to increase.¹⁰⁶ Mice with targeted deletion of the AQP2 gene have been made.¹⁰⁷ As expected, they have nephrogenic diabetes insipidus that is unresponsive to treatment with vasopressin.

Hypercalciuria is associated with polyuria despite adequate vasopressin levels. The activation by luminal calcium, of the calcium-sensing receptor (CaSR) on the apical membranes of the collecting duct cells of the kidney, is associated with resistance to vasopressin action.¹⁰⁸ The production of dilute urine when there is increased urinary concentration of calcium, has been hypothesized to be a protective mechanism to mitigate the risk of precipitation of calcium in the kidneys. Mechanisms by which the activation of the CaSR decreases AQP2 levels in the collecting duct cells include reduced synthesis of AQP2 by the generation of AQP2 targeting microribonucleic acid (miRNA), and the inactivation and degradation of AQP2 through ubiquitination and phosphorylation at serine261.^{109,110}

In addition to AQP2, different aquaporins appear to be involved in other aspects of renal water handling. In contrast to the apical localization of AQP2, aquaporin-3 and aquaporin-4 are expressed on the basolateral membrane of

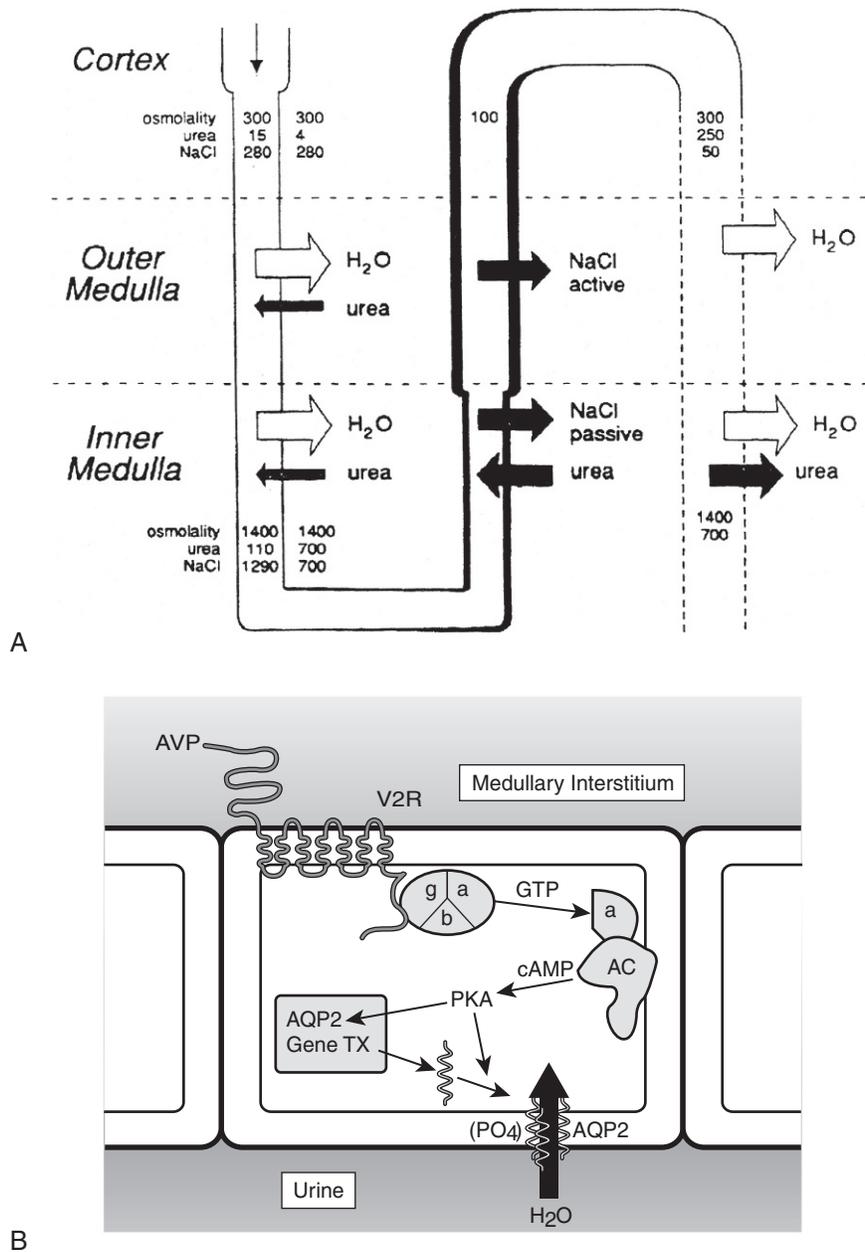


Fig. 12.9 Vasopressin action in the kidney. A, Solute and water handling in the kidney. B, Action of vasopressin in the collecting duct cell. Vasopressin (AVP) binds to the V2 receptor (V2R), causing the binding of guanosine triphosphate (GTP) to the stimulatory alpha G protein subunit (α). This activates adenylate cyclase (AC), resulting in an increase in cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA). The catalytic subunit of PKA, via phosphorylation of serine 256 of the water channel, aquaporin-2 (AQP2), causes aggregation of AQP2 homotetramers in membrane vesicles and their fusion with the collecting duct luminal membrane, resulting in an increase in water flow from the urine into the renal medullary interstitium. Demeclocycline, lithium, high calcium, and low potassium interfere with these processes, possibly at the level of cAMP generation and AQP2 synthesis or action. a, Gs alpha; b, Gs beta; g, Gs gamma. (A from van Laar, T., Lammers, G-J., Roos, R.A.C., Gerritsen, J.J., Meinders, A.E. (1998). Antiparkinsonian drugs causing inappropriate antidiuretic hormone secretion. *Mov Disord*, 13, 176–178. With permission.)

the collecting duct epithelium. They are involved in the flow of water and urea from the inside of the collecting duct cell into the extracellular renal medullary space. Mice made genetically deficient in aquaporin-4 demonstrate a mild urinary concentrating defect,¹¹¹ whereas those with deficiency of aquaporin-3 alone, or together with aquaporin-4, demonstrate more severely impaired urinary concentrating ability.¹¹² Mice made genetically deficient in aquaporin-1 demonstrate a urinary concentrating defect caused by decreased water permeability in the proximal tubule.¹¹³

Volume Sensor and Effector Pathways Renin-Angiotensin-Aldosterone System

In contrast to the vasopressin system, the classic, or peripheral, renin-angiotensin system primarily affects maintenance of intravascular volume as opposed to plasma tonicity. In addition to the well-established endocrine regulatory system, several local renin-angiotensin systems have emerged, with both autocrine and paracrine effects in their tissue of synthesis, whose regulation is independent of the classic system. Finally, brain and

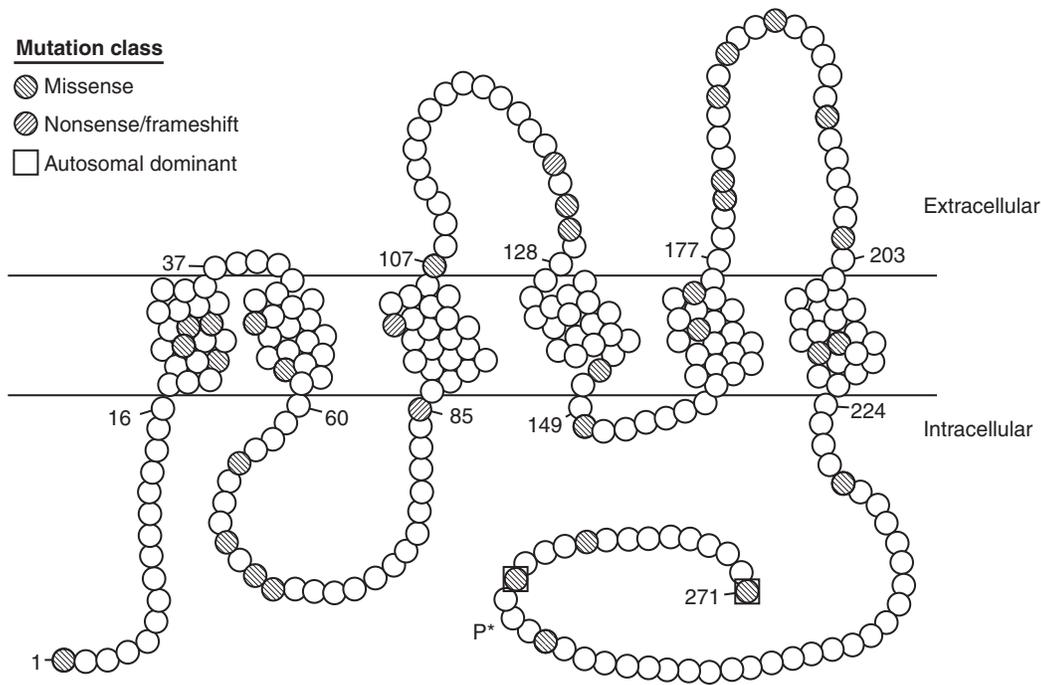


Fig. 12.10 Structure of the aquaporin-2 (AQP2) protein inserted into the luminal membrane of the distal tubule and AQP2 mutations that cause autosomal nephrogenic diabetes insipidus. Depicted, are predicted membrane topology, with the extracellular domain at the top of the figure. The 271-amino-acid protein consists of five transmembrane domains, four intracellular domains, and three extracellular domains. Amino acid mutations are classified as missense or nonsense/frameshift. Most mutations are transmitted with an autosomal recessive pattern, with autosomal dominant transmission noted by boxed amino acids. Vasopressin-dependent, protein kinase A-mediated phosphorylation of serine at amino acid 256 (P^{*}) is noted.

pituitary angiotensin systems involved in blood pressure, autonomic function, and fluid balance have been characterized with extensive interaction with the vasopressin system, and vasopressin has been found to play a role in the normal action of aldosterone on the renal tubular epithelium.¹¹⁴

Endocrine Renin-Angiotensin-Aldosterone System

Anatomy and Biochemistry. Renin, which is synthesized by the renal juxtaglomerular apparatus, is a proteolytic enzyme that catalyzes the cleavage of angiotensinogen, synthesized by hepatocytes, into the decapeptide angiotensin I. Angiotensin I possesses no intrinsic vasoreactive or mineralocorticoid secretagogue activity, but is efficiently cleaved by angiotensin-converting enzyme in the lungs, as well as other peripheral sites, to generate the octapeptide angiotensin II. Angiotensin II is further metabolized to the heptapeptide angiotensin III by removal of one amino-terminal amino acid. Angiotensin II possesses greater vasopressor activity and is present in approximately a 4-fold greater amount than angiotensin III. Angiotensins II and III possess equivalent mineralocorticoid secretory activity on the adrenal glomerulosa cells.

Angiotensin II and III act through cell surface receptors (AT₁) on the adrenal glomerulosa cells to activate the phospholipase C/protein kinase C pathway.¹¹⁵ This activation results in increased production of pregnenolone from cholesterol by side-chain cleavage enzyme (20,22-desmolase) and of aldosterone from corticosterone by the glomerulosa-specific corticosterone methyl oxidase I and II activities (18-hydroxylation and dehydrogenation, respectively).¹¹⁶ A distinct receptor subtype for angiotensin II and III, the AT₂ receptor, is not G protein coupled and is of unclear physiologic significance in the periphery,^{117–119} but may counteract AT₁ effects by inducing natriuresis.¹²⁰ Aldosterone, the primary and most potent endogenous mineralocorticoid released by the zona

glomerulosa, acts on target tissues expressing the nuclear mineralocorticoid (or type I glucocorticoid) receptor to promote sodium absorption and potassium excretion. For control of intravascular volume, the primary target of action of aldosterone is the distal nephron. Here, aldosterone increases synthesis of apical membrane sodium channels, mitochondrial enzymes involved in adenosine triphosphate production, and components of Na⁺, K⁺ adenosine triphosphatase to cause increased sodium reabsorption and potassium excretion.

Regulation of Secretion. Decreased intravascular volume as sensed by the renal juxtaglomerular apparatus results in release of renin.^{121,122} Increased plasma renin activity then allows increased conversion of angiotensinogen to angiotensin I, which in turn is converted peripherally to angiotensins II and III. Increased angiotensin II activity causes vasoconstriction and blood pressure elevation, whereas both angiotensins II and III stimulate aldosterone release from the zona glomerulosa and subsequent salt and water retention and potassium excretion by the distal tubule of the kidney. Conversely, expanded intravascular volume causes decreased renin output and less sodium and water resorption in the kidney, serving to decrease intravascular volume and restore homeostasis.

Changes in vascular volume are not the only regulators of the renin-angiotensin-aldosterone system. Serum potassium concentration directly modulates aldosterone release by the adrenal glomerulosa by its effects on plasma membrane potential and activation of voltage-gated calcium channels.¹²³ By membrane depolarization, increased serum potassium leads to increased aldosterone synthesis, which promotes renal potassium excretion, whereas low serum potassium reduces aldosterone synthesis and decreases urinary potassium losses. Pituitary adrenocorticotropic hormone and vasopressin act through their respective receptors on the glomerulosa cells to increase acute aldosterone secretion. These effects are of short

duration because long-term chronic infusions do not chronically elevate aldosterone concentrations. Direct inhibitors of aldosterone secretion, and thus promoters of natriuresis, include atrial natriuretic peptide (ANP),^{124,125} somatostatin,¹²⁶ and dopamine.¹²⁷

Local Renin-Angiotensin Systems

Anatomy and Biochemistry. In addition to the well-defined endocrine circuit, the components of the renin-angiotensin system have been found in a wide variety of tissues, including brain, pituitary, arterial wall, heart, ovary, kidney, and adrenal, where paracrine and autocrine regulatory functions^{122,128} have been postulated, undergoing regulation independent of the systemic counterpart. From the standpoint of regulation of water and volume homeostasis, the brain renin-angiotensin system merits further description. It has long been known that peripherally synthesized angiotensin II could increase blood pressure by effects on the brain outside the blood-brain barrier, at sites such as the OVLT, SFO, area postrema, and median eminence, as revealed by ligand-binding studies. Over the past decade, it has become clear that the complete system for generation of angiotensin II is present within the brain. Angiotensinogen has been localized to astrocytes by both immunohistochemical peptide localization and *in situ* hybridization analysis of messenger RNA. In contrast, renin has been found in high concentration in nerve terminals, with enhanced release on nerve depolarization. Angiotensin-converting enzyme has been found within vascular, choroid plexus, and neuronal components of the central nervous system, most notably the SFO and many hypothalamic nuclei, sites of endogenous angiotensin II receptor expression, primarily of the AT₁ subtype, as well as sites not expressing the angiotensin II receptor, such as the basal ganglia. The primary effector molecule, angiotensin II, has been localized specifically to neurons and subcellularly to synaptic vesicles. Two of the most significant sites include the circumventricular organs and the paraventricular nucleus of the hypothalamus. Within the paraventricular nucleus, angiotensin II immunoreactivity colocalizes with magnocellular vasopressin, whereas its receptors are within the parvocellular region of the paraventricular nucleus.

Regulation of Secretion. The forebrain angiotensin II pathway, of which the paraventricular nucleus is one component, and circumventricular organ angiotensin II pathway are important control centers for maintenance of osmotic and volume homeostasis. Increased concentration of peripheral angiotensin II, as would be expected in intravascular volume depletion, stimulates drinking behavior. This action of peripheral angiotensin II can be abolished by destruction of the OVLT or SFO, regions whose destruction has long been recognized as causing adipsia. Further effects of central angiotensin II action include augmentation of sodium appetite and stimulation of vasopressin release, all serving, as with peripheral angiotensin II, to restore intravascular volume and maintain blood pressure. The signal of hypovolemia is transduced through the vagal nerve from volume sensors to the brain stem and the region of the nucleus tractus solitarius. Efferents from these brain-stem centers project to the median preoptic nucleus and paraventricular nucleus, as does the forebrain angiotensin II pathway, where drinking and pressor effects, as well as vasopressin release, are elicited.

Separate pathways for vasopressin release mediate the response to either peripheral angiotensin II or purely osmotic stimulation of the osmosensors.¹²⁹ The release of vasopressin in response to osmotic stimulation is not increased by peripheral angiotensin II, and pure osmotic stimulation does not increase salt appetite. Central angiotensin II, in contrast, may function as a transmitter in the osmosensing circuit, leading to vasopressin release.

The Natriuretic Peptide System

In addition to the classic vasopressin and renin-angiotensin-aldosterone systems, the natriuretic peptide families of ligands and their receptors add further potential for modulation of salt and water balance. The interaction of the natriuretic peptide system occurs both in the central nervous system through effects on vasopressin secretion, and peripherally, through its ability to both directly promote natriuresis in the kidney and indirectly inhibit adrenal aldosterone production.

Anatomy and Biochemistry. ANP was initially discovered as a component of cardiac atrial muscle that was able to induce natriuresis, a decrease in blood pressure, and an increase in hematocrit when injected into rats.¹³⁰

The biologically active form of ANP consists of a 28-amino-acid peptide that includes a 17-amino-acid ring structure¹³¹ (Fig. 12.11). The primary sequence of the peptide has been conserved among mammalian species and, in addition to synthesis in cardiac atrial tissue,¹³² has been detected in brain, spinal cord, pituitary, and adrenal gland.^{133,134} Within the brain, ANP synthesis occurs at several critical neuroendocrine regulatory sites, including the periventricular, arcuate, anteroventral preoptic, and lateral hypothalamic nuclei. ANP is synthesized as a 151-amino-acid preprohormone and is stored as a 126-amino-acid prohormone after removal of the signal peptide sequence. Coupled with secretion of pro-ANP is its cleavage between amino acids 98 and 99 to yield the mature 28-amino-acid 99–126 fragment.

Subsequent investigation defined a second peptide from porcine brain with structural homology to ANP.¹³⁵ This peptide, designated brain natriuretic peptide (BNP), was later found to be secreted by the heart as well, in this case, from both ventricular and atrial tissue. Human BNP consists of a 32-amino-acid processed from a larger preprohormone¹³⁶ sharing a central ring structure with ANP (see Fig. 12.11), although it is less conserved between species than ANP.

A third member of this family, C-type natriuretic peptide (CNP), was also isolated from porcine brain.¹³⁷ In brain, CNP is the most abundant member of the natriuretic peptide family. Within the hypothalamus, specific sites of synthesis largely overlap sites of ANP expression. Little CNP can be detected in plasma, and in marked contrast to ANP and BNP, CNP does not increase in plasma in the setting of cardiac failure.¹³⁸ Outside the brain, CNP is synthesized in endothelial and vascular smooth muscle. In tissues capable of CNP gene expression, two forms of the peptide are produced, a 53-amino-acid peptide and a less abundant 22-amino-acid molecule (see Fig. 12.11).

Three distinct endogenous receptors exist for the natriuretic peptides. The first of these receptors isolated (natriuretic peptide receptor [NPR]-A or guanylyl cyclase [GC]-A) was cloned by virtue of its homology to sea urchin sperm guanylyl cyclase and was later found to have ANP and BNP as its normal ligands.¹³⁹ A second guanylyl cyclase type receptor (NPR-B) has substantial homology to NPR-A; however, it binds CNP with substantially greater affinity than ANP or BNP.¹⁴⁰ A third receptor (NPR-C¹⁴¹) does not possess guanylyl cyclase activity and probably functions to clear all three natriuretic peptides from the circulation.¹⁴² *In situ* hybridization studies using probes capable of distinguishing the different receptor types have revealed some interspecies discrepancy in distribution. The NPR-A receptor has been localized to kidney, adrenal, pituitary, brain, and heart in monkey, with NPR-B limited to adrenal, pituitary, and brain.¹⁴³ In rat, broad tissue distribution of both NPR-A and NPR-B has been described.¹⁴⁴ The NPR-C receptor has similarly been found in adrenal, heart, brain, and pituitary.¹⁴³

stimulates thirst.¹⁵⁴ The overall importance of the CNP central pathways in modulation of water balance in humans remains to be defined.

APPROACH TO THE PATIENT: DIFFERENTIAL DIAGNOSIS OF DISORDERS OF WATER METABOLISM

Hyponatremia (Fig. 12.12)

Hyponatremia (serum sodium <135 mEq/L) in children is usually associated with severe systemic disorders. It is most often caused by intravascular volume depletion or excessive salt loss and is also encountered with hypotonic fluid overload, especially in infants. Inappropriate vasopressin excess is one of the least common causes of hyponatremia in children, except after vasopressin administration for treatment of diabetes insipidus.

In evaluating the cause of hyponatremia, one should first determine whether the patient is dehydrated and hypovolemic. This is usually evident from the physical examination (decreased weight, skin turgor, central venous pressure) and laboratory data (high blood urea nitrogen, renin, aldosterone, uric acid). With a decrease in the glomerular filtration rate, proximal tubular reabsorption of sodium and water will be high, leading to a urinary sodium value often less than 10 mEq/L. Patients with decreased “effective” intravascular volume from congestive heart failure, cirrhosis, nephrotic syndrome, or lung disease will present with similar laboratory data, but will also have obvious signs of their underlying disease, which often includes peripheral edema. Patients with primary salt loss will also appear volume depleted. If the salt loss is from the kidney (e.g., diuretic therapy or polycystic kidney disease), the urine sodium level will be elevated, as may urine volume. Salt loss from other regions (e.g., the gut in gastroenteritis or the skin in cystic fibrosis) will cause urine sodium to be low, as in other forms of systemic dehydration. Cerebral salt wasting has been invoked to occur with central nervous system insults, and results in high serum ANP concentrations, leading

to high urine sodium and urine excretion and systemic dehydration.^{155–157} Its existence is controversial, and believed by many experts to be rare or not exist.^{158–162}

The syndrome of inappropriate antidiuresis (SIAD) exists when a primary elevation in vasopressin secretion or inappropriate activation of the vasopressin V2 receptor is the cause of hyponatremia.^{163,164} It is characterized by hyponatremia, an inappropriately concentrated urine (>100 mOsm/kg), normal or slightly elevated plasma volume, and a normal-to-high urine sodium (because of volume-induced suppression of aldosterone and elevation of ANP). Serum uric acid is low in patients with SIAD, whereas it is high in those with hyponatremia caused by systemic dehydration or other causes of decreased intravascular volume.¹⁶⁵ Measurement of plasma vasopressin is often not very useful because it is elevated in nearly all causes of hyponatremia, except for primary hypersecretion of ANP¹⁶⁶ or mutations in the vasopressin receptor that lead to its inappropriate activation. Because cortisol and thyroid deficiency cause hyponatremia by several mechanisms, discussed subsequently, they should be considered in all hyponatremic patients. Drug-induced hyponatremia should be considered in patients on potentially offending medications, as discussed later. In children with SIAD who do not have an obvious cause, a careful search for a tumor (thymoma, glioma, bronchial carcinoid) should be considered. Recently, the measurement of copeptin, coupled with hypertonic saline infusion, has been used to classify SIAD into different subtypes.¹⁶⁷

Polyuria, Polydipsia, and Hypernatremia (Fig. 12.13)

In children, it must first be determined whether pathologic polyuria or polydipsia (either exceeding 2 L/m²/day) is present. The following questions are asked: Is there a psychosocial reason for either polyuria or polydipsia? Can either be quantitated? Has either polyuria or polydipsia interfered with normal activities? Is nocturia or enuresis present? If so, does

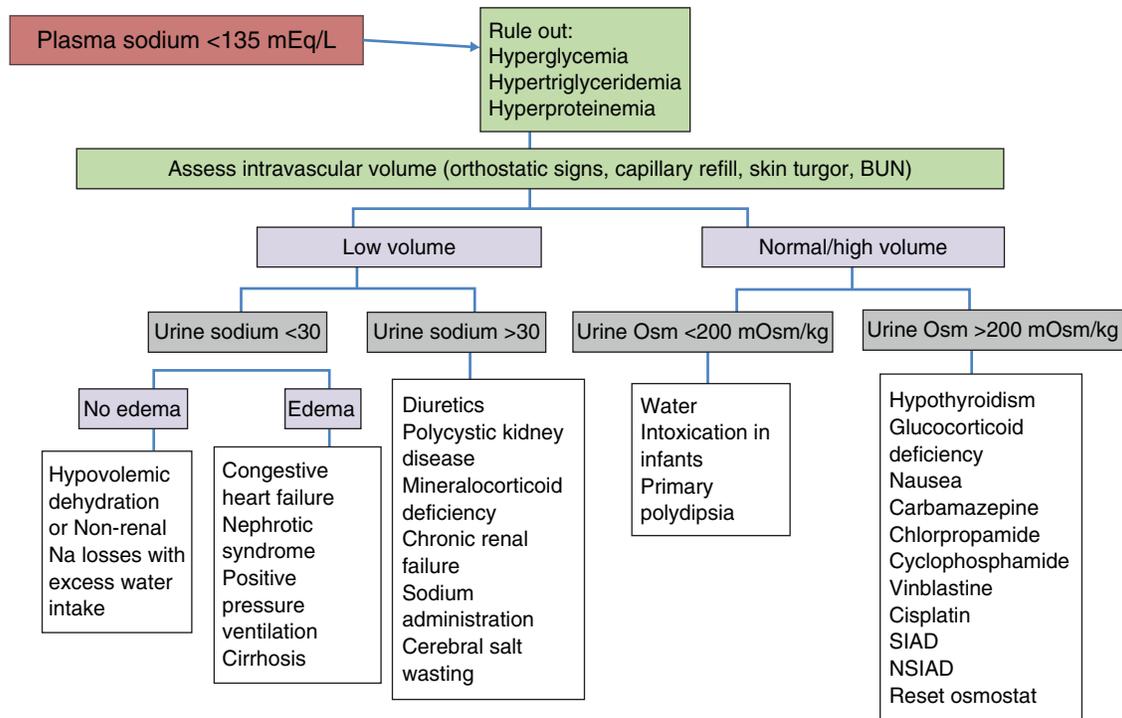


Fig. 12.12 Diagnostic algorithm for hyponatremia.

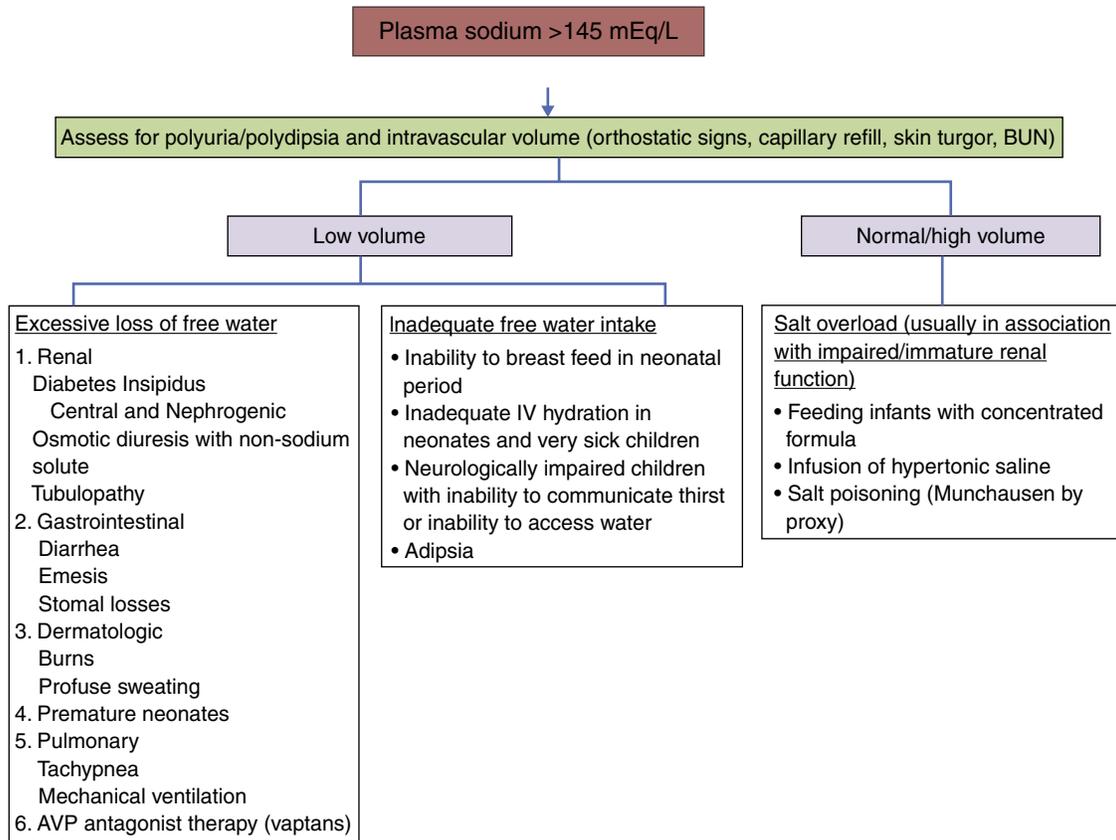


Fig. 12.13 Diagnostic algorithm for hypernatremia.

the patient also drink after nocturnal awakening? Does the history (including longitudinal growth data) or physical examination suggest other deficient or excessive endocrine secretion or an intracranial neoplasm?

If pathologic polyuria or polydipsia is present, the following should be obtained. In the outpatient setting: serum osmolality; serum concentrations of sodium, potassium, glucose, calcium, and blood urea nitrogen; and urinalysis, including measurement of urine osmolality, specific gravity, and glucose concentration. A serum osmolality greater than 300 mOsm/kg, with urine osmolality less than 300 mOsm/kg, establishes the diagnosis of diabetes insipidus. If serum osmolality is less than 270 mOsm/kg, or urine osmolality is greater than 600 mOsm/kg, the diagnosis of diabetes insipidus is unlikely. If, on initial screening, the patient has a serum osmolality less than 300 mOsm/kg, but the intake/output record at home suggests significant polyuria and polydipsia that cannot be attributed to primary polydipsia (i.e., the serum osmolality is greater than 270 mOsm/kg), the patient should undergo a water deprivation test to establish a diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes.

After a maximally tolerated overnight fast (based on the outpatient history), the child is admitted to the outpatient testing center in the early morning of a day when an 8- to 10-hour test can be carried out, and the child is deprived of water.^{168,169} The physical signs and biochemical parameters shown in the accompanying protocol are measured (Fig. 12.14). If at any time during the test, the urine osmolality exceeds 1000 mOsm/kg, or 600 mOsm/kg and is stable over 1 hour, the patient does not have diabetes insipidus. If at any time the serum osmolality exceeds 300 mOsm/kg and the urine osmolality is less than 600 mOsm/kg, the patient has diabetes insipidus. If the serum osmolality is less than 300 mOsm/kg and

the urine osmolality is less than 600 mOsm/kg, the test should be continued unless vital signs disclose hypovolemia. This may be difficult in a young child who may not tolerate a long period of fasting.

A common error is to stop a test too soon, based on the amount of body weight lost, before either urine osmolality has plateaued above 600 mOsm/kg or a serum osmolality above 300 mOsm/kg has been achieved. Unless the serum osmolality increases above the threshold for vasopressin release, a lack of vasopressin action (as inferred by a nonconcentrated urine) cannot be deemed pathologic. As an alternative to water deprivation, hypertonic saline can be infused intravenously to raise serum osmolality above the threshold for vasopressin release.¹⁷⁰ If the diagnosis of diabetes insipidus is made, aqueous vasopressin (Pitressin, 1 U/m²) should be given subcutaneously. If the patient has central diabetes insipidus, urine volume should fall and osmolality should at least double during the next hour, compared with the value before vasopressin therapy. If there is less than a 2-fold increase in urine osmolality after vasopressin administration, the patient probably has nephrogenic diabetes insipidus. Desmopressin should not be used for this test, because it has been associated with water intoxication in small children in this setting.¹⁷¹ Patients with long-standing primary polydipsia may have mild nephrogenic diabetes insipidus because of dilution of their renal medullary interstitium. This should not be confused with primary nephrogenic diabetes insipidus, because patients with primary polydipsia should have a tendency toward hyponatremia, rather than hypernatremia, in the basal state. Moreover, this form of secondary nephrogenic diabetes insipidus will reverse after cessation of overdrinking. Patients with a family history of X-linked nephrogenic diabetes insipidus, or more rarely, autosomal recessive mutations in the AQP2 water

Water Deprivation Test

ENDOCRINE FUNCTION TEST					Patient Name _____							
DIAGNOSIS: <u>Suspected Diabetes Insipidus</u>					_____							
TEST: <u>Water Deprivation</u>					_____							
Present Health _____ Good _____ Fair _____ Poor												
Diet for previous two days (attach diet history), to avoid tobacco, ethanol: _____												
Initial period of fast _____ hr												
Initial body weight _____ kg Recent Medications: _____												
Thirst sensation normal? _____												
No	Hour	Interval Minutes	Body weight	Vital signs	Serum			Plasma	Urine			
					Na	OSM	BUN	VP	Na	OSM	S.G.	vol/hr
		-30	Place IV hep lock									
		0	X	X	X	X	X	X	X	X	X	X
		60	X	X	X	X				X	X	X
		120	X	X	X	X			X	X	X	X
		180	X	X	X	X				X	X	X
		240	X	X	X	X		X	X	X	X	X
		300	X	X	X	X				X	X	X
		360	X	X	X	X			X	X	X	X
		420	X	X	X	X				X	X	X
		480	X	X	X	X	X	X*	X	X	X	X
*If patient has DI, last VP sample at last time point before VP administration (see below)												
AT ANY TIME DURING TEST:												
If serum osm <300 (Na<145), urine osm <600, continue test unless vital signs disclose hypovolemia												
If urine osm >1000, or >600 and stable (<30 mosm change for 2 time points), stop test = NORMAL												
If serum osm >300 and urine osm<600=DIABETES INSIPIDUS. Give Pitressin, 1U/m2 SQ and measure:												
TIME AFTER PITRESSIN ADMINISTRATION:												
		0		X						X	X	X
		30		X						X	X	X
		60		X					X	X	X	X
COMMENTS: _____												

Fig. 12.14 Protocol for evaluation of diabetes insipidus using water deprivation. *IV*, Intravenous; *OSM*, osmolality; *S.G.*, urinary specific gravity; *SQ*, subcutaneous.

channel (most commonly but not always found in consanguineous families¹⁷²), can be evaluated for these disorders in the prenatal or perinatal period by DNA sequence analysis, thus allowing therapy to be initiated without delay.¹⁷³

The water deprivation test should be sufficient in most patients to establish the diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes. Plasma

vasopressin concentrations may be obtained during the procedure (see Fig. 12.14), although they are rarely needed for diagnostic purposes in children.¹⁷⁴ They are particularly helpful in differentiating between partial central diabetes insipidus and nephrogenic diabetes insipidus, in that they are low in the former and high in the latter situation.¹⁷⁵ If urine osmolality concentrates normally, but only after serum osmolality is well

above 300 mOsm/kg, the patient may have an altered threshold for vasopressin release, also termed a *reset osmostat*. This may occur after head trauma, neurosurgery, or brain tumors.¹⁷⁶ More recently, an immunoassay for copeptin, the carboxy-terminus of the vasopressin precursor, has been developed, which may replace the measurement of vasopressin in the evaluation of diabetes insipidus.¹⁴ Copeptin is more stable than vasopressin, and blood concentrations of the two peptides are highly correlated.¹⁷⁷ Copeptin levels correlate well with vasopressin levels and show the same response as vasopressin levels to osmotic and to hemodynamic changes.¹⁷⁰

The diagnostic accuracy of using a copeptin cut-off value of 4.9 pmol/L (central diabetes insipidus if ≤ 4.9 pmol/L; primary polydipsia if > 4.9 pmol/L), after increasing the plasma sodium level to above 150 mmol/L with a bolus followed by an intravenous (IV) infusion of 3% saline, to differentiate between primary polydipsia, central diabetes insipidus and nephrogenic diabetes insipidus, was found to be significantly higher than that of the indirect water deprivation test (96.5% vs. 76.6%). The hypertonic saline infusion test correctly differentiated between partial diabetes insipidus and primary polyuria in 95.2% of the cases, in contrast to the 73.3% success rate with the use of the indirect water deprivation test in the same study.¹⁴ Nephrogenic diabetes insipidus could be diagnosed without the need for water deprivation, when the baseline copeptin level was greater than 21.4 pmol/L.¹⁷⁰

Magnetic resonance imaging (MRI) is not very helpful in distinguishing central diabetes insipidus from nephrogenic diabetes insipidus.¹⁷⁸ Normally, the posterior pituitary is seen as an area of enhanced brightness in T1-weighted images after administration of gadolinium.¹⁷⁹ The posterior pituitary "bright spot" is diminished or absent in both forms of diabetes insipidus, presumably because of decreased vasopressin synthesis in central, and increased vasopressin release in nephrogenic disease.^{180–182} In primary polydipsia, the bright spot is normal, probably because vasopressin accumulates in the posterior pituitary during chronic water ingestion,¹⁸² whereas it is decreased in SIAD, presumably because of increased vasopressin secretion.¹⁷⁸ Presence of hyperintensity upon T1-weighted MRI in the pituitary stalk, and its absence in the posterior pituitary region, has been associated with acute postneurosurgical diabetes insipidus.¹⁸³ Dynamic, fast-frame, MRI analysis has allowed estimation of blood flow to the posterior pituitary.¹⁸⁴ With this technique, both central and nephrogenic diabetes insipidus are associated with delayed enhancement in the area of the neurohypophysis.¹⁸⁵

In the inpatient postneurosurgical setting, central diabetes insipidus is likely if hyperosmolality (serum osmolality > 300 mOsm/kg) is associated with urine osmolality less than serum osmolality. One must beware of intraoperative fluid expansion with subsequent hypoosmolar polyuria masquerading as diabetes insipidus. Elevated copeptin levels within 24 hours after neurosurgery, in the hypothalamic-pituitary region, predict the unlikelihood of subsequent central diabetes insipidus, whereas low copeptin levels favor it.¹⁸⁶

SPECIFIC DISORDERS OF WATER METABOLISM

Hyponatremia With Normal Regulation of Vasopressin

Hyponatremia With Appropriate Decreased Secretion of Vasopressin

Increased Water Ingestion (Primary Polydipsia). In a hypoosmolar state with vasopressin secretion normally suppressed, the kidney can excrete urine with an osmolality as low as

50 mOsm/kg. Under these conditions, a daily solute load of 500 mOsm/m² could be excreted in 10 L/m² of urine per day. Neonates cannot dilute their urine to this degree and are prone to develop water intoxication at levels of water ingestion above 4 L/m²/day (approximately 60 mL/h in a newborn). This may happen when concentrated infant formula is diluted with excess water, either by accident or in a misguided attempt to make it last longer.¹⁸⁷ A primary increase in thirst, without apparent cause, leading to hyponatremia, has been reported in infants as young as 5 weeks of age.¹⁸⁸ In older children, with a normal kidney and the ability to suppress vasopressin secretion, hyponatremia does not occur unless water intake exceeds 10 L/m²/day, a feat that is almost impossible to accomplish. Long-standing ingestion of large volumes of water will decrease the hypertonicity within the renal medullary interstitium, which will impair water reabsorption and guard against water intoxication.¹⁸⁹ Hyponatremia will occur at lower rates of water ingestion when renal water clearance is impaired, either because of inappropriately elevated vasopressin secretion or for other reasons.

The rare patient in whom the osmotic thresholds for thirst and vasopressin release are reversed illustrates the importance of the normal relationship between these two responses to osmotic stimulation.¹⁹⁰ If thirst is activated below the threshold for vasopressin release, water intake and hypoosmolality will occur and suppress vasopressin secretion, leading to persistent polydipsia and polyuria. As long as daily fluid intake is less than 10 L/m², hyponatremia will not occur. Despite the presence of polyuria and polydipsia, this entity should not be confused with diabetes insipidus because of the absence of hypernatremia, although desmopressin treatment of such a patient may lower serum osmolality below the threshold for thirst, suppressing water ingestion and the consequent polyuria.

Decreased Renal Free Water Clearance. Adrenal insufficiency, either primary or secondary, has long been known to result in compromised free water excretion.^{34,191} The mechanisms by which glucocorticoids and mineralocorticoids modulate water diuresis have been the subject of substantial investigation. Some studies have demonstrated increased plasma vasopressin activity in the context of glucocorticoid insufficiency,^{192,193} consistent with more recent molecular biologic evidence that glucocorticoids inhibit transcription of the vasopressin gene.¹⁹⁴ Other investigators, however, have failed to detect vasopressin in plasma of patients with adrenal insufficiency and abnormal water clearance.¹⁹⁵ Consistent with vasopressin-independent actions of adrenal steroids on water metabolism, Brattleboro rats with hypothalamic diabetes insipidus manifest impaired excretion of a water load after adrenalectomy.¹⁹¹ In adrenalectomized Brattleboro rats, glucocorticoid administration restored urine flow rate but did not restore maximal urinary diluting capacity. Conversely, mineralocorticoid administration restored maximal urinary diluting capacity but not flow rate. Thus both mineralocorticoids and glucocorticoids are required for normal free water clearance. In part, these vasopressin-independent actions of mineralocorticoids and glucocorticoids have been attributed to the increased glomerular filtration rate arising from reexpansion of extracellular fluid volume (reduced owing to salt wasting) and improved cardiovascular tone, respectively.^{35,196,197} By restoring the glomerular filtration rate, more free water is delivered to the distal tubule for excretion. In addition, volume repletion reduces the nonosmotic stimuli for vasopressin release of volume depletion and hypotension. Recently, nitric oxide has been found to stimulate cyclic guanosine monophosphate-dependent membrane insertion of AQP2 into renal epithelial cells.¹⁹⁸ Because glucocorticoid has been shown to

inhibit endothelial nitric oxide synthase,¹⁹⁹ it is possible that under conditions of glucocorticoid deficiency, high levels of nitric oxide synthase result in elevated levels of endothelial nitric oxide in the renal vasculature, which in the distal renal tubule stimulate increased, vasopressin-independent, AQP2 activity and decreased free water clearance.

Direct effects of glucocorticoid or mineralocorticoid insufficiency on aquaporin expression and function have not been reported. In addition to impairing maximal renal diluting capacity, adrenal insufficiency compromises maximal urine-concentrating capacity.²⁰⁰ This effect has been shown to result from reduced tubular response to vasopressin.

Thyroid hormone is also required for normal free water clearance, and its deficiency likewise results in decreased renal water clearance and hyponatremia. Although some studies suggest that vasopressin mediates the hyponatremia of hypothyroidism because ethanol increases free water excretion in hypothyroid patients, this effect has not been found in other reports.²⁰¹ In addition, in severe hypothyroidism, hypovolemia is not present and hyponatremia is accompanied by appropriate suppression of vasopressin.²⁰² Similar to the consequences of isolated glucocorticoid deficiency described earlier, hypothyroidism impairs free water clearance more than maximal urine diluting capacity.²⁰³ This decrease in free water clearance may result from diminished glomerular filtration rate and delivery of free water to the diluting segment of distal nephron, as suggested by both animal²⁰⁴ and human studies.²⁰⁵

Given the often-subtle clinical findings associated with adrenal and thyroid deficiency, all patients with hyponatremia should be suspected of having these disease states and have appropriate diagnostic tests performed if indicated. Moreover, patients with coexisting adrenal failure and diabetes insipidus may have no symptoms of the latter until glucocorticoid therapy unmasks the need for vasopressin replacement.^{206,207} Similarly, resolution of diabetes insipidus in chronically polyuric and polydipsic patients may suggest inadequate glucocorticoid supplementation or noncompliance with glucocorticoid replacement.

Some drugs may cause hyponatremia by inhibiting renal water excretion without stimulating secretion of vasopressin

(Table 12.1), an action that could be called *nephrogenic SIAD*. In addition to augmenting vasopressin release, both carbamazepine^{208,209} and chlorpropamide^{210,211} increase the cellular response to vasopressin. Acetaminophen also increases the response of the kidney to vasopressin;²¹⁰ however, this has not been found to cause hyponatremia. High-dose cyclophosphamide treatment (15 to 20 mg/kg IV bolus) is often associated with hyponatremia, particularly when it is followed by a forced water diuresis to prevent hemorrhagic cystitis.^{212–214} Plasma vasopressin concentrations are normal, suggesting a direct effect of the drug to increase water resorption.²¹⁵ Similarly, vinblastine, independent of augmentation of plasma vasopressin concentration or vasopressin action,²¹⁶ and cisplatin^{217,218} cause hyponatremia. These drugs may damage the collecting duct tubular cells, which are normally highly impermeable to water, or may enhance AQP2 water channel activity and thereby increase water reabsorption down its osmotic gradient into the hypertonic renal interstitium.

Treatment. Hyponatremia caused by cortisol or thyroid hormone deficiency reverses promptly after institution of hormone replacement. Because the hyponatremia is often chronic, too rapid an increase in the serum sodium concentration should be avoided if possible, as will be discussed subsequently. When drugs that impair free water excretion must be used, water intake should be limited, as if the patient has SIAD, to 1 L/m²/24 h, using the regimen discussed.

Hyponatremia With Appropriate Increased Secretion of Vasopressin

Increased vasopressin secretion causing hyponatremia may be either an appropriate response or an inappropriate response to a pathologic state. Inappropriate secretion of vasopressin or V2 receptor activity (SIAD) is the much less common of the two entities.^{219,220} Whatever the cause, hyponatremia is a worrisome sign often associated with increased morbidity and mortality.²²¹

Systemic Dehydration. Systemic dehydration (water in excess of salt depletion) initially results in hypernatremia,

TABLE 12.1 Drugs Impairing Free Water Clearance

Class	Drug	Increases AVP Secretion	Increases AVP Effect	AVP-Independent Renal Effects	Hyponatremia
Angiotensin-converting enzyme inhibitors	Lisinopril				Yes
Anticonvulsants	Carbamazepine/ oxcarbazepine	Yes	Yes	Possibly	Yes
Antineoplastics	Valproic Acid				Yes
	Cis-platinum			Yes	Yes
	Cyclophosphamide	No		Yes	Yes
	Vinblastine	Yes		Yes	Yes
	Vincristine	Yes			Yes
Antiparkinsonian	Amantadine, trihexyphenidyl	Yes			Yes
Antipsychotics	Haloperidol, thioridazine				Yes
Antipyretics	Acetaminophen		Yes		
Hypolipidemics	Clofibrate	Yes	No		
Oral Hypoglycemics	Chlorpropamide, tolbutamide	Yes	Yes	No	Yes
Selective serotonin uptake inhibitors	Fluoxetine, sertraline, others	Likely			Yes
Tricyclic antidepressants	Imipramine, amitriptyline	Yes			Yes

AVP, Arginine vasopressin.

Proven actions of the drugs, if known, and whether the drugs have resulted in hyponatremia in humans, are indicated.

hyperosmolality, and activation of vasopressin secretion, as discussed earlier. In addition, the associated fall in the renal glomerular filtration rate results in an increase in proximal tubular sodium and water reabsorption, with a concomitant decrease in distal tubular water excretion. This limits the ability to form a dilute urine and, along with the associated stimulation of the renin-angiotensin-aldosterone system and suppression of ANP secretion, results in the excretion of urine that is very low in sodium. As dehydration progresses, hypovolemia and/or hypotension become major stimuli for vasopressin release, much more potent than hyperosmolality. This effect, by attempting to preserve volume, decreases free water clearance further and may lead to water retention and hyponatremia, especially if water replacement in excess of salt is given. Other nonosmotic factors may stimulate vasopressin release in a sick child, including pain, nausea, stress and specific conditions, such as pneumonia or meningitis. In many cases, hyponatremia caused by intravascular volume depletion is evident from physical and laboratory signs, such as decreased skin turgor, low central venous pressure, hemoconcentration, and elevated blood urea nitrogen levels. The diagnosis may be subtle, however. For example, patients with meningitis or pneumonia may present with hyponatremia, for which water restriction has been advocated in the belief that it is caused by central SIAD. Several studies have found that volume depletion, rather than SIAD, is often the cause of the hyponatremia,^{222–224} and that it resolves more readily when supplemental, rather than restricted, fluid and solute are administered.²²⁵ In patients with hyponatremia after head trauma, volume depletion rather than central SIAD is the cause in approximately one-half of cases.²²⁶ Similarly, many patients with gastroenteritis who present with mild hyponatremia and elevated plasma vasopressin levels²²⁷ have these on the basis of systemic dehydration rather than SIAD, and benefit from volume expansion rather than fluid restriction.²²⁸ More generally, most hospitalized pediatric patients with hyponatremia benefit from isotonic rather than hypotonic fluid replacement, suggesting that the underlying cause of the electrolyte disturbance is dehydration.^{229–231} Meta-analyses of several studies have revealed that the incidence of hyponatremia in hospitalized children that received hypotonic maintenance IV fluids was significantly higher than those that received isotonic maintenance IV fluids. As a result, the current guidelines for fluid management of hospitalized children recommend the use of isotonic IV fluids except in special clinical situations.²³²

Primary Loss of Sodium Chloride. Salt can be lost from the kidney, such as in patients with congenital polycystic kidney disease, acute interstitial nephritis, and chronic renal failure. Mineralocorticoid deficiency, pseudohypoaldosteronism (sometimes seen in children with urinary tract obstruction or infection), diuretic use, and gastrointestinal disease (usually gastroenteritis with diarrhea and/or vomiting) can also result in excess loss of sodium chloride. Hyponatremia can also result from salt loss in sweat in cystic fibrosis, although obstructive lung disease with elevation of plasma vasopressin probably plays a more prominent role, as has been discussed. With the onset of salt loss, any tendency toward hyponatremia will initially be countered by suppression of vasopressin and increased water excretion. With continuing salt loss, hypovolemia and/or hypotension ensues, causing nonosmotic stimulation of vasopressin. This, plus increased thirst, which leads to ingestion of hypotonic fluids with low solute content, results in hyponatremia. Weight loss is usually evident, as is the source of sodium wasting. If it is the kidney, it is accompanied by a rate of urine output and a urine sodium content greater than those associated with most other causes of hyponatremia, except a primary increase in ANP secretion.

Decreased Effective Plasma Volume. Congestive heart failure, cirrhosis, nephrotic syndrome, positive-pressure mechanical ventilation,²³³ severe burns,²³⁴ lung disease (bronchopulmonary dysplasia^{235–237} [in neonates]), cystic fibrosis with obstruction,^{238,239} and severe asthma^{240,241} are all characterized by a decrease in “effective” intravascular volume.^{201,242} This occurs because of impaired cardiac output, an inability to keep fluid within the vascular space, or impaired blood flow into the heart, respectively. As with systemic dehydration, in an attempt to preserve intravascular volume, water and salt excretion by the kidney are reduced; and decreased barosensor stimulation results in a compensatory, appropriate increase in vasopressin secretion, leading to an antidiuretic state and hyponatremia.²⁴³ Because of the associated stimulation of the renin-angiotensin-aldosterone system, these patients also have an increase in the total-body content of sodium chloride and may have peripheral edema, which distinguishes them from those with systemic dehydration. In patients with impaired cardiac output and elevated atrial volume (e.g., congestive heart failure or lung disease), ANP concentrations are elevated, which contributes to hyponatremia by promoting natriuresis.

Treatment. Patients with systemic dehydration and hypovolemia should be rehydrated with salt-containing fluids, such as normal saline or lactated Ringer’s solution. Because of activation of the renin-angiotensin-aldosterone system, the administered sodium will be avidly conserved and a water diuresis will quickly ensue, as volume is restored and vasopressin concentrations fall.²⁴⁴ Under these conditions, caution must be taken to prevent too rapid a correction of hyponatremia, which may itself result in brain damage.

Hyponatremia caused by a decrease in effective plasma volume from cardiac, hepatic, renal, or pulmonary dysfunction is more difficult to reverse. The most effective therapy is the least easily achieved: treatment of the underlying systemic disorder. Patients weaned from positive-pressure ventilation undergo a prompt water diuresis and resolution of hyponatremia, as cardiac output is restored and vasopressin concentrations fall. The only other effective route is to limit water intake to that required for the renal excretion of the obligate daily solute load of approximately 500 mOsm/m² and to replenish insensible losses. In a partial antidiuretic state with a urine osmolality of 750 mOsm/kg H₂O and insensible losses of 500 mL/m², oral intake would have to be limited to approximately 1200 mL/m²/day. Because of concomitant hyperaldosteronism, the dietary restriction of sodium chloride needed to control peripheral edema in patients with heart failure may reduce the daily solute load and further limit the amount of water that can be ingested without exacerbating hyponatremia. Hyponatremia in these settings is often slow to develop, rarely causes symptoms, and usually does not need treatment. If the serum sodium falls below 125 mEq/L, water restriction to 1 L/m²/day is usually effective in preventing a further decline. Because water retention in these disorders is a compensatory response to decreased intravascular volume, an attempt to reverse it with drugs, such as demeclocycline or specific V2 receptor antagonists (which induce nephrogenic diabetes insipidus as discussed subsequently) could result in worsening hypovolemia, with potentially dire consequences.²⁴⁵

In general, patients with hyponatremia caused by salt loss require ongoing supplementation with sodium chloride and fluids. Initially, IV replacement of urine volume with fluid containing sodium chloride (150 to 450 mEq/L depending on the degree of salt loss) may be necessary; oral salt supplementation may be required subsequently.¹⁶⁶ This treatment contrasts to that of SIAD, in which water restriction without sodium supplementation is the mainstay.

Precautions in the Emergency Treatment of Hyponatremia. Most children with hyponatremia develop the disorder gradually, are asymptomatic, and should be treated with water restriction alone. The development of acute hyponatremia, or a serum sodium concentration below 120 mEq/L, may be associated with lethargy, psychosis, coma, or generalized seizures, especially in younger children. Acute hyponatremia causes cell swelling owing to the entry of water into cells (Fig. 12.15), which can lead to neuronal dysfunction from alterations in the ionic environment or to cerebral herniation because of the encasement of the brain in the cranium. If present for more than 24 hours, cell swelling triggers a compensatory decrease in intracellular organic osmolytes, resulting in the partial restoration of normal cell volume in chronic hyponatremia.²⁴⁶

The proper emergency treatment of cerebral dysfunction depends on whether the hyponatremia is acute or chronic.^{247,248} In all cases, water restriction should be instituted. If hyponatremia is acute, and therefore probably not associated with a decrease in intracellular organic osmolyte concentration, rapid correction with hypertonic 3% sodium chloride, administered intravenously, may be indicated. As a general guide, this solution, given in the amount of 12 mL/kg, will result in an increase in serum sodium concentration of approximately 10 mEq/L. If hyponatremia is chronic, hypertonic saline treatment must be undertaken with caution, because it may result in both cell shrinkage (see Fig. 12.15) and the associated syndrome of central pontine myelinolysis.²⁴⁹ This syndrome, affecting the central portion of the basal pons, as well as other brain regions, is characterized by axonal demyelination, with sparing of neurons. It becomes evident within 24 to 48 hours after too rapid correction of hyponatremia, has a characteristic appearance by computed tomography and MRI, and often causes irreversible brain damage.²⁴⁹⁻²⁵¹ If hypertonic saline treatment is undertaken, the serum sodium concentration should be raised only high enough to cause an improvement in mental status, and in no case faster than 0.5 mEq/L/h or 12 mEq/L/day.^{247,249-251} In the case of systemic dehydration, the increase in serum sodium level may occur very rapidly using this regimen. The associated hyperaldosteronism will cause avid retention of the administered sodium, leading to rapid restoration of volume and suppression of vasopressin

secretion and resulting in a brisk water diuresis and an increase in the serum sodium concentration.²⁴⁴

Acute treatment of hyponatremia is more difficult in patients with decreased effective plasma volume. This is both because the underlying disorder makes it difficult to maintain the administered fluid within the intravascular space and because an associated increase in ANP promotes natriuresis and loss of the administered salt. Furthermore, patients with cardiac disease who are administered hypertonic saline may require concomitant treatment with a diuretic, such as furosemide, to prevent worsening of heart failure, which will also increase natriuresis.

Hyponatremia With Abnormal Regulation of Vasopressin

Hyponatremia With Inappropriate Increased Secretion of Vasopressin or Increased Vasopressin V2 Receptor Activity (Syndrome of Inappropriate Antidiuresis)

Causes of SIAD. SIAD is uncommon in children.^{219,220,252} It can occur with encephalitis, brain tumor,²⁵³ head trauma,^{226,254} brain malformations,²⁵⁵ in psychiatric disease,²⁵⁶ and in the postictal period after generalized seizures;²⁵⁷ after prolonged nausea;^{258,259} pneumonia;^{260,261} or acquired immunodeficiency syndrome.²⁶² Many drugs have been associated with impaired free water clearance as indicated in Table 12.1. Impaired free water clearance can result from alteration in vasopressin release, increased vasopressin effect at the same plasma vasopressin concentration, or vasopressin-independent changes in distal collecting tubule water permeability. Common drugs that have been shown to result in hyponatremia include carbamazepine,²⁰⁹ chlorpropamide,²⁶³ vinblastine,²¹⁶ vincristine,²⁶⁴ and tricyclic antidepressants.^{265,266} Newer sulfonylurea agents, including glyburide, are not associated with SIAD.²⁶⁷ Other rarer causes of SIAD in children are listed in Table 12.2. Although it has been believed to be the cause of hyponatremia associated with viral meningitis, volume depletion is more commonly the etiology.^{222,225} In contrast, the majority of children with tuberculous meningitis have hyponatremia

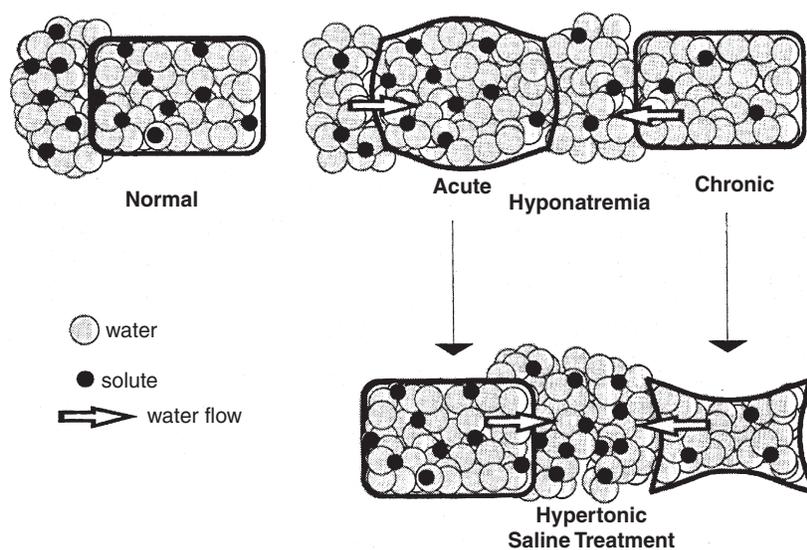


Fig. 12.15 Changes in organic osmolytes with hyponatremia and after its correction. Under normal conditions, osmotic balance exists between extracellular and intracellular compartments. With acute hyponatremia, water enters cells, causing cell swelling. After approximately 24 hours of continued hyponatremia, intracellular organic osmolytes decrease, restoring cell volume toward normal. Hypertonic saline treatment of acute hyponatremia results in restoration of normal cell volume, whereas the same treatment of chronic hyponatremia results in cell shrinkage. Large circle, water; closed smaller circle, solute; arrow, direction of water flow.

TABLE 12.2 Causes of Syndrome of Inappropriate Secretion of Vasopressin

Central Nervous System	Cancer	Infections	Pulmonary
Head trauma	Small cell of lung	Herpes zoster	Viral pneumonia
Subarachnoid hemorrhage	Duodenum	Respiratory syncytial virus	Bacterial pneumonia
Brain abscess	Pancreas	Tuberculosis	Abscess
Guillain-Barre syndrome	Thymoma	Aspergillosis	
Hydrocephalus	Bladder	Botulism	
Meningitis	Ureter		
	Lymphoma		
	Ewing sarcoma		

and SIAD (in one report attributed to cerebral salt wasting²⁶⁸), which predict more severe disease and poor outcome.^{269–271} SIAD is the cause of the hyponatremic second phase of the triple-phase response seen after hypothalamic-pituitary surgery. Hyponatremia with elevated vasopressin secretion is found in up to 35% of patients 1 week after transsphenoidal pituitary surgery.^{272,273} The mechanism is most likely retrograde neuronal degeneration with cell death and vasopressin release. Secondary adrenal insufficiency causing stimulation of vasopressin release²⁷⁴ may also play a role, because hyponatremia most commonly follows the removal of adrenocorticotropic hormone-secreting corticotroph adenomas.²⁷³ In the vast majority of children with SIAD, the cause is the excessive administration of vasopressin, whether to treat central diabetes insipidus,^{171,275} or less commonly, bleeding disorders²⁷⁶ (as has been discussed previously), or, most uncommonly, following dDAVP therapy for enuresis.

Two unrelated infants with mutations in the vasopressin V2 receptor that presented with severe hyponatremia in the first months of life heralded a new genetic cause of hyponatremia.²⁷⁷ These two infants had missense mutations at codon 137 that converted arginine to cysteine or leucine and led to constitutive activation of the V2 receptor with appropriately suppressed arginine vasopressin plasma concentration. This genetic disorder has been termed “*nephrogenic syndrome of inappropriate antidiuresis (NSIAD)*.” It remains unclear what portion of isolated early-onset chronic SIAD results from activating mutations of the V2 receptor, although the incidence is likely to be very low. Interestingly, this same codon is also the site of a loss-of-function mutation (R137H), which leads to X-linked nephrogenic diabetes insipidus.²⁷⁸ Over one dozen patients with NSIAD have been reported,^{279–281} including one patient presenting at the age of 38 years.²⁸²

Treatment of SIAD. Chronic SIAD is best treated by chronic oral fluid restriction. Under full vasopressin antidiuretic effect (urine osmolality of 1,000 mOsm/L), a normal daily obligate renal solute load of 500 mOsm/m² would be excreted in 500 mL/m² of water. This, plus a daily nonrenal water loss of 500 mL/m², would require that oral fluid intake be limited to 1000 mL/m²/day to avoid hyponatremia, as has been discussed more fully. In young children, this degree of fluid restriction may not provide adequate calories for growth. In this situation, the creation of nephrogenic diabetes insipidus may be indicated to allow sufficient fluid intake for normal growth.^{283–285} Lithium and demeclocycline were previously recommended to induce nephrogenic diabetes insipidus in this situation but are associated with significant toxicities, which limits their use in pediatric patients. Oral urea has been

effectively used to treat adult patients with chronic SIAD by virtue of its ability to induce an effective osmotic diuresis. This therapy was also demonstrated to be safe and effective in four children with chronic SIAD, including two with mutations in the vasopressin V2 receptor.^{279,280,285,286} Specific nonpeptide V2 receptor antagonists (vaptans) have also been developed for use in subacute or chronic SIAD caused by inappropriate increased vasopressin secretion.²⁸⁷ Their use is not US Food and Drug Administration (FDA) approved in children. Side effects include increased thirst, dry mouth, elevated hepatic transaminase levels, liver toxicity, and a significant overcorrection of hyponatremia. The aquaretic effects of the vaptans, after either parental or oral administration, have a rapid onset of action, exert peak effects within a few hours, and subside within 24 hours.^{43,164,288,289} In a large series of adult patients with euvolemic or hypervolemic hyponatremia resulting from cirrhosis, heart failure, or SIAD, these vasopressin receptor antagonists were effective in sustained elevation of serum sodium concentration. Another study in adults, limited to subjects with SIAD arising from inappropriate vasopressin secretion, demonstrated the efficacy of long-term treatment with an orally active vaptan, in conjunction with fluid restriction to 1.5 L per day.²⁹⁰ Substantial variability in the degree of serum sodium elevation was observed, however. This variability resulted from both interindividual differences in drug efficacy/disposition, and the failure to adequately restrict water consumption. The primary adverse effect from these agents is inflammation at infusion sites, although rises in serum sodium above rates recommended to prevent myelinolysis have also been found.⁴³ Very limited experience with vaptans has been reported in children,^{283–285,289,290} though they have been used to promote hydration during chemotherapy, with malignancy-associated SIAD.²⁹¹ These agents have not been effective in treating activating mutations of the V2 receptor,²⁹² although low-dose urea has been useful.²⁷⁹ Because the predictability of hypertonic saline administration for acute, severe forms of SIAD is greater than that of vaptans, hypertonic saline infusion for symptomatic hyponatremia because of inappropriate vasopressin secretion remains the recommended intervention.⁴³ Of note, some of these V2 receptor antagonists facilitate the proper transport of loss-of-function V2 receptor mutants to the cell surface.

Acute treatment of hyponatremia caused by SIAD is only indicated if cerebral dysfunction is present. In that case, treatment is dictated by the duration of hyponatremia and the extent of cerebral dysfunction. Because patients with SIAD have volume expansion, salt administration is not very effective in raising the serum sodium because it is rapidly excreted in the urine because of suppressed aldosterone and elevated ANP concentrations.

Hyponatremia With Inappropriate Decreased Secretion of Vasopressin, Caused by Increased Secretion of Atrial Natriuretic Peptide

Although ANP does not usually play a primary role in the pathogenesis of disorders of water metabolism, it may have an important secondary role.^{235,293–295} Patients with SIAD have elevated ANP concentrations, probably caused by hypervolemia, which may contribute to the elevated natriuresis of SIAD and which decrease as water intake is restricted.²⁹³ Likewise, the suppressed ANP concentrations found in central diabetes insipidus, probably caused by the associated hypovolemia, rise after dDAVP therapy.²⁹³ However, hyponatremia in some patients, primarily those with central nervous system disorders, including brain tumor, head trauma, hydrocephalus, neurosurgery, cerebral vascular accidents, and brain death, has been postulated to be caused by the primary hypersecretion of ANP.^{159,166,283–285,289,290,296,297} This syndrome, called *cerebral salt wasting*, is defined by hyponatremia accompanied by

elevated urinary sodium excretion (often more than 150 mEq/L), excessive urine output, hypovolemia, suppressed vasopressin, and elevated ANP concentrations (>20 pmol/L). Thus it is distinguished from SIAD, in which normal or decreased urine output, euvolemia, only modestly elevated urine sodium concentration, and elevated vasopressin concentration occur. Direct measurement of intravascular volume status with a central venous line is often helpful. The distinction is important because the therapies of the two disorders are markedly different. As noted previously, there is controversy regarding the prevalence of cerebral salt wasting,^{158–161} with most patients actually having either SIAD, dehydration, elevated glucocorticoids, or a combination of these. In patients in an intensive care setting, with the initial diagnosis of cerebral salt wasting, none of them upon further investigation were hypovolemic, one of the cardinal criteria of the syndrome.²⁹⁸

Treatment of Cerebral Salt Wasting. Treatment of patients with cerebral salt wasting consists of restoring intravascular volume with sodium chloride and water, as with the treatment of other causes of systemic dehydration. The underlying cause of the disorder, which is usually caused by acute brain injury, should also be treated if possible. A common error is to replace sodium with IV normal saline in the setting of hyperosmolar urine. If urine osmolality is higher than that of the infused saline, hyponatremia may worsen. Instead, saline hypertonic to urine, or enteral sodium chloride, should be used to avoid this issue.

Other Causes of True and Factitious Hyponatremia

True hyponatremia is associated with hyperglycemia, which causes the influx of water into the intravascular space. Serum sodium will decrease by around 1.6 mEq/L for every 100 mg/dL increment in blood glucose above 100 mg/dL. Glucose is not ordinarily an osmotically active agent, and does not stimulate vasopressin release, probably because it is able to equilibrate freely across plasma membranes. However, in the presence of insulin deficiency and hyperglycemia, glucose acts as an osmotic agent, presumably because its normal intracellular access to osmosensor sites is prevented.²⁹⁹ Under these circumstances, an osmotic gradient exists, and this stimulates vasopressin release. In diabetic ketoacidosis, this, together with the hypovolemia caused by the osmotic diuresis secondary to glycosuria, results in marked stimulation of vasopressin secretion.^{300–303} Rapid correction of hyponatremia may follow soon after the institution of fluid and insulin therapy. Whether this contributes to the pathogenesis of cerebral edema, occasionally seen following treatment of diabetic ketoacidosis, is not known. Elevated concentrations of triglycerides may cause factitious hyponatremia, as can obtaining a blood sample downstream from an IV infusion of hypotonic fluid.

Hypernatremia With Inappropriate Decreased Vasopressin Secretion or Action

Central Diabetes Insipidus

Central (hypothalamic, neurohypophyseal, neurogenic, or vasopressin-sensitive) diabetes insipidus can be caused by disorders of vasopressin gene structure; syndromes that result in loss of vasopressinergic neurons; accidental or surgical trauma to vasopressin neurons; congenital anatomical hypothalamic or pituitary defects; neoplasms; infiltrative, autoimmune, and infectious diseases affecting vasopressin neurons or fiber tracts; and increased metabolism of vasopressin.^{304,305} The etiology of central diabetes insipidus is not apparent in anywhere between 9% and 55% of children and young adults in different

series published in the literature.^{306–309} Long-term surveillance can identify an underlying cause that is not apparent at the time of initial diagnosis, sometimes after as long as 21 years.³¹⁰

Genetic Causes. Familial, autosomal dominant central diabetes insipidus is manifest within the first half of the first decade of life.^{311–317} Vasopressin secretion, initially normal, gradually declines until diabetes insipidus of variable severity ensues.³¹⁸ Patients respond well to vasopressin replacement therapy. The disease has a high degree of penetrance, but may be of variable severity within a family,³¹⁹ and may spontaneously improve in middle age.^{319,320} Vasopressin-containing neurons are absent from the magnocellular paraventricular neurons,³²¹ but present in parvocellular regions.³²² Several different oligonucleotide mutations in the vasopressin structural gene have been found to cause the disease.³²³ To date, there have been over 75 mutations detected in the coding region of the vasopressin gene, with the majority being missense mutations in or around cysteines (see Fig. 12.3).³²⁴ Most mutations are in the neurophysin portion of the vasopressin precursor, except for five in either the signal peptide or vasopressin peptide regions of the gene. This suggests that mutant neurophysin has a deleterious function, possibly being misfolded and blocking the proper intracellular sorting or packaging of vasopressin into secretory granules (see later). There are no disease-causing mutations in the copeptin region of the vasopressin precursor. This suggests either that this region has a low mutation rate, that it does not serve a critical function in vasopressin biology, or mutations in it are not disruptive to cellular health.

A family with a missense mutation within the vasopressin peptide region (Proline→Leucine at amino acid 7) of the gene, causing markedly reduced biological activity, was reported.³²⁵ The disease in this family is transmitted with an autosomal recessive pattern. This indicates that haploinsufficiency is not the basis for the autosomal dominant nature of the disease in families with the more common mutations in the neurophysin region of the gene. Another patient with autosomal recessive central diabetes insipidus presented at a young age (5 months), with compound heterozygous mutations in both copies of *AVP*, one inherited from each normal parent, again indicating that only one functioning copy is required for normal physiology.³²⁶

On the other hand, with autosomal dominant familial central diabetes insipidus, the abnormal gene product may interfere with the processing and secretion of the product of the normal allele,³²⁷ or cause neuronal degeneration and cell death.³²⁸ In support of this, mutant vasopressin precursors impair the secretion of the normal protein in cell models,³²⁷ and in a transgenic mouse model of the disease, a progressive loss of hypothalamic vasopressin-containing neurons occurs as the mice develop diabetes insipidus.³²⁹ Heterozygous mice with a mutation (C67X) in the vasopressin gene known to cause autosomal dominant familial neurohypophyseal diabetes insipidus in humans, developed diabetes insipidus by 2 months of age. They were found to have retention of the precursor of vasopressin within the neurons and the induction of an endoplasmic reticulum chaperone protein (BiP).³³⁰ However, humans presenting at less than 1 year of life with central diabetes insipidus are less likely to have autosomal dominant central diabetes insipidus, and more likely to have a structural brain defect.

Vasopressin deficiency is also found in the DIDMOAD syndrome, consisting of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness.^{313,331–335} The gene for this syndrome complex, also known as *Wolfram syndrome*, was localized to human chromosome 4p16 by polymorphic linkage analysis. The *Wolfram syndrome* gene, *WFS1*, encodes an 890-amino-acid tetrameric transmembrane protein that primarily localizes

to the endoplasmic reticulum. It is thought to function as a calcium channel or regulator of a calcium channel.^{336–342} Patients with mutations in proconvertase *PCSK1*, required for cleavage of the vasopressin precursor into mature vasopressin and neurophysin, have multiple endocrinopathies including central diabetes insipidus.³⁴³

Trauma. The axons of vasopressin-containing magnocellular neurons extend uninterrupted to the posterior pituitary over a distance of approximately 10 mm. Trauma to the base of the brain can cause swelling around or severance of these axons, resulting in either transient or permanent diabetes insipidus.³⁴⁴ Permanent diabetes insipidus can occur after seemingly minor trauma. Approximately one-half of patients with fractures of the sella turcica will develop permanent diabetes insipidus,³⁴⁵ which may be delayed as long as 1 month following the trauma, during which time neurons of severed axons may undergo retrograde degeneration.³⁴⁶ Septic shock³⁴⁷ and postpartum hemorrhage, associated with pituitary infarction (Sheehan syndrome),^{348,349} may involve the posterior pituitary with varying degrees of diabetes insipidus. Diabetes insipidus is never associated with cranial irradiation of the hypothalamic-pituitary region, although this treatment can cause deficits in all of the hypothalamic releasing hormones carried by the portal-hypophyseal system to the anterior pituitary. Because vasopressin is carried directly to the posterior pituitary via magnocellular axonal transport, it may be that radiation affects hypothalamic releasing hormone function by interruption of the portal-hypophyseal circulation, which is absent from the vasopressin circuitry.

Neurosurgical Intervention. One of the most common causes of central diabetes insipidus is the neurosurgical destruction of vasopressin neurons following pituitary-hypothalamic surgery. It is important to distinguish polyuria associated with the onset of acute postsurgical central diabetes insipidus from polyuria caused by the normal diuresis of fluids given during surgery. In both cases, the urine may be very dilute and of high volume, exceeding 200 mL/m²/h. However, in the former case, serum osmolality will be high, whereas in the latter case it will be normal. A careful examination of the intraoperative record should also help distinguish between these two possibilities. Vasopressin axons traveling from the hypothalamus to the posterior pituitary terminate at various levels within the stalk and gland (see Fig. 12.4). Because surgical interruption of these axons can result in retrograde degeneration of hypothalamic neurons, lesions closer to the hypothalamus will affect more neurons and cause greater permanent loss of hormone secretion. Not infrequently, a “triple phase” response is seen.³⁵⁰ Although the exact incidence of this phenomenon remains unknown, in a small study, nearly one in three children who underwent surgery for a craniopharyngioma developed it.³⁵¹ Following surgery, an initial phase of transient diabetes insipidus is observed, lasting 1.5 to 2 days, and possibly caused by edema in the area interfering with normal vasopressin secretion. If significant vasopressin cell destruction has occurred, this is often followed by a second phase of SIAD, which may last up to 10 days, and is caused by the unregulated release of vasopressin by dying neurons. A third phase of permanent diabetes insipidus may follow if more than 90% of vasopressin cells are destroyed. Usually, a marked degree of SIAD in the second phase portends significant permanent diabetes insipidus in the final phase of this response. In patients with coexisting vasopressin and cortisol deficits (e.g., in combined anterior and posterior hypopituitarism, following neurosurgical treatment of craniopharyngioma), symptoms of diabetes insipidus may be masked because cortisol deficiency impairs renal free water clearance, as discussed subsequently. In such cases, institution of

glucocorticoid therapy alone may precipitate polyuria, leading to the diagnosis of diabetes insipidus.

Congenital Anatomic Defects. Midline brain anatomic abnormalities, such as septo-optic dysplasia with agenesis of the corpus callosum,³⁵² the Kabuki syndrome,³⁵³ holoprosencephaly,³⁵⁴ and familial pituitary hypoplasia with absent stalk³⁵⁵ may be associated with central diabetes insipidus.^{255,356} These patients need not have external evidence of craniofacial abnormalities.³⁵⁴ Central diabetes insipidus caused by midline brain abnormalities is often accompanied by defects in thirst perception,^{352,356} suggesting that a common osmosensor may control both vasopressin release and thirst perception. Some patients with suspected defects in osmosensor function but with intact vasopressin neurons may have recumbent diabetes insipidus, with baroreceptor-mediated release of vasopressin, while upright, and vasopressin-deficient polyuria, while supine.³⁵⁷

Neoplasms. Several important clinical implications follow from knowledge of the anatomy of the vasopressin system. Because hypothalamic vasopressin neurons are distributed over a large area within the hypothalamus, tumors that cause diabetes insipidus must either be very large or infiltrative or be strategically located at the point of convergence of the hypothalamo-neurohypophyseal axonal tract in the infundibulum. Germinomas and pinealomas typically arise near the base of the hypothalamus where vasopressin axons converge before their entry into the posterior pituitary, and for this reason, are among the most common primary brain tumors associated with diabetes insipidus.³⁵⁸ Germinomas causing the disease can be very small^{359,360} and undetectable by MRI for several years following the onset of polyuria.³⁶¹ For this reason, quantitative measurement of the beta subunit of human chorionic gonadotropin, often secreted by germinomas and pinealomas, and regularly repeated MRI scans should be performed in children with idiopathic or unexplained diabetes insipidus. Empty sella syndrome, possibly caused by unrecognized pituitary infarction, can be associated with diabetes insipidus in children.³⁶² Craniopharyngiomas and optic gliomas can also cause central diabetes insipidus when very large, although this is more often a postoperative complication of the treatment for these tumors.³⁶⁸ Hematologic malignancies can cause diabetes insipidus. In some cases, such as with acute myelocytic leukemia, the cause is infiltration of the pituitary stalk and sella.^{363–365} However, more than 30 patients with monosomy or deletion of chromosome 7 associated with acute blast transformation of myelodysplastic syndrome presented with central diabetes insipidus,^{366–373} without evidence of infiltration of the posterior pituitary by neoplastic cells, leaving the cause of the diabetes insipidus unresolved.

Infiltrative, Autoimmune, and Infectious Diseases.

Langerhans cell histiocytosis and lymphocytic hypophysitis are the most common types of infiltrative disorders causing central diabetes insipidus. Approximately 10% of patients with histiocytosis will have diabetes insipidus. These patients tend to have more serious, multisystem disease for longer periods of time than those without diabetes insipidus,^{374,375} and anterior pituitary deficits often accompany posterior pituitary deficiency.³⁷⁶ MRI characteristically shows thickening of the pituitary stalk.³⁷⁷ One report suggests that in patients with Langerhans cell histiocytosis, radiation treatment to the pituitary region within 14 days of onset of symptoms of diabetes insipidus may result in return of vasopressin function in more than one-third of affected patients.³⁷⁸

Lymphocytic infundibuloneurohypophysitis may account for over one-half of patients with “idiopathic” central diabetes insipidus.^{379,380} This entity may be associated with other

autoimmune diseases.³⁸¹ Image analysis discloses an enlarged pituitary and thickened stalk,^{379,382} and biopsy of the posterior pituitary reveals lymphocytic infiltration of the gland, stalk, and magnocellular hypothalamic nuclei.³⁸³ A necrotizing form of this entity has been described, which also causes anterior pituitary failure and responds to steroid treatment.³⁸⁴ Diabetes insipidus can also be associated with pulmonary granulomatous diseases,³⁸⁵ including sarcoidosis.³⁸⁶ The use of checkpoint inhibitors as anticancer immunotherapy causes an autoimmune hypophysitis that can not only result in anterior pituitary dysfunction, but also central diabetes insipidus, which may be reversible upon discontinuation of the drug.^{387,388}

Whether antibody-mediated destruction of vasopressin cells occurs is controversial. Over one-half of patients with central diabetes insipidus of a nontraumatic cause have antibodies directed against vasopressin-containing cells,³⁸⁹ and patients with other autoimmune diseases have such antibodies without evidence of diabetes insipidus.³⁹⁰ Many patients with central diabetes insipidus also have antivasopressin peptide antibodies, although their appearance usually follows institution of vasopressin treatment.³⁹¹ It is very possible that antibodies directed against vasopressin-containing cells or vasopressin are not pathogenetic, but instead are markers of prior neuronal cell destruction.

Infections involving the base of the brain, such as meningococcal,³⁹² *Streptococcus pneumoniae*,³⁹³ cryptococcal, listeria,³⁹⁴ toxoplasmosis meningitis,³⁹⁵ congenital cytomegalovirus infection,³⁹⁶ congenital syphilis,³⁹⁷ and nonspecific inflammatory disease of the brain³⁹⁸ can cause central diabetes insipidus. The disease is often transient, suggesting that it is caused by inflammation rather than destruction of vasopressin-containing neurons.

Brain Death. Central diabetes insipidus can appear in the setting of hypoxic brain death.³⁹⁹ Although its presence has been suggested as a marker for brain death in children,⁴⁰⁰ in some studies, only a minority of patients with brain death manifest the disorder,⁴⁰¹ and up to 15% of patients with cerebral insults and diabetes insipidus ultimately recover brain function.⁴⁰² Polyuria in the setting of brain death can be accompanied by high concentrations of plasma vasopressin,⁴⁰³ suggesting that some cases mistaken for diabetes insipidus are actually caused by other causes, such as cerebral salt wasting with polyuria, as discussed subsequently.

Increased Metabolism of Vasopressin. The metabolic clearance rate of vasopressin increases 4-fold during pregnancy because of the elaboration of a vasopressinase by the placenta.⁶⁰ If the mother cannot respond with a concomitant increase in vasopressin action because of preexisting subclinical central or nephrogenic diabetes insipidus,^{59,61} overt, transient disease will appear, usually early in the third trimester (although occasionally earlier⁴⁰⁴), and resolve within 1 week of delivery.^{64,405} Even without prior defects vasopressin function, an extreme elevation in vasopressinase concentrations in primigravidas with either preeclampsia, liver dysfunction, or multiple gestation,^{62,63,66,406–408} may result in development of the syndrome. The central diabetes insipidus responds to dDAVP, which cannot be metabolized by vasopressinase, and has no oxytocic or hypertensive activity, because it is specific for AVPR2.

Drugs. The most common agent associated with inhibition of vasopressin release and impaired urine concentrating ability is ethanol.⁴⁰⁹ Because inhibition of vasopressin release by ethanol can be overcome in the setting of concurrent hypovolemia, clinically important diabetes insipidus caused by ethanol ingestion is uncommon.⁴¹⁰ Phenytoin, opiate antagonists, ketamine, halothane, and alpha-adrenergic agents have also been associated with impaired vasopressin release.^{411–413}

Children With Primary Enuresis. Although normal children have a nocturnal rise in plasma vasopressin, associated with an increase in urine osmolality, and a decrease in urine volume, those with primary enuresis have a blunted or absent rise in vasopressin, and excrete a higher urine volume of lower tonicity.^{414,415} This has suggested that enuretic children have a primary deficiency in vasopressin secretion, although the same outcome could be caused solely by excessive water intake in these children. The use of the V2 agonist dDAVP is highly effective in abolishing bed wetting episodes, although relapse is high once therapy is stopped.^{416–418} Fluid intake must be limited while a child is exposed to the antidiuretic action of dDAVP to guard against water intoxication. It is therefore best that the medication (only available in tablet in the United States) be taken at bedtime, with no further drinking until the morning, after the antidiuretic effect has waned. Nasal spray dDAVP is no longer approved for use in enuretic children because of this safety concern, whereas dDAVP tablets are FDA approved. The efficacy of dDAVP compared with enuretic alarms is similar, as is their relapse rate.⁴¹⁹ Because of safety concerns, enuretic alarms may be preferable.

Treatment of Central Diabetes Insipidus

Fluid Therapy. Patients with otherwise untreated diabetes insipidus crave cold fluids, especially water. With complete central diabetes insipidus, maximum urine concentrating ability is approximately 100 mOsm/kg. Because 5 L of urine would be required to excrete an average daily solute load of 500 mOsm/m², fluid intake must match this to maintain normal plasma tonicity. With an intact thirst mechanism and free access to oral fluids, a person with complete diabetes insipidus can maintain plasma osmolality and sodium in the high normal range, although at great inconvenience. Furthermore, long-standing intake of these volumes of fluid in children can lead to hydrourerter,⁴²⁰ and even hyperfluorosis in communities that provide fluoridated water.⁴²¹ With fluid management alone or with the use of medications other than vasopressin or its analogues, these children can develop nonobstructive hydrourerteronephrosis, bladder wall thickening and trabeculation, overflow incontinence and impaired renal function requiring a drainage procedure.⁴²² These complications are more likely to occur in children with nephrogenic, as opposed to central, diabetes insipidus because treatment with vasopressin analogs is used most of the time in children with central diabetes mellitus.

There are two situations in which central diabetes insipidus may be treated solely with high levels of fluid intake, without vasopressin. Vasopressin therapy coupled with excessive fluid intake (usually greater than 1 L/m²/day as discussed subsequently) can result in unwanted hyponatremia. Because neonates and young infants receive all of their nutrition in liquid form, the obligatory high oral fluid requirements for this age (3 L/m²/day), combined with vasopressin treatment are likely to lead to this dangerous complication.²⁷⁵ In infants, oral tablet and intranasal liquid desmopressin are not only difficult to administer accurately, but also their use is associated with significant fluctuations in the serum sodium levels. Such neonates may be better managed with fluid therapy alone. A reduced solute load diet will aid in this regard. Human milk is best for this purpose (75 mOsm/kg H₂O), whereas cow's milk is worst (230 mOsm/kg H₂O). For example, in an infant with diabetes insipidus with a fixed urine osmolality of 100 mOsm/kg H₂O, 300 mL of urine per day is required to excrete the amount of solute consumed in human milk, whereas 900 mL of urine per day is required to excrete the higher amount of solute consumed in cow's milk. The Similac PM 60/40 formula has a renal solute load of 92 mOsm/kg H₂O. In addition, supplementation free water may be needed, depending on the severity of

the diabetes insipidus. Options, such as 20 to 30 mL of supplemental free water for every 120 to 160 mL of formula, or dilution of the formula with free water have been used. Although children managed with such a regimen may be chronically thirsty, parents may have difficulty keeping up with the voluminous fluid intake and urine output, and poor growth may occur if adequate calories are not provided along with water,¹⁸⁸ these problems are more easily addressed than is life-threatening hyponatremia. Alternatively, thiazide (chlorothiazide, 5–10 mg/kg/dose), twice or thrice daily, and/or amiloride diuretics may be added to facilitate renal proximal tubular sodium and water reabsorption⁴²³ and thereby decrease oral fluid requirements. This therapy may be accompanied by a mild degree of dehydration. More recently, parenteral desmopressin (0.02–0.08 mcg/dose given once or twice daily) has been administered subcutaneously in infants with good results, although this is not approved by the FDA.⁴²⁴ The successful use of subcutaneous desmopressin in conjunction with a sliding scale fluid regimen has been reported to treat an infant with central diabetes insipidus and an absent thirst mechanism. The desmopressin dose, which was titrated on the basis of plasma sodium levels monitored at home, ranged between 0.01 mcg and 0.024 mcg per dose, given twice daily.⁴²⁵ Parenteral desmopressin was originally formulated at a concentration of 4 mcg/mL, to be used at a dose of 0.3 mcg/kg/dose to treat bleeding diatheses, such as hemophilia A and von Willebrand disease type 1. Thus care must be taken if it is used at 1/40 to one-fourth of this dose to treat infants with diabetes insipidus. Buccal administration of a diluted intranasal formulation of dDAVP (10 mcg/mL; 1–5 mcg twice daily) in infants with central diabetes insipidus, whose mean age was 4.5 months, resulted in normalization and stabilization of sodium levels within days of starting the treatment.⁴²⁶ Neonates with central diabetes insipidus have been successfully treated with orally administered diluted formulation of intranasal dDAVP (10 mcg/mL) at doses ranging from 2 to 5 mcg twice daily. There was no need to restrict their feed volume, while on this treatment regimen.⁴²⁷

In the acute postoperative management of central diabetes insipidus occurring after neurosurgery in children, vasopressin therapy may be successfully used,^{428–430} but extreme caution must be exerted with its use. While under the full antidiuretic effect of vasopressin, a patient will have a urine osmolality of approximately 1000 mOsm/kg and become hyponatremic if she or he receives an excessive amount of fluids, depending on the solute load and nonrenal water losses. With a solute excretion of 500 mOsm/m²/day, normal renal function, and nonrenal fluid losses of 500 mL/m²/day, fluid intake of greater than 1 L/m²/day (two-thirds of the normal maintenance fluid requirement) will result in hyponatremia. In addition, vasopressin therapy will mask the emergence of the SIAD phase of the triple phase neurohypophyseal response to neurosurgical injury (as has been discussed).

Because of the concerns associated with perioperative vasopressin administration, two different approaches to managing central diabetes insipidus in the surgical patient have been used. The first approach may be particularly useful to manage acute postoperative diabetes insipidus in young children. It uses fluids alone and avoids the use of vasopressin.⁴³¹ This method consists of matching input and output hourly using between 1 and 3 L/m²/day (40–120 mL/m²/h). If IV therapy is used, a basal 40 mL/m²/h should be given as 5% dextrose (D5) in one-fourth normal saline (normal saline = 0.9% sodium chloride) and the remainder, depending on the urine output, as 5% dextrose in water. Potassium chloride (40 mEq/L) may be added if oral intake is to be delayed for several days. No additional fluid should be administered for hourly urine volumes under 40 mL/m²/h. For hourly urine volumes

above 40 mL/m²/h, the additional volume should be replaced with 5% dextrose up to a total maximum of 120 mL/m²/h. For example, in a child with a surface area of 1 m² (approximately 30 kg), the basal infusion rate would be 40 mL/h of 5% dextrose in one-fourth normal saline. For an hourly urine output of 60 mL, an additional 20 mL/h 5% dextrose would be given, for a total infusion rate of 60 mL/h. For urine outputs above 120 mL/h, the total infusion rate would be 120 mL/h. In the presence of diabetes insipidus, this will result in a serum sodium in the 150 mEq/L range and a mildly volume contracted state, which will allow one to assess both thirst sensation, as well as the return of normal vasopressin function or the emergence of SIAD. Patients may become mildly hyperglycemic with this regimen, particularly if they are also receiving postoperative glucocorticoids. However, because it does not use vasopressin, this fluid management protocol prevents any chance of hyponatremia.

Vasopressin and Vasopressin Analogs. Evidence suggests that perioperative use of IV vasopressin in children with central diabetes insipidus may be the treatment modality of choice in most situations, resulting in excursions of serum sodium of smaller magnitude and few adverse sequelae.⁴³⁰ Although IV therapy with synthetic aqueous vasopressin (Pitressin) had been shown to be useful in the management of central diabetes insipidus of acute onset,^{428,429} concern existed as to the safety of its administration in the complex, rapidly changing course of the child recovering from hypothalamic/pituitary surgery. If continuous vasopressin is administered, fluid intake must be limited to 1 L/m²/day or two-thirds maintenance fluid administration (assuming normal solute intake and nonrenal water losses as described). The potency of synthetic vasopressin is still measured using a bioassay and is expressed in bioactive units, with one milliunit (mU) equivalent to approximately 2.5 ng of vasopressin. For IV vasopressin therapy, 1.5 mU/kg/h results in a blood vasopressin concentration of approximately 10 pg/mL,⁴³² twice that needed for full antidiuretic activity.⁴³³ Vasopressin's effect is maximal within 2 hours of the start of infusion,⁴³³ and one must beware of it sticking to IV bottles and tubing. Occasionally, following hypothalamic (but not transphenoidal) surgery, higher initial concentrations of vasopressin are required to treat acute diabetes insipidus, which may be attributable to the release of a substance related to vasopressin from the damaged hypothalamo-neurohypophyseal system, which acts as an antagonist to normal vasopressin activity.⁴³⁴ Much higher rates of vasopressin infusion, resulting in plasma concentrations above 1000 pg/mL, should be avoided, as they may cause cutaneous necrosis,⁴³⁵ rhabdomyolysis,^{435,436} and cardiac rhythm disturbances.⁴³⁷

In light of the considerations described in the previous paragraph, an effective and safe algorithm for management of perioperative central diabetes insipidus with IV infusion of vasopressin has been used with encouraging results.⁴³⁰ This algorithm starts with the child receiving an IV infusion of normal saline at two-thirds maintenance or 1 L/m²/day. A tentative diagnosis of intraoperative or postoperative central diabetes insipidus is made by documenting a serum sodium of greater than 145 mEq/L, along with urine output of greater than 4 mL/kg/h. Additional confirmatory evidence includes plasma osmolality above 300 mOsm/kg H₂O and relatively hypotonic urine. When documentation of parameters consistent with central diabetes insipidus is obtained, an IV infusion of aqueous vasopressin begins at 0.5 mU/kg/h, with no change in IV fluid administration. The dose of vasopressin is titrated upward in 0.5 mU/kg/h increments to establish a urine output rate of less than 2 mL/kg/h at approximately 10-minute intervals. The vasopressin and IV fluid administration then remain stable at these rates, with additional normal saline, or equivalent volume expanding solutions, given only to replace ongoing

blood loss or to maintain hemodynamic stability. Postoperatively, this management paradigm requires intensive care unit monitoring, with frequent assessment of electrolytes (hourly initially), urine output and osmolality/specific gravity, and vital signs. This management scheme can also be followed for patients with established central diabetes insipidus requiring general surgery and prolonged restriction of oral intake. In this situation, the usual dose of chronic, long-acting vasopressin is withheld or reduced immediately before surgery, depending upon the timing of surgery in relation to the usual administration times. In preparation for surgery, normal saline is infused at two-thirds maintenance ($1 \text{ L/m}^2/\text{day}$). When measures consistent with emergence of central diabetes insipidus caused by termination of efficacy of the presurgical dose are obtained, the IV vasopressin is initiated and titrated as described earlier.

Patients treated with vasopressin for postneurosurgical diabetes insipidus should be switched from IV to oral fluid intake at the earliest opportunity, because thirst sensation, if intact, will help regulate blood osmolality, as discussed. With the concomitant absence of vasopressin secretion and thirst, the clinician must perform administration fluids and an antidiuretic agent. This must be done with utmost care, for the therapeutic window for fluid administration is narrow when the patient cannot modulate their own antidiuretic activity.⁵⁰ Either the patient can be put into a persistent antidiuretic state, with dDAVP or vasopressin, and fluid strictly limited to $1 \text{ L/m}^2/\text{day}$, or the patient can be given intermittent dDAVP with clear urinary "breakthrough" between doses, and a fixed amount of fluid empirically determined to result in normal blood electrolyte concentrations. IV dDAVP (desmopressin) should not be used in the acute management of postoperative central diabetes insipidus, for it offers no advantage over vasopressin, and its long half-life (8–12 hours) compared with that of vasopressin (5–10 minutes) is a distinct disadvantage, as it may increase the chance of causing water intoxication.¹⁷¹ In fact, the use of IV dDAVP, 0.3 mcg/kg , to shorten the bleeding time in a variety of bleeding disorders (as has been discussed), has been associated with water intoxication,²⁷⁶ particularly in young children who have high obligate oral fluid needs.^{438–440}

A special problem arises when a patient with established central diabetes insipidus must receive a high volume of fluid for therapeutic reasons, for example, accompanying cancer chemotherapy.⁴⁴¹ Such patients can be managed by discontinuing antidiuretic therapy and increasing fluid intake to 3 to $5 \text{ L/m}^2/\text{day}$ (rendering the patient moderately hypernatremic). This would be similar to what would occur in a patient with intact vasopressin secretion, in whom overhydration to this extent would suppress endogenous vasopressin secretion. However, although $5 \text{ L/m}^2/\text{day}$ is typically adequate to maintain serum sodium concentration in the range of 150 mEq/L in children with central diabetes insipidus, this rate may not be adequate in the setting of chemotherapy administration when solute excretion increases because of cell death and release of cellular contents. By using a low dose of IV vasopressin ($0.08\text{--}0.1 \text{ mU/kg/h}$, approximately one-eighth the full antidiuretic dose, titrated upward as needed), a partial antidiuretic effect allows the administration of higher amounts of fluid without causing hyponatremia.⁴⁴² Whereas management of diabetes insipidus, with fluids alone, may be used for chemotherapy with ifosfamide (which is associated with nephrotoxicity and hemorrhagic cystitis), recent data suggest that excretion of a hypotonic urine, as would occur in patients with diabetes insipidus management with fluids alone, increases the risk of developing nephrotoxicity during therapy with platinum-based antineoplastic agents.⁴⁴³ By allowing administration of 0.45% saline at rates of approximately $3 \text{ L/m}^2/\text{day}$, the low-dose vasopressin infusion yields a urine osmolality higher than

that achievable with fluids alone,⁴⁴² and may have the additional benefit of conferring renal protection.

In the outpatient setting, treatment of central diabetes insipidus in older children should begin with oral (see later) or intranasal dDAVP ($10 \text{ mcg}/0.1 \text{ mL}$, 0.025 mL (2.5 mcg) given by rhinal tube at bedtime and the dose increased to the lowest amount that gives an antidiuretic effect. If the dose is effective, but has too short a duration, it should be increased further, or a second, morning dose should be added. Patients should escape from the antidiuretic effect for at least 1 hour before the next dose, to ensure that any excessive water will be excreted. Otherwise, water intoxication may occur. dDAVP is also available as a nasal spray in the same concentration, with each spray delivering 10 mcg (0.1 mL). This is the standard preparation that was used for treatment of primary enuresis before this indication was removed. Oral dDAVP tablets and dissolvable wafers are now in widespread use and have largely replaced intranasal therapy.⁴⁴⁴ Although, when given orally, dDAVP is at least 20-fold less potent than when given via the intranasal route, oral dDAVP in doses of 25 to 300 mcg every 8 to 12 hours is reported to be highly effective and safe in children.^{445–447} Lysine vasopressin (Diapid) nasal spray (50 units/mL) may be used if a duration less than that of dDAVP is desired. One spray delivers 2 units (0.04 mL), with a duration of action between 2 and 8 hours.

As noted previously, cortisol deficiency may cause decreased free water clearance by stimulating a nitric oxide-mediated pathway, which results in the insertion of AQP2 channels into the apical membranes of collecting duct cells, in a vasopressin-independent fashion.^{198,199} Conversely, it is possible that excessive amounts of cortisol, caused by endogenous release during stress, or to treatment with exogenous drug, may inhibit the insertion of water channels. This may explain why patients, with central diabetes insipidus treated with desmopressin, become "resistant," and require an increased dosage, during times of stress or treatment with glucocorticoids.⁴⁴⁸

In addition to polyuria and polydipsia, decreased bone mineral density has been reported in patients with central diabetes insipidus.⁴⁴⁹ The decreased bone density was not corrected by vasopressin analog treatment alone, suggesting that institution of bisphosphonate or other therapies designed to prevent bone loss may be of long-term benefit in the treatment of diabetes insipidus.

Adipsic Hypernatremia

A subset of patients with central diabetes insipidus also have disorders of thirst mechanism. Lesions that affect the sensory circumventricular organs of the hypothalamus, including the organum vasculosum of the lamina terminalis, subfornical organ, and the area postrema, can result in either absent thirst or an elevated osmotic threshold for thirst perception. These areas lie outside the blood-brain barrier and constantly measure osmolality and sodium levels. Patients with diabetes insipidus and an intact thirst mechanism rarely have a significant hypernatremia because the thirst mechanism ensures that their water intake matches their excessive urinary water losses. However, patients with diabetes insipidus and adipsia can have severe hypernatremia and complications, such as rhabdomyolysis, acute renal failure, and venous thrombosis. Etiologies for adipsic hypernatremia include congenital malformations, such as holoprosencephaly and septooptic dysplasia sequence; vascular lesions, such as anterior communicating artery aneurysm; neoplasms, such as craniopharyngiomas and suprasellar germinomas; granulomatous conditions, such as histiocytosis and sarcoidosis; and miscellaneous conditions, such as arachnoidal cysts and hydrocephalus. Recently, there has been a case series reported of adipsic hypernatremia, without any abnormality on MRI of the hypothalamus and the pituitary gland. The affected children had associated hyperprolactinemia,

childhood obesity, and impaired growth hormone secretion. The presence of antibodies targeting the subfornical organ in these patients strongly suggests an autoimmune etiology for their adipsic hypernatremia.^{450,451} The treatment of adipsic hypernatremia involves giving a fixed daily dose of an dDAVP with controlled daily fluid intake and strict monitoring of fluid balance, body weight, and plasma sodium levels. Successful treatment of an infant with diabetes insipidus and absent thirst has been reported using a sliding scale fluid regimen and subcutaneous dDAVP, guided by the frequent home monitoring of plasma sodium levels.⁴²⁵

Nephrogenic Diabetes Insipidus

Causes of Nephrogenic Diabetes Insipidus. Nephrogenic (vasopressin-resistant) diabetes insipidus can be caused by genetic or acquired causes. Genetic causes are less common but more severe than acquired forms of the disease, although genetic etiologies are more common in children than in adults.

Genetic Causes

Congenital, X-Linked Diabetes Insipidus: V2 Receptor Mutations. Congenital, X-linked nephrogenic diabetes insipidus is caused by inactivating mutations of the vasopressin V2 receptor.¹⁷³ Approximately 250 mutations, mostly missense, have been reported to be associated with nephrogenic diabetes insipidus.³²⁴ Because of its mode of transmission, it is a disease of males, although rarely females may be affected, presumably caused by extreme lyonization during X chromosome inactivation.^{452,453} In keeping with a germline, as opposed to somatic mutation in the V2 receptor, these patients are deficient in all systemic V2 receptor-mediated actions,^{454,455} and have intact V1-receptor-mediated responses.^{456,457} As expected, the V2 receptor defect is proximal to the activation of renal adenylate cyclase.^{458,459} Unlike the function of other G protein-coupled seven transmembrane receptors, such as the parathyroid hormone and thyroid-stimulating hormone receptors, that of the V2 receptor is unaffected in patients with pseudohypoparathyroidism, who have inactivating mutations in the alpha subunit of G_s.⁴⁶⁰

Because of vasopressin resistance in congenital nephrogenic diabetes insipidus, the kidney elaborates large volumes of hypotonic urine, with osmolality ranging between 50 and 100 mOsm/kg. Manifestations of the disease are usually present within the first several weeks of life,^{461,462} but may only become apparent after weaning from the breast. The predominant symptoms are polyuria and polydipsia. Thirst may be more difficult to satisfy than in central diabetes insipidus. Many infants initially present with fever, vomiting, and dehydration, often leading to an evaluation for infection. Growth failure in the untreated child may be secondary to the ingestion of large amounts of water, which the child may prefer over milk and other higher caloric substances.⁴⁶³ Mental retardation of variable severity may result from repeated episodes of dehydration.⁴⁶⁴ Intracerebral calcification of the frontal lobes and basal ganglia is not uncommon in children with X-linked nephrogenic diabetes insipidus.^{465–468} Because this appears early and is not seen in children with central diabetes insipidus of equivalent severity, cerebral calcification is probably unrelated to the level of dehydration or therapeutic intervention. It is possible that elevated vasopressin concentrations, acting via intact V1 or V3 receptors, contribute to some of the unique manifestations of X-linked nephrogenic diabetes insipidus, such as cerebral calcification, intense thirst, vomiting, and growth failure. Older children may present with enuresis or nocturia. They may learn to reduce food intake (and therefore solute load) to decrease polyuria, which may contribute to growth failure. After long-standing ingestion and excretion of

large volumes of water, patients may develop nonobstructive hydronephrosis, hydroureter, and megabladder.⁴²⁰

Although one founder (arriving in North America from Scotland in 1761 on the ship *Hopewell*) was initially postulated to be the ancestor of most North American subjects with congenital, X-linked nephrogenic diabetes insipidus,⁴⁶⁹ hundreds of independent mutations in the V2 receptor have been found, with some appearing to have arisen independently more than once^{470–486} (Fig. 12.16). These are mostly single base mutations that either result in amino acid substitutions, translational frameshifts, or termination of peptide synthesis, and are distributed fairly evenly throughout the receptor protein. Mutations may affect either vasopressin binding, cyclic AMP generation, or possibly transcriptional regulation.^{487–491} Patients with different mutations will likely be found to exhibit phenotypic heterogeneity, including in severity of disease and response to treatment. Genetic heterogeneity may underlie the variable response of patients with X-linked diabetes insipidus to dDAVP treatment. In a family with a known mutation, prenatal or early postnatal DNA screening can unambiguously identify affected males, allowing the institution of appropriate therapy.⁴⁹²

Congenital, Autosomal Nephrogenic Diabetes Insipidus: AQP2 Mutations. After the initial description of X-linked nephrogenic diabetes insipidus,⁴⁹³ several patients were reported with similar clinical findings except for autosomal recessive transmission of the disease,⁴⁹⁴ or normal V2 receptor function outside of the kidney.⁴⁹⁵ With the cloning of the complementary DNA for the renal water channel, AQP2, many patients with autosomal recessive nephrogenic diabetes insipidus have been reported who have a total of 57 mutations different in this gene.^{324,488} Most are missense mutations, although four are nonsense or frameshift mutations.¹⁷² They are scattered throughout the molecule, including within four of the five transmembrane domains, two of three extracellular domains, and two of four intracellular domains (see Fig. 12.10). Recently, an autosomal dominant mode of inheritance for nephrogenic diabetes insipidus has been described, associated with mutations in AQP2. One of these dominant mutations results in mixed tetramers of the wild type and mutant alleles being retained in the Golgi apparatus.⁴⁹⁶ AQP2 mutations impair the ability of the luminal membrane to undergo an increase in water permeability, following signaling through the V2 receptor. They could include patients previously described who had a normal rise in urinary cyclic AMP in response to vasopressin, without a concomitant increase in urine osmolality.⁴⁵⁸ AQP2 protein is excreted in the urine in both soluble and membrane-bound forms. AQP2 excretion is low in untreated central and nephrogenic diabetes insipidus, but following dDAVP administration, increases markedly in the former, but not latter, disease.¹⁰⁶ For this reason, its measurement in urine has been suggested as an aid in the differential diagnosis of diabetes insipidus.¹⁰⁶

Acquired Causes

Acquired causes of nephrogenic diabetes insipidus are more common and less severe than genetic causes. Nephrogenic diabetes insipidus may be caused by drugs, such as lithium and demeclocycline, both of which are thought to interfere with vasopressin-stimulated cyclic AMP generation or action. Approximately 50% of patients receiving lithium have impaired urinary concentrating ability, although only 10% to 20% of them develop symptomatic nephrogenic diabetes insipidus, which is almost always accompanied by a reduction in the glomerular filtration rate.^{497,498} The risk increases with duration of therapy. Lithium impairs the ability of vasopressin to stimulate adenylate cyclase,⁴⁹⁹ resulting in a 90% fall in AQP2 messenger RNA expression in renal collecting duct,⁵⁰⁰

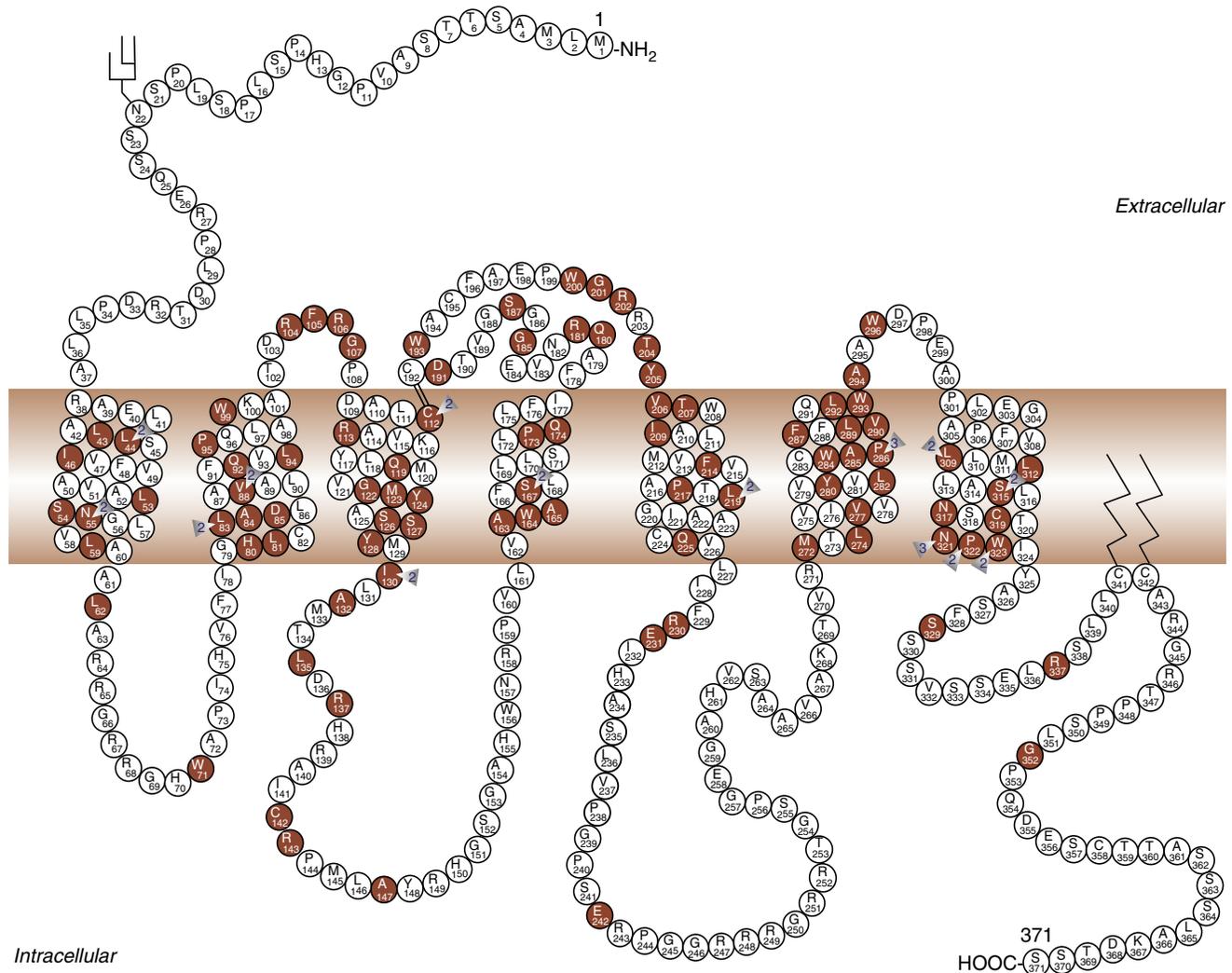


Fig. 12.16 Predicted structure of the V2 vasopressin receptor (AVPR2) and mutations, which cause X-linked nephrogenic diabetes insipidus. Depicted are predicted membrane topology, with the extracellular domain at the top of the figure and amino acids in the one-letter code. Shaded amino acids depict mutations, with numbers indicating more than one mutation at that position. (From Bichet, D.G., Bockenhauer, D. (2016). Genetic forms of nephrogenic diabetes insipidus (NDI): vasopressin receptor defect (X-linked) and aquaporin defect (autosomal recessive and dominant). *Best Pract Res Clin Endocrinol Metabol*, 30, 263–276. With permission.)

which may be the basis for its causing nephrogenic diabetes insipidus.

Demeclocycline treatment causes nephrogenic diabetes insipidus by inhibiting transepithelial water transport.⁵⁰¹ For this reason, it is useful in the treatment of dilutional hyponatremia associated with inappropriate secretion of vasopressin, as discussed previously. Other agents that cause nephrogenic diabetes insipidus include hypercalcemia, hyperkalemia, and therapy with foscarnet (used in treatment of cytomegalovirus infection in immunosuppressed patients),^{502,503} clozapine,⁵⁰³ amphotericin,⁵⁰⁴ methicillin,⁵⁰⁵ or rifampin.⁵⁰⁶ Whether any of these agents cause nephrogenic diabetes insipidus by interfering with the expression or insertion into apical collecting duct membranes of AQP2 water channels is not yet known. Ureteral obstruction,⁵⁰⁷ chronic renal failure, polycystic kidney disease, medullary cystic disease, Sjogren syndrome,⁵⁰⁸ and sickle cell disease can also impair renal concentrating ability.⁵⁰⁹ Osmotic diuresis caused by glycosuria in diabetes mellitus, or to sodium excretion with diuretic therapy, will interfere with renal water conservation. Primary polydipsia can result in secondary nephrogenic diabetes insipidus because the chronic excretion of a dilute urine lowers the osmolality of the

hypertonic renal interstitium, thus decreasing renal concentrating ability. Finally, decreased protein or sodium intake also can lead to diminished tonicity of the renal medullary interstitium and nephrogenic diabetes insipidus.

Treatment of Nephrogenic Diabetes Insipidus. The treatment of acquired nephrogenic diabetes insipidus focuses on elimination, if possible, of the underlying disorder, such as offending drugs, hypercalcemia, hypokalemia, or ureteral obstruction. Congenital nephrogenic diabetes insipidus is often difficult to treat. The main goals should be to ensure the intake of adequate calories for growth and to avoid severe dehydration. Foods with the highest ratio of caloric content to osmotic load should be ingested, to maximize growth and minimize the urine volume required to excrete urine solute. However, even with the early institution of therapy, growth and mental retardation are not uncommon.⁵¹⁰

Thiazide diuretics, in combination with amiloride or indomethacin, are the most useful pharmacologic agents in the treatment of nephrogenic diabetes insipidus.⁵¹¹ Thiazides work both by enhancing sodium excretion at the expense of water, as well as by causing a fall in glomerular filtration rate, which

results in proximal tubular sodium and water reabsorption.^{423,512} Indomethacin, 2 mg/kg/day, further enhances proximal tubular sodium and water reabsorption,^{423,513,514} although this effect is not mediated by inhibition of cyclooxygenase.⁵¹⁵ The combination of thiazide and amiloride diuretics is the most commonly used regimen for the treatment of congenital, X-linked nephrogenic diabetes insipidus, because amiloride counteracts thiazide-induced hypokalemia,⁴⁶¹ avoids the nephrotoxicity associated with indomethacin therapy, and is well tolerated, even in infants.⁵¹⁶ In addition, amiloride decreases the uptake of lithium by renal epithelial cells, and for this additional reason has been proposed in combination with thiazide as treatment for lithium-induced nephrogenic diabetes insipidus.⁵¹⁷ High-dose dDAVP therapy, in combination with indomethacin, has been reported to be helpful in treating some subjects with nephrogenic diabetes insipidus.⁵¹⁸ This treatment may prove to be useful in patients with genetic defects in the V2 receptor, which reduce the binding affinity for vasopressin.

A therapy that has thus far only been used in mice and not yet available for humans, in which abnormal stop codons are bypassed,⁵¹⁹ may prove useful to treat patients with nonsense mutations in AVPR2 and AQP2.

CONCLUDING REMARKS

Precise regulation of water balance is necessary for the proper function of multiple cellular pathways. Vasopressin released from the posterior pituitary, stimulated by both hyperosmolar and nonosmotic factors, acts via the kidney V2 vasopressin receptor to stimulate both an increase in AQP2 expression and its insertion into collecting duct luminal membrane, thereby enhancing renal water reabsorption to minimize subsequent water loss. Thirst controls the second major physiologic response to hyperosmolality and results in increased water intake to make up for past water loss. The renin-angiotensin-aldosterone and ANP systems also make important contributions to water and volume regulation by modulation of sodium intake and output.

The proper diagnosis of disorders caused by deficient and excessive action of vasopressin requires a thorough understanding of the physiologic regulation of this hormone. Recent advances in molecular medicine have revealed mutations in the vasopressin gene, and the V2 receptor or AQP2 genes, responsible for familial central and nephrogenic diabetes insipidus, respectively. Molecular methods allow the diagnosis of these disorders in the prenatal or early postnatal periods. Nevertheless, the most frequent cause of central diabetes insipidus remains a destructive lesion of the central nervous system caused by tumor or neurosurgical insult, and pharmacologic toxicity remains the most common cause of nephrogenic diabetes insipidus.

Hyponatremia is a common occurrence in childhood, but is rarely caused by a primary increase in vasopressin secretion or increase in intrinsic activity of the V2 receptor (SIAD). It is more commonly caused by hypovolemia (either primary or secondary to decreased effective vascular volume), salt loss, excessive ingestion of hypotonic fluids, or cortisol deficiency. Hyponatremia resulting from increased vasopressin action is most commonly caused by excessive vasopressin administration during the treatment of central diabetes insipidus or coagulopathies.

In infants, central diabetes insipidus is best treated with fluid therapy, which avoids the administration of vasopressin or its V2 receptor analog, dDAVP, whereas in older children, dDAVP is the drug of choice. Nephrogenic diabetes insipidus remains a therapeutic challenge. Hyponatremia caused by SIAD is best managed by restricting water intake, whereas salt and water replacement are indicated when hyponatremia is caused

by hypovolemia or excessive secretion of ANP, as occurs in cerebral salt wasting. Hyponatremia causing central nervous system dysfunction is a medical emergency. Blood sodium must be raised promptly, but at a rate not greater than 0.5 mEq/L/h to avoid the occurrence of central pontine myelinolysis.

REFERENCES

1. Knepper MA, Kwon TH, Nielsen S. Molecular physiology of water balance. *N Engl J Med*. 2015;372:1349–1358.
2. Acher R. Molecular evolution of biologically active peptides. *Proc R Soc*. 1980;210:21–43.
3. Du Vigneaud V. Hormones of the posterior pituitary gland: oxytocin and vasopressin. *Harvey Lect*. 1954;50:1–26.
4. Oliver G, Schafer EA. On the action of extracts of pituitary body and certain other glandular organs. *J Physiol (London)*. 1895;18:277–279.
5. Du Vigneaud V, Gish DT, Katsoyannis PG. A synthetic preparation possessing biological properties associated with arginine-vasopressin. *J Am Chem Soc*. 1984;76:4751–4752.
6. Du Vigneaud V, Ressler C, Swan JM, Roberts CW, Katsoyannis PG, Gordon S. The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J Am Chem Soc*. 1953;75:4879–4880.
7. Vavra I, Machova A, Holecck V, Cort JH, Zaoral M, Sorm F. Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. *Lancet*. 1968;1:948–952.
8. Richardson DW, Robinson AG. Desmopressin. *Ann Int Med*. 1985;103:228–239.
9. Osborne WA, Vincent S. A contribution to the study of the pituitary body. *Br J Med, Part I*. 1900;502–503.
10. Acher R, Fromageot C. The relationship of oxytocin and vasopressin to active proteins of posterior pituitary origin. In: Heller H, ed. *The Neurohypophysis*. London: Butterworths; 1957:39–50.
11. Pickering BT, Jones CW. The neurophysins. *Horm Proteins Peptides*. 1978;5:103–158.
12. Chen L, Rose JP, Breslow E, et al. Crystal structure of a bovine neurophysin II dipeptide complex at 2.8 Å determined from the single-wave length anomalous scattering signal of an incorporated iodine atom. *Proc Natl Acad Sci U S A*. 1991;88:4240–4244.
13. Rose JP, Breslow E, Huang HB, Wang BC. Crystallographic analysis of the neurophysin-oxytocin complex: a preliminary report. *J Mol Biol*. 1991;221:43–45.
14. Fenske W, Refardt J, Christ-Crain M. Copeptin in the diagnosis of diabetes insipidus. *N Engl J Med*. 2018;379:1785–1786.
15. Sachs H, Fawcett P, Takabatake Y, et al. Biosynthesis and release of vasopressin and neurophysin. *Recent Prog Horm Res*. 1969;25:447–484.
16. Lindheimer MD, Barron WM, Davison JM. Osmoregulation of thirst and vasopressin release in pregnancy [published erratum appears in *Am J Physiol* 1989 Oct;257(4 Pt 2):preceding F503]. [Review]. *Am J Physiol*. 1989;257:F159–F169.
17. Brownstein MJ, Russell JT, Gainer H. Synthesis, transport, and release of posterior pituitary hormones. *Science*. 1980;207:373–378.
18. Wallis M. Molecular evolution of the neurohypophysial hormone precursors in mammals: comparative genomics reveals novel mammalian oxytocin and vasopressin analogues. *Gen Comp Endocrinol*. 2012;179:313–318.
19. Summar ML, Phillips JA, Battey J, et al. Linkage relationships of human arginine vasopressin-neurophysin-II and oxytocin-neurophysin-I to prodynorphin and other loci on chromosome 20. *Mol Endocrinol*. 1990;4:947–950.
20. Ruppert SD, Schere G, Schutz G. Recent gene conversion involving bovine vasopressin and oxytocin precursor genes suggested by nucleotide sequence. *Nature*. 1984;308:554–557.
21. Sawchenko PE, Swanson LW, Vale WW. Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci U S A*. 1984;81:1883–1887.
22. Whitnall MH, Mezey E, Gainer H. Co-localization of corticotropin-releasing factor and vasopressin in median eminence neurosecretory vesicles. *Nature*. 1985;317:248–252.
23. Majzoub JA, Herman M. Adrenocorticotropin. In: Melmed S, ed. *The Pituitary*. 2nd ed. Cambridge, England: Blackwell Scientific; 2002.

24. Verney EB. The antidiuretic hormone and the factors which determine its release. *Proc R Soc.* 1947;135:25–105.
25. Kaur C, Ling EA. The circumventricular organs. *Histol Histopathol.* 2017;32:879–892.
26. Bealer SL, Crofton JT, Share L. Hypothalamic knife cuts alter fluid regulation, vasopressin secretion and natriuresis during water deprivation. *Neuroendocrinology.* 1983;36:364–370.
27. Thrasher TN, Keil LC, Ramsay DJ. Lesions of the organum vasculosum of the lamina terminalis (OVLT) attenuate osmotically-induced drinking and vasopressin secretion in the dog. *Endocrinology.* 1982;110:1837–1839.
28. Phillips MI. Functions of angiotensin in the central nervous system. *Annu Rev Physiol.* 1987;49:413–435.
29. Simpson JB. The circumventricular organs and the central action of angiotensin. *Neuroendocrinology.* 1981;32:248–256.
30. Yamaguchi K, Koike M, Hama H. Plasma vasopressin response to peripheral administration of angiotensin in conscious rats. *Am J Physiol.* 1985;248:R249–R256.
31. Robertson GL, Shelton RL, Athar S. The osmoregulation of vasopressin. *Kidney Int.* 1976;10:25–37.
32. Robertson GL. The regulation of vasopressin function in health and disease. *Recent Prog Horm Res.* 1977;33:333–385.
33. Davison JM, Gilmore EA, Durr J, Robertson GL, Lindheimer MD. Altered osmotic thresholds for vasopressin secretion and thirst in human pregnancy. *Am J Physiol.* 1984;246:F105–F109.
34. Boykin J, DeTorrente A, Erickson A, Robertson G, Schrier RW. Role of plasma vasopressin in impaired water excretion of glucocorticoid deficiency. *J Clin Invest.* 1978;62:738–744.
35. Linas SL, Berl T, Robertson GL, Aisenbrey GA, Schrier RW, Anderson RJ. Role of vasopressin in the impaired water excretion of glucocorticoid deficiency. *Kidney Int.* 1980;18:58–67.
36. Spruce BA, Baylis PH, Burd J, Watson MJ. Variation in osmoregulation of arginine vasopressin during the human menstrual cycle. *Clin Endocrinol (Oxf).* 1985;22:37–42.
37. Vokes TJ, Weiss NM, Schreiber J, Gaskill MB, Robertson GL. Osmoregulation of thirst and vasopressin during normal menstrual cycle. *Am J Physiol.* 1988;254:R641–R647.
38. Davison JM, Shiells EA, Phillips PR, Lindheimer MD. Serial evaluation of vasopressin release and thirst in human pregnancy. Role of human chorionic gonadotrophin in the osmoregulatory changes of gestation. *J Clin Invest.* 1988;81:798–806.
39. Kucharczyk J, Morgenson GJ. Separate lateral hypothalamic pathways for extracellular and intracellular thirst. *Am J Physiol.* 1975;228:295–301.
40. Zimmerman CA, Leib DE, Knight ZA. Neural circuits underlying thirst and fluid homeostasis. *Nat Rev Neurosci.* 2017;18:459–469.
41. Coble JP, Grobe JL, Johnson AK, Sigmund CD. Mechanisms of brain renin angiotensin system-induced drinking and blood pressure: importance of the subfornical organ. *Am J Physiol Regul Integr Comp Physiol.* 2015;308:R238–R249.
42. Gruber KA, Wilkin LD, Johnson AK. Neurohypophysial hormone release and biosynthesis in rats with lesions of the anteroventral third ventricle (AV3V) region. *Brain Res.* 1986;378:115–119.
43. Robertson GL. Vaptans for the treatment of hyponatremia. *Nat Rev Endocrinol.* 2011;7:151–161.
44. Thompson CJ, Burd JM, Baylis PH. Acute suppression of plasma vasopressin and thirst after drinking in hypernatremic humans. *Am J Physiol.* 1987;252:R1138–R1142.
45. Salata RA, Verbalis JG, Robinson AG. Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. *J Clin Endocrinol Metab.* 1987;65:561–567.
46. Williams TD, Seckl JR, Lightman SL. Dependent effect of drinking volume on vasopressin but not atrial peptide in humans. *Am J Physiol.* 1989;257:R762–R764.
47. Figaro MK, Mack GW. Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation. *The Am J Physiol.* 1997;272:R1740–R1746.
48. Zimmerman CA, Lin YC, Leib DE, et al. Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature.* 2016;537:680–684.
49. Mandelblat-Cerf Y, Kim A, Burgess CR, et al. Bidirectional anticipation of future osmotic challenges by vasopressin neurons. *Neuron.* 2017;93:57–65.
50. Janus DM, Wojcik M, Zygmunt-Gorska A, Wyrobek L, Urbanik A, Starzyk JB. Adipsic diabetes insipidus in pediatric patients. *Indian J Pediatr.* 2014;81:1307–1314.
51. Sawchenko PE, Swanson LW. Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science.* 1981;214:685.
52. Robertson GL, Athar S. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J Clin Endocrinol Metab.* 1976;42:613–620.
53. Fujiwara Y, Tanoue A, Tsujimoto G, Koshimizu TA. The roles of V1a vasopressin receptors in blood pressure homeostasis: a review of studies on V1a receptor knockout mice. *Clin Exp Nephrol.* 2012;16:30–34.
54. Mikkelsen JD, Jacobsen J, Kiss A. Differential sensitivity to nicotine among hypothalamic magnocellular neurons. *Endocr Regul.* 2012;46:13–20.
55. Sivukhina EV, Jirikowski GF. Magnocellular hypothalamic system and its interaction with the hypothalamo-pituitary-adrenal axis. *Steroids.* 2016;111:21–28.
56. Bouley R, Hasler U, Lu HA, Nunes P, Brown D. Bypassing vasopressin receptor signaling pathways in nephrogenic diabetes insipidus. *Semin Nephrol.* 2008;28:266–278.
57. Ong SL, Whitworth JA. How do glucocorticoids cause hypertension: role of nitric oxide deficiency, oxidative stress, and eicosanoids. *Endocrinol Metab Clin North Am.* 2011;40:393–407, ix.
58. Viinamaki O, Erkkola R, Kanto J. Plasma vasopressin concentrations and serum vasopressinase activity in pregnant and nonpregnant women. *Biol Res Pregn Perinatol.* 1986;7:17–19.
59. Chanson P, Salenave S. Diabetes insipidus and pregnancy. *Ann Endocrinol.* 2016;77:135–138.
60. Davison JM, Shiells EA, Barron WM, Robinson AG, Lindheimer MD. Changes in the metabolic clearance of vasopressin and in plasma vasopressinase throughout human pregnancy. *J Clin Invest.* 1989;83:1313–1318.
61. Iwasaki Y, Oiso Y, Kondo K, et al. Aggravation of subclinical diabetes insipidus during pregnancy [see comments]. *N Engl J Med.* 1991;324:522–526.
62. Kennedy S, Hall PM, Seymour AE, Hague WM. Transient diabetes insipidus and acute fatty liver of pregnancy. *Br J Obstet Gynaecol.* 1994;101:387–391.
63. Katz VL, Bowes Jr WA. Transient diabetes insipidus and pre-eclampsia. *Southern Med J.* 1987;80:524–525.
64. Durr JA, Hoggard JG, Hunt JM, Schrier RW. Diabetes insipidus in pregnancy associated with abnormally high circulating vasopressinase activity. *N Engl J Med.* 1987;316:1070–1074.
65. Davison JM, Shiells EA, Phillips PR, Barron WM, Lindheimer MD. Metabolic clearance of vasopressin and an analogue resistant to vasopressinase in human pregnancy. *Am J Physiol.* 1993;264:F348–F353.
66. Kregel J, Katz VL, Bowes Jr WA. Transient diabetes insipidus of pregnancy. *Obstet Gynecol Surv.* 1989;44:789–795.
67. Hirasawa A, Shibata K, Kotosai K, Tsujimoto G. Cloning, functional expression and tissue distribution of human cDNA for the vascular-type vasopressin receptor. *Biochem Biophys Res Comm.* 1994;203:72–79.
68. Morel A, O'Carroll AM, Brownstein MJ, Lolait SJ. Molecular cloning and expression of a rat V1a arginine vasopressin receptor. *Nature.* 1992;356:523–526.
69. Ostrowski NL, Young WS, Knepper MA, Lolait SJ. Expression of vasopressin V1a and V2 receptor messenger ribonucleic acid in the liver and kidney of embryonic, developing, and adult rats. *Endocrinology.* 1993;133:1849–1859.
70. Thibonnier M, Auzan C, Madhun Z, Wilkins P, Berti-Mattera L, Clauser E. Molecular cloning, sequencing, and functional expression of a cDNA encoding the human V1a vasopressin receptor. *J Biol Chem.* 1994;269:3304–3310.
71. Ostrowski NL, Lolait SJ, Bradley DJ, O'Carroll AM, Brownstein MJ. Distribution of V1a and V2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology.* 1992;131:533–535.
72. Johnson EM, Theler JM, Capponi AM, Vallotton MB. Characterization of oscillations in cytosolic free Ca²⁺ concentration and measurement of cytosolic Na⁺ concentration changes evoked by angiotensin II and vasopressin in individual rat aortic smooth

- muscle cells. Use of microfluorometry and digital imaging. *J Biol Chem.* 1991;266:12618–12626.
73. Takeuchi K, Abe K, Yasujima M, et al. Phosphoinositide hydrolysis and calcium mobilization induced by vasopressin and angiotensin II in cultured vascular smooth muscle cells. *Tohoku J Exp Med.* 1992;166:107–122.
 74. Spruce BA, McCulloch AJ, Burd J, Orskov H, Heaton A, Baylis PH. The effect of vasopressin infusion on glucose metabolism in man. *Clin Endocrinol (Oxf).* 1985;22:463–468.
 75. Inaba K, Umeda Y, Yamane Y, Urakami M, Inada M. Characterization of human platelet vasopressin receptor and the relation between vasopressin-induced platelet aggregation and vasopressin binding to platelets. *Clin Endocrinol (Oxf).* 1988;29:377–386.
 76. Briley EM, Lolait SJ, Axelrod J, Felder CC. The cloned vasopressin V1a receptor stimulates phospholipase A2, phospholipase C, and phospholipase D through activation of receptor-operated calcium channels. *Neuropeptides.* 1994;27:63–74.
 77. Montani JP, Liard JF, Schouin J, Mohring J. Hemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. *Circ Res.* 1980;47:346–355.
 78. Altura BM, Altura BT. Actions of vasopressin, oxytocin, and synthetic analogs on vascular smooth muscle. *Federation Proc.* 1984;43:80–86.
 79. Kimura T, Tanizawa O, Mori K, Brownstein MJ, Okayama H. Structure and expression of a human oxytocin receptor. *Nature.* 1992;356:526–529.
 80. Rozen F, Russo C, Banville D, Zingg HH. Structure, characterization, and expression of the rat oxytocin receptor gene. *Proc Natl Acad Sci U S A.* 1995;92:200–204.
 81. Aoyagi T, Birumachi J, Hiroshima M, et al. Alteration of glucose homeostasis in V1a vasopressin receptor-deficient mice. *Endocrinology.* 2007;148:2075–2084.
 82. Baertschi AJ, Friedli M. A novel type of vasopressin receptor on anterior pituitary corticotrophs. *Endocrinology.* 1985;116:499–502.
 83. de Keyser Y, Auzan C, Lenne F, Beldjord C, Thibonnier M, Bertagna X. Cloning and characterization of the human V3 pituitary vasopressin receptor. *FEBS Lett.* 1994;356:215–220.
 84. Tanoue A, Ito S, Honda K, et al. The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *J Clin Invest.* 2004;113:302–309.
 85. Sugimoto T, Saito M, Mochizuki S, Watanabe Y, Hashimoto S, Kawashima H. Molecular cloning and functional expression of a cDNA encoding the human V1b vasopressin receptor. *J Biol Chem.* 1994;269:27088–27092.
 86. Lolait SJ, Stewart LQ, Jessop DS, Young 3rd WS, O'Carroll AM. The hypothalamic-pituitary-adrenal axis response to stress in mice lacking functional vasopressin V1b receptors. *Endocrinology.* 2007;148:849–856.
 87. Roberts EM, Pope GR, Newson MJ, Lolait SJ, O'Carroll AM. The vasopressin V1b receptor modulates plasma corticosterone responses to dehydration-induced stress. *J Neuroendocrinol.* 2011;23:12–19.
 88. Wersinger SR, Kelliher KR, Zufall F, Lolait SJ, O'Carroll AM, Young 3rd WS. Social motivation is reduced in vasopressin 1b receptor null mice despite normal performance in an olfactory discrimination task. *Horm Behav.* 2004;46:638–645.
 89. Lolait SJ, O'Carroll AM, McBride OW, König M, Morel A, Brownstein MJ. Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. *Nature.* 1992;357:336–339.
 90. Hirsch AT, Dzau VJ, Majzoub JA, Creager MA. Vasopressin-mediated forearm vasodilation in normal humans. Evidence for a vascular vasopressin V2 receptor. *J Clin Invest.* 1989;84:418–426.
 91. Tagawa T, Imaizumi T, Endo T, Shiramoto M, Hirooka Y, Ando S. Vasodilatory effect of arginine vasopressin is mediated by nitric oxide in human forearm vessels. *J Clin Invest.* 1993;92:1483–1490.
 92. Kobrinsky NL, Israels ED, Gerrard JM, et al. Shortening of bleeding time by 1-deamino-8-D-arginine vasopressin in various bleeding disorders. *Lancet.* 1984;1:1145–1148.
 93. Birnbaumer M, Seibold A, Gilbert S, et al. Molecular cloning of the receptor for human antidiuretic hormone. *Nature.* 1992;357:333–335.
 94. Frattini A, Zucchi I, Villa A, et al. Type 2 vasopressin receptor gene, the gene responsible nephrogenic diabetes insipidus, maps to Xq28 close to the LICAM gene. *Biochem Biophys Res Comm.* 1993;193:864–871.
 95. Yun J, Schoneberg T, Liu J, et al. Generation and phenotype of mice harboring a nonsense mutation in the V2 vasopressin receptor gene. *J Clin Invest.* 2000;106:1361–1371.
 96. Harris HW, Strange K, Zeidel ML. Current understanding of the cellular biology and molecular structure of the antidiuretic hormone-stimulated water transport pathway. *J Clin Invest.* 1991;88:1–8.
 97. Brown D. The discovery of water channels (aquaporins). *Ann Nutr Metab.* 2017;70(Suppl 1):37–42.
 98. Nielsen S, Marples D, Birn H, et al. Expression of VAMP2-like protein in kidney collecting duct intracellular vesicles. *J Clin Invest.* 1995;96:1834–1844.
 99. Mandon B, Chou CL, Nielsen S, Knepper MA. Syntaxin-4 is localized to the apical plasma membrane of rat renal collecting duct cells: possible role in aquaporin-2 trafficking. *J Clin Invest.* 1996;98:906–913.
 100. Knepper MA. The aquaporin family of molecular water channels. *Proc Natl Acad Sci U S A.* 1994;91:6255–6258.
 101. Sasaki S, Fushimi K, Saito H, et al. Cloning, characterization, and chromosomal mapping of human aquaporin of collecting duct. *J Clin Invest.* 1994;93:1250–1256.
 102. Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science.* 1994;264:92–95.
 103. Stevens AL, Breton S, Gustafson CE, et al. Aquaporin 2 is a vasopressin-independent, constitutive apical membrane protein in rat vas deferens. *Am J Physiol.* 2000;278. C791–C802.
 104. Fushimi K, Sasaki S, Marumo F. Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J Biol Chem.* 1997;272:14800–14804.
 105. Valenti G, Procino G, Liebenhoff U, et al. A heterotrimeric G protein of the Gi family is required for cAMP-triggered trafficking of aquaporin 2 in kidney epithelial cells. *J Biol Chem.* 1998;273:22627–22634.
 106. Kanno K, Sasaki S, Hirata Y, et al. Urinary excretion of aquaporin-2 in patients with diabetes insipidus [see comments]. *N Engl J Med.* 1995;332:1540–1545.
 107. Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Neonatal mortality in an aquaporin-2 knock-in mouse model of recessive nephrogenic diabetes insipidus. *J Biol Chem.* 2001;276:2775–2779.
 108. Moeller HB, Fuglsang CH, Fenton RA. Renal aquaporins and water balance disorders. *Best Pract Res Clin Endocrinol Metabol.* 2016;30:277–288.
 109. Procino G, Mastrofrancesco L, Tamma G, et al. Calcium-sensing receptor and aquaporin 2 interplay in hypercalciuria-associated renal concentrating defect in humans. An in vivo and in vitro study. *PLoS One.* 2012;7(3):e33145.
 110. Ranieri M, Zahedi K, Tamma G, et al. CaSR signaling down-regulates AQP2 expression via a novel microRNA pathway in pendrin and NaCl cotransporter knockout mice. *FASEB J.* 2018;32:2148–2159.
 111. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J Clin Invest.* 1997;100:957–962.
 112. Ma T, Song Y, Yang B, et al. Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci USA.* 2000;97:4386–4391.
 113. Schnermann J, Chou CL, Ma T, Traynor T, Knepper MA, Verkman AS. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci USA.* 1998;95:9660–9664.
 114. Hori K, Nagai T, Izumi Y, et al. Vasopressin V1a receptor is required for nucleocytoplasmic transport of mineralocorticoid receptor. *Am J Physiol Renal Physiol.* 2012;303(7):F1080–F1088.
 115. Balla T, Baukal AJ, Eng S, Catt KJ. Angiotensin II receptor subtypes and biological responses in the adrenal cortex and medulla. *Mol Pharmacol.* 1991;40:401–406.

116. Aguilera G, Catt KJ. Loci of action of regulators of aldosterone biosynthesis in isolated glomerulosa cells. *Endocrinology*. 1979;104:1046–1052.
117. Bottari SP, de Gasparo M, Steckelings UM, Levens NR. Angiotensin II receptor subtypes: characterization, signalling mechanisms, and possible physiological implications. [Review]. *Front Neuroendocrinol*. 1993;14:123–171.
118. Bottari SP, Taylor V, King IN, Bogdal Y, Whitebread S, de Gasparo M. Angiotensin II AT2 receptors do not interact with guanine nucleotide binding proteins. *Eur J Pharmacol*. 1991;207:157–163.
119. Speth RC, Kim KH. Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine6 angiotensin II. *Biochem Biophys Res Comm*. 1990;169:997–1006.
120. Carey RM. Blood pressure and the renal actions of AT2 receptors. *Curr Hypertens Rep*. 2017;19:21.
121. Dzau VJ. Molecular and physiological aspects of tissue renin-angiotensin system: emphasis on cardiovascular control. [Review]. *J Hypertens Suppl*. 1988;6:S7–S12.
122. Vaidya A, Mulatero P, Baudrand R, Adler GK. The Expanding spectrum of primary aldosteronism: implications for diagnosis, pathogenesis, and treatment. *Endocr Rev*. 2018;39:1057–1088.
123. Chartier L, Schiffrin EL. Role of calcium in effects of atrial natriuretic peptide on aldosterone production in adrenal glomerulosa cells. *Am J Physiol*. 1987;252:E485–E491.
124. Chartier L, Schiffrin E, Thibault G, Garcia R. Atrial natriuretic factor inhibits the stimulation of aldosterone secretion by angiotensin II, ACTH and potassium in vitro and angiotensin II-induced steroidogenesis in vivo. *Endocrinology*. 1984;115:2026–2028.
125. Chartier L, Schiffrin EL. Atrial natriuretic peptide inhibits the effect of endogenous angiotensin II on plasma aldosterone in conscious sodium-depleted rats. *Clin Sci*. 1987;72:31–35.
126. Hausdorff WP, Aguilera G, Catt KJ. Inhibitory actions of somatostatin on cyclic AMP and aldosterone production in agonist-stimulated adrenal glomerulosa cells. *Cell Signal*. 1989;1:377–386.
127. Gallo-Payet N, Chouinard L, Balestre MN, Guillon G. Dual effects of dopamine in rat adrenal glomerulosa cells. *Biochem Biophys Res Comm*. 1990;172:1100–1108.
128. Dzau VJ, Hirsch AT. Emerging role of the tissue renin-angiotensin systems in congestive heart failure. *Eur Heart J*. 1990;11(Suppl B):65–71.
129. Ishikawa S, Saito T, Yoshida S. Effects of glucose and sodium chloride on the release of vasopressin in response to angiotensin II from the guinea pig. *Neuroendocrinology*. 1980;31:365–368.
130. de Bold AJ. Tissue fractionation studies on the relationship between an atrial natriuretic factor and specific atrial granules. *Can J Physiol Pharmacol*. 1982;60:324–330.
131. Yandle TG. Biochemistry of natriuretic peptides. [Review]. *J Int Med*. 1994;235:561–576.
132. Hamid Q, Wharton J, Terenghi G, et al. Localization of atrial natriuretic peptide mRNA and immunoreactivity in the rat heart and human atrial appendage. *Proc Natl Acad Sci U S A*. 1987;84:6760–6764.
133. Imura H, Nakao K, Itoh H. The natriuretic peptide system in the brain: implications in the central control of cardiovascular and neuroendocrine functions. *Front Neuroendocrinol*. 1992;13:217–249.
134. Ritter D, Chao J, Needeleman P, Tetens E, Greenwald JE. Localization, synthetic regulation, and biology of renal atriopeptin-like prohormone. *Am J Physiol*. 1992;263:F503–F509.
135. Sudoh T, Kanagawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. *Nature*. 1988;332:78–81.
136. Sudoh T, Maekawa K, Kojima M, Minamino N, Kangawa K, Matsuo H. Cloning and sequence analysis of cDNA encoding a precursor for human brain natriuretic peptide. *Biochem Biophys Res Comm*. 1989;159:1427–1434.
137. Sudoh T, Minamino N, Kanagawa K, Matsuo H. C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. *Biochem Biophys Res Comm*. 1990;168:863–870.
138. Espiner EA. Physiology of natriuretic peptides. [Review]. *J Int Med*. 1994;235:527–541.
139. Garbers DL, Koesling D, Schultz G. Guanylyl cyclase receptors. *Mol Biol Cell*. 1994;5:1–5.
140. Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H. Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science*. 1991;252:120–123.
141. Fuller F, Porter JG, Arfsten AE, et al. Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. *J Biol Chem*. 1988;263:9395–9401.
142. Maack T. Receptors of atrial natriuretic factor. *Annu Rev Physiol*. 1992;54:11–27.
143. Wilcox JN, Augustine A, Goeddel DV, Lowe DG. Differential regional expression of three natriuretic peptide genes within primate tissues. *Mol Cell Biol*. 1991;11:3454–3462.
144. Tallerico-Melnyk T, Yip CC, Watt VM. Widespread colocalization of messenger RNAs encoding the guanylate cyclase-coupled natriuretic peptide receptors in rat tissues. *Biochem Biophys Res Comm*. 1992;189:610–616.
145. Fujii T, Kojima S, Imanishi M, et al. Different mechanisms of polyuria and natriuresis associated with paroxysmal supraventricular tachycardia. *Am J Cardiol*. 1991;68:343.
146. Fujii T, Kojima S, Ohe T, et al. Dominance of blood pressure in natriuresis associated with supraventricular tachycardia. *Nephron*. 1991;57:262.
147. Kaye GC, Bayliss P, Lowry PJ, et al. Effect of induced supraventricular tachycardias on changes in urine and plasma hormone levels in man. *Clin Sci*. 1992;82:33.
148. Williams TD, Walsh KP, Lightman SL, Sutton R. Atrial natriuretic peptide inhibits postural release of renin and vasopressin in humans. *Am J Physiol*. 1988;255:R368–R372.
149. Bahr V, Sander-Bahr C, Ardevol R, Tuchelt H, Beland B, Oelkers W. Effects of atrial natriuretic factor on the renin-aldosterone system: in vivo and in vitro studies. *J Steroid Biochem Mol Biol*. 1993;45:173–178.
150. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*. 2012;122:1022–1036.
151. Wei C, Aarhus LL, Miller VM, Burnett JC. Action of C-type natriuretic peptide in isolated canine arteries and veins. *Am J Physiol*. 1993;264: H71–H73.
152. Clavell A, Stingo A, Wei C, Heublein D, Burnett J. C-type natriuretic peptide: a selective cardiovascular peptide. *Am J Physiol*. 1993;264:R290–R295.
153. Charles CJ, Richards AM, Espiner EA. Central C-type natriuretic peptide but not atrial natriuretic factor lowers blood pressure and adrenocortical secretion in normal conscious sheep. *Endocrinology*. 1992;131:1721–1726.
154. Samson WK, Skala KD, Huang FLS. CNP-22 stimulates, rather than inhibits, water drinking in the rat: evidence for a unique biological action of the C-type natriuretic peptides. *Brain Res*. 1991;568:285–288.
155. Peters JP, Welt LG, Sims EA, Orloff J, Needham J. A salt-wasting syndrome associated with cerebral disease. *Trans Assoc Am Physicians*. 1950;63:57–64.
156. Nelson PB, Seif SM, Maroon JC, Robinson AG. Hyponatremia in intracranial disease: perhaps not the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). *J Neurosurg*. 1981;55:938–941.
157. Wu X, Zhou X, Gao L, et al. Diagnosis and management of combined central diabetes insipidus and cerebral salt wasting syndrome after traumatic brain injury. *World Neurosurg*. 2016;88:48–37.
158. Rivkees SA. Differentiating appropriate antidiuretic hormone secretion, inappropriate antidiuretic hormone secretion and cerebral salt wasting: the common, uncommon, and misnamed. *Curr Opin Pediatr*. 2008;20:448–452.
159. Wijdicks EF, Ropper AH, Hunnicutt EJ, Richardson GS, Nathanson JA. Atrial natriuretic factor and salt wasting after aneurysmal subarachnoid hemorrhage. *Stroke*. 1991;22:1519–1524.
160. Verbalis JG, Goldsmith SR, Greenberg A, et al. Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. *Am J Med*. 2013;126: S1–S42.
161. Hannon MJ, Behan LA, O'Brien MM, et al. Hyponatremia following mild/moderate subarachnoid hemorrhage is due to SIAD and glucocorticoid deficiency and not cerebral salt wasting. *J Clin Endocrinol Metab*. 2014;99:291–298.

162. Brimioulle S, Orellana-Jimenez C, Aminian A, Vincent JL. Hyponatremia in neurological patients: cerebral salt wasting versus inappropriate antidiuretic hormone secretion. *Intensive Care Med.* 2008;34:125–131.
163. Jones DP. Syndrome of inappropriate secretion of antidiuretic hormone and hyponatremia. *Pediatr Rev.* 2018;39:27–35.
164. Moritz ML. Syndrome of inappropriate antidiuresis. *Pediatr Clin North Am.* 2019;66:209–226.
165. Assadi FK, John EG. Hypouricemia in neonates with syndrome of inappropriate secretion of antidiuretic hormone. *Pediatr Res.* 1985;19:424–427.
166. Ganong CA, Kappy MS. Cerebral salt wasting in children. The need for recognition and treatment [published erratum appears in *Am J Dis Child* 1993 Apr;147(4):369]. *Am J Dis Child.* 1993;147:167–169.
167. Fenske WK, Christ-Crain M, Horning A, et al. A copeptin-based classification of the osmoregulatory defects in the syndrome of inappropriate antidiuresis. *J Am Soc Nephrol.* 2014;25:2376–2383.
168. Frasier SD, Kutnik LA, Schmidt RT, Smith Jr FG. A water deprivation test for the diagnosis of diabetes insipidus in children. *Am J Dis Child.* 1967;114:157–160.
169. Richman RA, Post EM, Notman DD, Hochberg Z, Moses AM. Simplifying the diagnosis of diabetes insipidus in children. *Am J Dis Child.* 1981;135:839–841.
170. Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-dependent disorders of fluid homeostasis. *Nat Rev Endocrinol.* 2016;12:168–176.
171. Koskimies O, Pyllkanen J. Water intoxication in infants caused by the urine concentration test with the vasopressin analogue (DDAVP). *Acta Paediatr Scand.* 1984;73:131–132.
172. Bircan Z, Karacayir N, Cheong HI. A case of aquaporin 2 R85X mutation in a boy with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol.* 2008;23:663–665.
173. Bichet DG, Bockenhauer D. Genetic forms of nephrogenic diabetes insipidus (NDI): vasopressin receptor defect (X-linked) and aquaporin defect (autosomal recessive and dominant). *Best Pract Res Clin Endocrinol Metab.* 2016;30:263–276.
174. Milles JJ, Spruce B, Baylis PH. A comparison of diagnostic methods to differentiate diabetes insipidus from primary polyuria: a review of 21 patients. *Acta Endocrinol.* 1983;104:410–416.
175. Zerbe RL, Robertson GL. A comparison of plasma vasopressin measurements with a standard indirect test in the differential diagnosis of polyuria. *N Engl J Med.* 1981;305:1539–1546.
176. Andersson B, Leksell LG, Rundgren M. Regulation of water intake. [Review]. *Ann Rev Nutr.* 1982;2:73–89.
177. Fenske W, Allolio B. Current state and future perspectives in the diagnosis of diabetes insipidus: a clinical review. *J Clin Endocrinol Metab.* 2012;97:3426–3437.
178. Papapostolou C, Mantzoros CS, Evagelopoulou C, Moses AC, Kleefeld J. Imaging of the sella in the syndrome of inappropriate secretion of antidiuretic hormone. *J Int Med.* 1995;237:181–185.
179. Adams NC, Farrell TP, O’Shea A, et al. Neuroimaging of central diabetes insipidus—when, how and findings. *Neuroradiology.* 2018;60:995–1012.
180. Halimi P, Sigal R, Doyon D, Delivet S, Bouchard P, Pigeau I. Post-traumatic diabetes insipidus: MR demonstration of pituitary stalk rupture. *J Comput Assist Tomogr.* 1988;12:135–137.
181. Maghnie M, Villa A, Arico M, et al. Correlation between magnetic resonance imaging of posterior pituitary and neurohypophyseal function in children with diabetes insipidus. *J Clin Endocrinol Metab.* 1992;74:795–800.
182. Moses AM, Clayton B, Hochhauser L. Use of T1-weighted MR imaging to differentiate between primary polydipsia and central diabetes insipidus [comment] [see comments]. *Am J Neuroradiol.* 1992;13:1273–1277.
183. Hayashi Y, Kita D, Watanabe T, et al. Prediction of postoperative diabetes insipidus using morphological hyperintensity patterns in the pituitary stalk on magnetic resonance imaging after transphenoidal surgery for sellar tumors. *Pituitary.* 2016;19:552–559.
184. Maghnie M, Genovese E, Arico M, Villa A, Beluffi G, Campani R. Evolving pituitary hormone deficiency is associated with pituitary vasculopathy: dynamic MR study in children with hypopituitarism, diabetes insipidus, and Langerhans cell histiocytosis. *Radiology.* 1994;193:493–499.
185. Sato N, Ishizaka H, Yagi H, Matsumoto M, Endo K. Posterior lobe of the pituitary in diabetes insipidus: dynamic MR imaging. *Radiology.* 1993;186:357–360.
186. Winzeler B, Zweifel C, Nigro N, et al. Postoperative copeptin concentration predicts diabetes insipidus after pituitary surgery. *J Clin Endocrinol Metab.* 2015;100:2275–2282.
187. Medani CR. Seizures and hypothermia due to dietary water intoxication in infants. *Southern Med J.* 1987;80:421–425.
188. Davidson S, Frand M, Rotem Y. Primary polydipsia in infancy: a benign disorder simulating diabetes insipidus. *Clin Pediatr.* 1978;17:419–420.
189. Kovacs L, Sulyok E, Lichardus B, Mihajlovskij N, Bircak J. Renal response to arginine vasopressin in premature infants with late hyponatraemia. *Arch Dis Child.* 1986;61:1030–1032.
190. Robertson GL. Dipsogenic diabetes insipidus: a newly recognized syndrome caused by a selective defect in the osmoregulation of thirst. *Trans Assoc Am Physicians.* 1987;100:241–249.
191. Green HH, Harrington AR, Valtin H. On the role of antidiuretic hormone in the inhibition of acute water diuresis in adrenal insufficiency and the effects of gluco- and mineralocorticoids in reversing the inhibition. *J Clin Invest.* 1970;49:1724–1736.
192. Ahmed AB, George BC, Gonzalez-Auvert C, Dingman JF. Increased plasma arginine vasopressin in clinical adrenocortical insufficiency and its inhibition by glucosteroids. *J Clin Invest.* 1967;46:111–123.
193. Dingman JF, Despointes RH. Adrenal steroid inhibition of vasopressin release from the neurohypophysis of normal subjects and patients with Addison’s disease. *J Clin Invest.* 1960;39:1851–1863.
194. Iwasaki Y, Oiso Y, Saito H, Majzoub JA. Positive and negative regulation of the rat vasopressin gene promoter. *Endocrinology.* 1997;138:5266–5274.
195. Kleeman CR, Czaczkes JW, Cutler R. Mechanisms of impaired water excretion in adrenal and pituitary insufficiency. IV. Antidiuretic hormone in primary and secondary adrenal insufficiency. *J Clin Invest.* 1964;43:1641–1648.
196. Kamoi K, Tamura T, Tanaka K, Ishibashi M, Yamaji T. Hyponatremia and osmoregulation of thirst and vasopressin secretion in patients with adrenal insufficiency. *J Clin Endocrinol Metab.* 1993;77:1584–1588.
197. Laczfi F, Janaky T, Ivanyi T, Julesz J, Laszlo FA. Osmoregulation of arginine-8-vasopressin secretion in primary hypothyroidism and in Addison’s disease. *Acta Endocrinol.* 1987;114:389–395.
198. Bouley R, Breton S, Sun T, et al. Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. *J Clin Invest.* 2000;106:1115–1126.
199. Wallerath T, Witte K, Schafer SC, et al. Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proc Natl Acad Sci U S A.* 1999;96:13357–13362.
200. Schwartz MJ, Kokko JP. Urinary concentrating defect of adrenal insufficiency. Permissive role of adrenal steroids on the hydro-osmotic response across the rabbit cortical collecting tubule. *J Clin Invest.* 1980;66:234–242.
201. Schrier RW, Berl T. Nonosmolar factors affecting renal water excretion (first of two parts). [Review]. *N Engl J Med.* 1975;292:81–88.
202. Iwasaki Y, Oiso Y, Yamauchi K, Takatsuki K, Kondo K, Hasegawa H. Osmoregulation of plasma vasopressin in myxedema. *J Clin Endocrinol Metab.* 1990;70:534–539.
203. Discala VA, Kinney MJ. Effects of myxedema in the renal diluting and concentrating mechanism. *Am J Med.* 1971;50:325–335.
204. Michael UF, Barenberg RL, Chavez R, Vaamonde CA, Papper S. Renal handling of sodium and water in the hypothyroid rat. Clearance and micropuncture studies. *J Clin Invest.* 1972;51:1405–1412.
205. Yount E, Little JM. Renal clearance in patients with myxedema. *J Clin Endocrinol Metab.* 1955;15:343–346.
206. Iwasaki Y, Kondo K, Hasegawa H, Oiso Y. Osmoregulation of plasma vasopressin in three cases with adrenal insufficiency of diverse etiologies. *Horm Res.* 1997;47:38–44.
207. Yamada K, Tamura Y, Yoshida S. Effect of administration of corticotropin-releasing hormone and glucocorticoid on arginine vasopressin response to osmotic stimulus in normal subjects and patients with hypocorticotropinism without overt diabetes insipidus. *J Clin Endocrinol Metab.* 1989;69:396–401.

208. Kamiyama T, Iseki K, Kawazoe N, Takishita S, Fukiyama K. Carbamazepine-induced hyponatremia in a patient with partial central diabetes insipidus. *Nephron*. 1993;64:142–145.
209. Van Amelsvoort T, Bakshi R, Devaux CB, Schwabe S. Hyponatremia associated with carbamazepine and oxcarbazepine therapy: a review. [Review]. *Epilepsia*. 1994;35:181–188.
210. Lozada ES, Gouaux J, Franki N, Appel GB, Hays RM. Studies of the mode of action of the sulfonylureas and phenylacetamides in enhancing the effect of vasopressin. *J Clin Endocrinol Metab*. 1972;34:704–712.
211. Moses AM, Numann P, Miller M. Mechanism of chlorpropamide-induced antidiuresis in man: evidence for release of ADH and enhancement of peripheral action. *Metabolism*. 1973;22:59–66.
212. Bressler RB, Huston DP. Water intoxication following moderate-dose intravenous cyclophosphamide. *Arch Int Med*. 1985;145:548–549.
213. Harlow PJ, DeClerck YA, Shore NA, Ortega JA, Carranza A, Heuser E. A fatal case of inappropriate ADH secretion induced by cyclophosphamide therapy. *Cancer*. 1979;44:896–898.
214. Larose P, Ong H, du Souich P. The effect of cyclophosphamide on arginine vasopressin and the atrial natriuretic factor. *Biochem Biophys Res Comm*. 1987;143:140–144.
215. Bode U, Seif SM, Levine AS. Studies on the antidiuretic effect of cyclophosphamide: vasopressin release and sodium excretion. *Med Pediatr Oncol*. 1980;8:295–303.
216. Zavagli G, Ricci G, Tataranni G, Mapelli G, Abbasciano V. Life-threatening hyponatremia caused by vinblastine. *Med Oncol Tumor Pharmacother*. 1988;5:67–69.
217. Hutchison FN, Perez EA, Gandara DR, Lawrence HJ, Kaysen GA. Renal salt wasting in patients treated with cisplatin. *Ann Int Med*. 1988;108:21–25.
218. Ritch PS. Cis-dichlorodiammineplatinum II-induced syndrome of inappropriate secretion of antidiuretic hormone. *Cancer*. 1988;61:448–450.
219. Gerigk M, Bald M, Feth F, Rascher W. Clinical settings and vasopressin function in hyponatraemic children. *Eur J Pediatr*. 1993;152:301–305.
220. Judd BA, Haycock GB, Dalton N, Chantler C. Hyponatraemia in premature babies and following surgery in older children. *Acta Paediatr Scand*. 1987;76:385–393.
221. Anderson RJ, Chung HM, Kluge R, Schrier RW. Hyponatremia: a prospective analysis of its epidemiology and the pathogenetic role of vasopressin. *Ann Int Med*. 1985;102:164–168.
222. Kanakriyeh M, Carvajal HF, Vallone AM. Initial fluid therapy for children with meningitis with consideration of the syndrome of inappropriate anti-diuretic hormone. *Clin Pediatr*. 1987;26:126–130.
223. Padilla G, Ervin MG, Ross MG, Leake RD. Vasopressin levels in infants during the course of aseptic and bacterial meningitis [see comments]. *Am J Dis Child*. 1991;145:991–993.
224. Tagarro A, Martin MD, Del-Amo N, et al. Hyponatremia in children with pneumonia rarely means SIADH. *Paediatr Child Health*. 2018;23:e126–e133.
225. Powell KR, Sugarman LI, Eskenazi AE, et al. Normalization of plasma arginine vasopressin concentrations when children with meningitis are given maintenance plus replacement fluid therapy [see comments]. *J Pediatr*. 1990;117:515–522.
226. Vingerhoets F, de Tribolet N. Hyponatremia hypo-osmolarity in neurosurgical patients. “Appropriate secretion of ADH” and “cerebral salt wasting syndrome”. *Acta Neurochir*. 1988;91:50–54.
227. Neville KA, Verge CF, O’Meara MW, Walker JL. High antidiuretic hormone levels and hyponatremia in children with gastroenteritis. *Pediatrics*. 2005;116:1401–1407.
228. Neville KA, Verge CF, Rosenberg AR, O’Meara MW, Walker JL. Isotonic is better than hypotonic saline for intravenous rehydration of children with gastroenteritis: a prospective randomised study. *Arch Dis Child*. 2006;91:226–232.
229. Choong K, Kho ME, Menon K, Bohn D. Hypotonic versus isotonic saline in hospitalised children: a systematic review. *Arch Dis Child*. 2006;91:828–835.
230. Cavari Y, Pitfield AF, Kisson N. Intravenous maintenance fluids revisited. *Pediatr Emerg Care*. 2013;29:1225–1228, quiz 9–31.
231. Oh GJ, Sutherland SM. Perioperative fluid management and postoperative hyponatremia in children. *Pediatr Nephrol*. 2016;31:53–60.
232. McNab S, Ware RS, Neville KA, et al. Isotonic versus hypotonic solutions for maintenance intravenous fluid administration in children. *Cochrane Database Syst Rev*. 2014. Cd009457.
233. Zanardo V, Ronconi M, Ferri N, Zacchello G. Plasma arginine vasopressin, diuresis, and neonatal respiratory distress syndrome. *Pediatr Padol*. 1989;24:297–302.
234. Potts FL, May RB. Early syndrome of inappropriate secretion of antidiuretic hormone in a child with burn injury. *Ann Emerg Med*. 1986;15:834–835.
235. Kojima T, Fukuda Y, Hirata Y, Matsuzaki S, Kobayashi Y. Changes in vasopressin, atrial natriuretic factor, and water homeostasis in the early stage of bronchopulmonary dysplasia. *Pediatr Res*. 1990;27:260–263.
236. Rao M, Eid N, Herrod L, Parekh A, Steiner P. Antidiuretic hormone response in children with bronchopulmonary dysplasia during episodes of acute respiratory distress. *Am J Dis Child*. 1986;140:825–828.
237. Sulyok E, Kovacs L, Lichardus B, et al. Late hyponatremia in premature infants: role of aldosterone and arginine vasopressin. *J Pediatr*. 1985;106:990–994.
238. Cohen LF, di Sant’Agnese PA, Taylor A, Gill Jr JR. The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis. *J Pediatr*. 1977;90:574–578.
239. Stegner H, Caspers S, Niggemann B, Commentz J. Urinary arginine-vasopressin (AVP) excretion in cystic fibrosis (CF). *Acta Endocrinol, Supplementum*. 1986;279:448–451.
240. Arisaka O, Shimura N, Hosaka A, Nakayama Y, Kaneko K, Maeda M. Water intoxication in asthma assessed by urinary arginine vasopressin. *Eur J Pediatr*. 1988;148:167–169.
241. Iikura Y, Odajima Y, Akazawa A, Nagakura T, Kishida M, Akimoto K. Antidiuretic hormone in acute asthma in children: effects of medication on serum levels and clinical course. *Allergy Proc*. 1989;10:197–201.
242. Schrier RW, Berl T. Nonosmolar factors affecting renal water excretion (second of two parts). *N Engl J Med*. 1975;292:141–145.
243. O’Rahilly S. Secretion of antidiuretic hormone in hyponatraemia: not always “inappropriate”. *Br Med J Clin Res Ed*. 1985;290:1803–1804.
244. Kamel KS, Bear RA. Treatment of hyponatremia: a quantitative analysis. *Am J Kidney Dis*. 1993;21:439–443.
245. Schrier RW. Treatment of hyponatremia (editorial). *N Engl J Med*. 1985;312:1121–1123.
246. Videen JS, Michaelis T, Pinto P, Ross BD. Human cerebral osmoles during chronic hyponatremia. A proton magnetic resonance spectroscopy study. *J Clin Invest*. 1995;95:788–793.
247. Ayus JC, Arieff AI. Pathogenesis and prevention of hyponatremic encephalopathy. [Review]. *Endocrinol Metabol Clin North Am*. 1993;22:425–446.
248. Strange K. Regulation of solute and water balance and cell volume in the central nervous system [editorial]. [Review]. *J Am Soc Nephrol*. 1992;3:12–27.
249. Sterns RH, Riggs JE, Schochet Jr SS. Osmotic demyelination syndrome following correction of hyponatremia. *N Engl J Med*. 1986;314:1535–1542.
250. Ayus JC, Krothapalli RK, Arieff AI. Changing concepts in treatment of severe symptomatic hyponatremia. Rapid correction and possible relation to central pontine myelinolysis. *Am J Med*. 1985;78:897–902.
251. Ayus JC, Krothapalli RK, Arieff AI. Treatment of symptomatic hyponatremia and its relation to brain damage. A prospective study. *N Engl J Med*. 1987;317:1190–1195.
252. Sklar C, Fertig A, David R. Chronic syndrome of inappropriate secretion of antidiuretic hormone in childhood. *Am J Dis Child*. 1985;139:733–735.
253. Tang TT, Whelan HT, Meyer GA, Strother DR, Blank EL, Camitta BM. Optic chiasm glioma associated with inappropriate secretion of antidiuretic hormone, cerebral ischemia, non-obstructive hydrocephalus and chronic ascites following ventriculoperitoneal shunting. *Childs Nervous Syst*. 1991;7:458–461.
254. Padilla G, Leake JA, Castro R, Ervin MG, Ross MG, Leake RD. Vasopressin levels and pediatric head trauma. *Pediatrics*. 1989;83:700–705.
255. Cizmeci MN, Kanburoglu MK, Akelma AZ, Donmez A, Duymaz S, Tatli MM. Syndrome of inappropriate antidiuretic hormone

- secretion refractory to treatment in a newborn with alobar holoprosencephaly. *Genet Couns (Geneva, Switzerland)*. 2013;24:313–318.
256. Goldman MB, Luchins DJ, Robertson GL. Mechanisms of altered water metabolism in psychotic patients with polydipsia and hyponatremia. *N Engl J Med*. 1988;318:397–403.
 257. Meierkord H, Shorvon S, Lightman SL. Plasma concentrations of prolactin, noradrenaline, vasopressin and oxytocin during and after a prolonged epileptic seizure. *Acta Neurol Scand*. 1994;90:73–77.
 258. Edwards CM, Carmichael J, Baylis PH, Harris AL. Arginine vasopressin—a mediator of chemotherapy induced emesis? *Br J Cancer*. 1989;59:467–470.
 259. Coslovsky R, Bruck R, Estrov Z. Hypo-osmolal syndrome due to prolonged nausea. *Arch Intern Med*. 1984;144:191–192.
 260. Dhawan A, Narang A, Singhi S. Hyponatraemia and the inappropriate ADH syndrome in pneumonia. *Ann Trop Paediatr*. 1992;12:455–462.
 261. van Steensel-Moll HA, Hazelzet JA, van der Voort E, Neijens HJ. Excessive secretion of antidiuretic hormone in infections with respiratory syncytial virus. *Arch Dis Child*. 1990;65:1237–1239.
 262. Tang WW, Kaptein EM, Feinstein EI, Massry SG. Hyponatremia in hospitalized patients with the acquired immunodeficiency syndrome (AIDS) and the AIDS-related complex. *Am J Med*. 1993;94:169–174.
 263. Weissman PN, Shenkman L, Gregerman RI. Chlorpropamide hyponatremia: drug-induced inappropriate antidiuretic-hormone activity. *N Engl J Med*. 1971;284:65–71.
 264. Escuro RS, Adelstein DJ, Carter SG. Syndrome of inappropriate secretion of antidiuretic hormone after infusional vincristine. *Cleve Clin J Med*. 1992;59:643–644.
 265. Liskin B, Walsh BT, Roose SP, Jackson W. Imipramine-induced inappropriate ADH secretion. *J Clin Psychopharmacol*. 1984;4:146–147.
 266. Parker WA. Imipramine-induced syndrome of inappropriate antidiuretic hormone secretion. *Drug Intelligence Clin Pharma*. 1984;18:890–894.
 267. Moses AM, Howanitz J, Miller M. Diuretic action of three sulfonylurea drugs. *Ann Int Med*. 1973;78:541–544.
 268. Misra UK, Kalita J, Bhoi SK, Singh RK. A study of hyponatremia in tuberculous meningitis. *J Neurol Sci*. 2016;367:152–157.
 269. Cotton MF, Donald PR, Schoeman JF, Aalbers C, Van Zyl LE, Lombard C. Plasma arginine vasopressin and the syndrome of inappropriate antidiuretic hormone secretion in tuberculous meningitis [see comments]. *Pediatr Infect Dis J*. 1991;10:837–842.
 270. Cotton MF, Donald PR, Schoeman JF, Van Zyl LE, Aalbers C, Lombard CJ. Raised intracranial pressure, the syndrome of inappropriate antidiuretic hormone secretion, and arginine vasopressin in tuberculous meningitis. *Child Nerv Syst*. 1993;9:10–15, discussion.
 271. Hill AR, Uribarri J, Mann J, Berl T. Altered water metabolism in tuberculosis: role of vasopressin [see comments]. *Am J Med*. 1990;88:357–364.
 272. Olson BR, Rubino D, Gumowski J, Oldfield EH. Isolated hyponatremia after transsphenoidal pituitary surgery. *J Clin Endocrinol Metab*. 1995;80:85–91.
 273. Sane T, Rantakari K, Poranen A, Tahtela R, Valimaki M, Pelkonen R. Hyponatremia after transsphenoidal surgery for pituitary tumors. *J Clin Endocrinol Metab*. 1994;79:1395–1398.
 274. Oelkers W. Hyponatremia and inappropriate secretion of vasopressin (antidiuretic hormone) in patients with hypopituitarism [see comments]. *N Engl J Med*. 1989;321:492–496.
 275. Crigler Jr JF. Commentary: On the use of pitressin in infants with neurogenic diabetes insipidus. *J Pediatr*. 1976;88:295–296.
 276. Smith TJ, Gill JC, Ambruso DR, Hathaway WE. Hyponatremia and seizures in young children given DDAVP. *Am J Hematol*. 1989;31:199–202.
 277. Feldman BJ, Rosenthal SM, Vargas GA, et al. Nephrogenic syndrome of inappropriate antidiuresis. *N Engl J Med*. 2005;352:1884–1890.
 278. Morello JP, Bichet DG. Nephrogenic diabetes insipidus. *Annu Rev Physiol*. 2001;63:607–630.
 279. Vanderghyest F, Brachet C, Heinrichs C, Decaux G. Long-term treatment of hyponatremic patients with nephrogenic syndrome of inappropriate antidiuresis: personal experience and review of published case reports. *Nephron Clin Pract*. 2012;120: c168–172.
 280. Greenberg JH, Tufro A, Marsenic O. Approach to the treatment of the infant with hyponatremia. *Am J Kidney Dis*. 2015;65:513–517.
 281. Brachet C, Vanderghyest F, Heinrichs C. Nephrogenic syndrome of inappropriate antidiuresis in a female neonate: review of the clinical presentation in females. *Horm Res Paediatr*. 2015;84:65–67.
 282. Powlson AS, Challis BG, Halsall DJ, Schoenmakers E, Gurnell M. Nephrogenic syndrome of inappropriate antidiuresis secondary to an activating mutation in the arginine vasopressin receptor AVPR2. *Clin Endocrinol (Oxf)*. 2016;85:306–312.
 283. Peters S, Kuhn R, Gardner B, Bernard P. Use of conivaptan for refractory syndrome of inappropriate secretion of antidiuretic hormone in a pediatric patient. *Pediatr Emerg Care*. 2013;29:230–232.
 284. Marx-Berger D, Milford DV, Bandhakavi M, et al. Tolvaptan is successful in treating inappropriate antidiuretic hormone secretion in infants. *Acta Paediatr (Oslo, Norway)*. 2016;105: e334–337.
 285. Tuli G, Tessaris D, Einaudi S, De Sanctis L, Matarazzo P. Tolvaptan treatment in children with chronic hyponatremia due to inappropriate antidiuretic hormone secretion: a report of three cases. *J Clin Res Pediatr Endocrinol*. 2017;9:288–292.
 286. Decaux G. Long-term treatment of patients with inappropriate secretion of antidiuretic hormone by the vasopressin receptor antagonist conivaptan, urea, or furosemide. *Am J Med*. 2001;110:582–584.
 287. Berl T. Vasopressin antagonists. *N Engl J Med*. 2015;372:2207–2216.
 288. Decaux G, Soupart A, Vassart G. Non-peptide arginine-vasopressin antagonists: the vaptans. *Lancet*. 2008;371:1624–1632.
 289. Imamura T, Kinugawa K. Update of acute and long-term tolvaptan therapy. *J Cardiol*. 2019;73:102–107.
 290. Soupart A, Gross P, Legros JJ, et al. Successful long-term treatment of hyponatremia in syndrome of inappropriate antidiuretic hormone secretion with satavaptan (SR121463B), an orally active nonpeptide vasopressin V2-receptor antagonist. *Clin J Am Soc Nephrol*. 2006;1:1154–1160.
 291. Rianthavorn P, Cain JP, Turman MA. Use of conivaptan to allow aggressive hydration to prevent tumor lysis syndrome in a pediatric patient with large-cell lymphoma and SIADH. *Pediatr Nephrol*. 2008;23:1367–1370.
 292. Decaux G. V2-antagonists for the treatment of hyponatraemia. *Nephrol Dial Transplant*. 2007;22:1853–1855.
 293. Kamoi K, Ebe T, Kobayashi O, et al. Atrial natriuretic peptide in patients with the syndrome of inappropriate antidiuretic hormone secretion and with diabetes insipidus. *J Clin Endocrinol Metab*. 1990;70:1385–1390.
 294. Manooogian C, Pandian M, Ehrlich L, Fisher D, Horton R. Plasma atrial natriuretic hormone levels in patients with the syndrome of inappropriate antidiuretic hormone secretion. *J Clin Endocrinol Metab*. 1988;67:571–575.
 295. Kojima T, Hirata Y, Umeda Y, et al. Role of atrial natriuretic peptide in the diuresis of a newborn infant with the syndrome of inappropriate antidiuretic hormone secretion. *Acta Paediatr Scand*. 1989;78:793–796.
 296. Isotani E, Suzuki R, Tomita K, et al. Alterations in plasma concentrations of natriuretic peptides and antidiuretic hormone after subarachnoid hemorrhage. *Stroke*. 1994;25:2198–2203.
 297. Dinger M, Ladenson PW, Borel C, Hart GK, Kirsch JR, Hanley DF. Sodium and water regulation in a patient with cerebral salt wasting. *Arch Neurol*. 1989;46:928–930.
 298. Brimiouille S, Orellana-Jimenez C, Aminian A, Vincent JL. Hyponatremia in neurological patients: cerebral salt wasting versus inappropriate antidiuretic hormone secretion. *Intensive Care Med*. 2008;34:125–131.
 299. Vokes TP, Aycinena PR, Robertson GL. Effect of insulin on osmoregulation of vasopressin. *Am J Physiol*. 1987;252:E538–E548.
 300. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. *Diabetes*. 1979;28:503–508.
 301. Ishikawa S, Saito T, Okada K, Nagasaka S, Kuzuya T. Prompt recovery of plasma arginine vasopressin in diabetic coma after intravenous infusion of a small dose of insulin and a large amount of fluid. *Acta Endocrinol*. 1990;122:455–461.

302. Tulassay T, Rascher W, Korner A, Miltenyi M. Atrial natriuretic peptide and other vasoactive hormones during treatment of severe diabetic ketoacidosis in children. *J Pediatr*. 1987;111:329–334.
303. Zerbe RL, Vinicor F, Robertson GL. Regulation of plasma vasopressin in insulin-dependent diabetes mellitus. *Am J Physiol*. 1985;249:E317–E325.
304. Di Iorgi N, Morana G, Napoli F, Allegrì AE, Rossi A, Maghnie M. Management of diabetes insipidus and adipsia in the child. *Best Pract Res Clin Endocrinol Metabol*. 2015;29:415–436.
305. Dabrowski E, Kadakia R, Zimmerman D. Diabetes insipidus in infants and children. *Best Pract Res Clin Endocrinol Metabol*. 2016;30:317–328.
306. Greger NG, Kirkland RT, Clayton GW, Kirkland JL. Central diabetes insipidus. 22 years' experience. *Am J Dis Child*. 1986;140:551–554.
307. Wang LC, Cohen ME, Duffner PK. Etiologies of central diabetes insipidus in children. *Pediatr Neurol*. 1990;11:273–277.
308. Werny D, Elfers C, Perez FA, Pihoker C, Roth CL. Pediatric central diabetes insipidus: brain malformations are common and few patients have idiopathic disease. *J Clin Endocrinol Metab*. 2015;100:3074–3080.
309. De Los Santos MA, Aguila CM, Rojas MI, et al. Central diabetes insipidus: clinical profile that suggests organicity in Peruvian children: Lima - Peru 2001-2013. *J Pediatr Endocrinol Metab*. 2016;29:1353–1358.
310. Richards GE, Thomsett MJ, Boston BA, DiMeglio LA, Shulman DI, Draznin M. Natural history of idiopathic diabetes insipidus. *J Pediatr*. 2011;159:566–570.
311. Pedersen EB, Lamm LU, Albertsen K, et al. Familial cranial diabetes insipidus: a report of five families. *Genetic, diagnostic and therapeutic aspects Q J Med*. 1985;57:883–896.
312. Tian D, Cen J, Nie M, Gu F. Identification of five novel arginine vasopressin gene mutations in patients with familial neurohypophyseal diabetes insipidus. *Int J Mol Med*. 2016;38:1243–1249.
313. Perrotta S, Di Iorgi N, Ragione FD, et al. Early-onset central diabetes insipidus is associated with de novo arginine vasopressin-neurophysin II or Wolfram syndrome 1 gene mutations. *Eur J Endocrinol*. 2015;172:461–472.
314. Rutishauser J, Spiess M, Kopp P. Genetic forms of neurohypophyseal diabetes insipidus. *Best Pract Res Clin Endocrinol Metabol*. 2016;30:249–262.
315. Hrckova G, Janko V, Kytarova J, et al. Two novel mutations in seven Czech and Slovak kindreds with familial neurohypophyseal diabetes insipidus-benefit of genetic testing. *Eur J Pediatr*. 2016;175:1199–1207.
316. Schernthaner-Reiter MH, Stratakis CA, Luger A. Genetics of diabetes insipidus. *Endocrinol Metab Clin North Am*. 2017;46:305–334.
317. Koufaris C, Alexandrou A, Sismani C, Skordis N. Identification of an AVP-NPII mutation within the AVP moiety in a family with neurohypophyseal diabetes insipidus: review of the literature. *Hormones (Athens, Greece)*. 2015;14:442–446.
318. Turkkahraman D, Saglar E, Karaduman T, Mergen H. AVP-NPII gene mutations and clinical characteristics of the patients with autosomal dominant familial central diabetes insipidus. *Pituitary*. 2015;18:898–904.
319. Os I, Aakesson I, Enger E. Plasma vasopressin in hereditary cranial diabetes insipidus. *Acta Med Scand*. 1985;217:429–434.
320. Toth EL, Bowen PA, Crockford PM. Hereditary central diabetes insipidus: plasma levels of antidiuretic hormone in a family with a possible osmoreceptor defect. *Can Med Assoc J*. 1984;131:1237–1241.
321. Nagai I, Li CH, Hsieh SM, Kizaki T, Urano Y. Two cases of hereditary diabetes insipidus, with an autopsy finding in one. *Acta Endocrinol*. 1984;105:318–323.
322. Bergeron C, Kovacs K, Ezrin C, Mizzen C. Hereditary diabetes insipidus: an immunohistochemical study of the hypothalamus and pituitary gland. *Acta Neuropathol*. 1991;81:345–348.
323. Babey M, Kopp P, Robertson GL. Familial forms of diabetes insipidus: clinical and molecular characteristics. *Nat Rev Endocrinol*. 2011;7:701–714.
324. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet*. 2017;136:665–677.
325. Willcutts MD, Felner E, White PC. Autosomal recessive familial neurohypophyseal diabetes insipidus with continued secretion of mutant weakly active vasopressin. *Hum Mol Genet*. 1999;8:1303–1307.
326. Bourdet K, Vallette S, Deladoey J, Van Vliet G. Early-onset central diabetes insipidus due to compound heterozygosity for AVP mutations. *Horm Res Paediatr*. 2016;85:283–287.
327. Ito M, Yu RN, Jameson JL. Mutant vasopressin precursors that cause autosomal dominant neurohypophyseal diabetes insipidus retain dimerization and impair the secretion of wild-type proteins. *J Biol Chem*. 1999;274:9029–9037.
328. Ito M, Jameson JL. Molecular basis of autosomal dominant neurohypophyseal diabetes insipidus. Cellular toxicity caused by the accumulation of mutant vasopressin precursors within the endoplasmic reticulum. *J Clin Invest*. 1997;99:1897–1905.
329. Russell TA, Ito M, Ito M, et al. A murine model of autosomal dominant neurohypophyseal diabetes insipidus reveals progressive loss of vasopressin-producing neurons. *J Clin Invest*. 2003;112:1697–1706.
330. Russell TA, Ito M, Yu RN, Martinson FA, Weiss J, Jameson JL. A murine model of autosomal dominant neurohypophyseal diabetes insipidus reveals progressive loss of vasopressin-producing neurons. *J Clin Invest*. 2003;112:1697–1706.
331. Thompson CJ, Charlton J, Walford S, et al. Vasopressin secretion in the DIDMOAD (Wolfram) syndrome. *Q J Med*. 1989;71:333–345.
332. Grosse Aldenhovel HB, Gallenkamp U, Sulemana CA. Juvenile onset diabetes mellitus, central diabetes insipidus and optic atrophy (Wolfram syndrome)—neurological findings and prognostic implications. *Neuropediatr*. 1991;22:103–106.
333. Papadimitriou DT, Manolagos E, Bothou C, et al. Maternal uniparental disomy of chromosome 4 and homozygous novel mutation in the WFS1 gene in a paediatric patient with Wolfram syndrome. *Diabetes Metabol*. 2015;41:433–435.
334. Akturk HK, Yasa S. Previously unreported abnormalities in Wolfram Syndrome Type 2. *Pediatr Endocrinol Diabetes Metabol*. 2017;23:107–110.
335. Khanim F, Kirk J, Latif F, Barrett TG. WFS1/wolframin mutations, Wolfram syndrome, and associated diseases. *Hum Mutat*. 2001;17:357–367.
336. Osman AA, Saito M, Makepeace C, Permutt MA, Schlesinger P, Mueckler M. Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J Biol Chem*. 2003;278:52755–52762.
337. Riggs AC, Bernal-Mizrachi E, Ohsugi M, et al. Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. *Diabetologia*. 2005;48:2313–2321.
338. Smith CJ, Crock PA, King BR, Meldrum CJ, Scott RJ. Phenotype-genotype correlations in a series of wolfram syndrome families. *Diabetes Care*. 2004;27:2003–2009.
339. Zalloua PA, Azar ST, Delepine M, et al. WFS1 mutations are frequent monogenic causes of juvenile-onset diabetes mellitus in Lebanon. *Hum Mol Genet*. 2008;17:4012–4021.
340. Farmer A, Ayme S, de Heredia ML, et al. EURO-WABB: an EU rare diseases registry for Wolfram syndrome, Alstrom syndrome and Bardet-Biedl syndrome. *BMC Pediatr*. 2013;13:130.
341. de Heredia ML, Cleries R, Nunes V. Genotypic classification of patients with Wolfram syndrome: insights into the natural history of the disease and correlation with phenotype. *Genet Med*. 2013;15:497–506.
342. Wiley SE, Andreyev AY, Divakaruni AS, et al. Wolfram Syndrome protein, Miner1, regulates sulphhydryl redox status, the unfolded protein response, and Ca²⁺ homeostasis. *EMBO Mol Med*. 2013;5:904–918.
343. Frank GR, Fox J, Candela N, et al. Severe obesity and diabetes insipidus in a patient with PCSK1 deficiency. *Mol Genet Metab*. 2013;110:191–194.
344. Labib M, McPhate G, Marks V. Post-traumatic diabetes insipidus combined with primary polydipsia. *Postgrad Med J*. 1987;63:33–35.
345. Defoer F, Mahler C, Dua G, Appel B. Posttraumatic diabetes insipidus. *Acta Anaesthesiol Belg*. 1987;38:397–399.

346. Hadani M, Findler G, Shaked I, Sahar A. Unusual delayed onset of diabetes insipidus following closed head trauma. *Case report J Neurosurg.* 1985;63:456–458.
347. Jenkins HR, Hughes IA, Gray OP. Cranial diabetes insipidus in early infancy. *Arch Dis Child.* 1988;63:434–435.
348. Piech JJ, Thieblot P, Haberer JP, Delatour M, Moinade S, Gaillard G. [Twin pregnancy with acute hepatic steatosis followed by antehypophyseal insufficiency and diabetes insipidus]. [French]. *Presse Med.* 1985;14:1421–1423.
349. Iwasaki Y, Oiso Y, Yamauchi K, et al. Neurohypophyseal function in postpartum hypopituitarism: impaired plasma vasopressin response to osmotic stimuli. *J Clin Endocrinol Metab.* 1989;68:560–565.
350. Seckl JR, Dunger DB, Lightman SL. Neurohypophyseal peptide function during early postoperative diabetes insipidus. *Brain.* 1987;110:737–746.
351. Finken MJ, Zwaveling-Soonawala N, Walenkamp MJ, Vulmsa T, van Trotsenburg AS, Rotteveel J. Frequent occurrence of the triphasic response (diabetes insipidus/hyponatremia/diabetes insipidus) after surgery for craniopharyngioma in childhood. *Horm Res Paediatr.* 2011;76:22–26.
352. Maserà N, Grant DB, Stanhope R, Preece MA. Diabetes insipidus with impaired osmotic regulation in septo-optic dysplasia and agenesis of the corpus callosum. *Arch Dis Child.* 1994;70:51–53.
353. Tawa R, Kaino Y, Ito T, Goto Y, Kida K, Matsuda H. A case of Kabuki make-up syndrome with central diabetes insipidus and growth hormone neurosecretory dysfunction. *Acta Paediatr Jpn.* 1994;36:412–415.
354. Van Gool S, de Zegher F, de Vries IS, Vanderschueren-Lodeweyckx M, Casaer P, Eggermont E. Alobar holoprosencephaly, diabetes insipidus and coloboma without craniofacial abnormalities: a case report. *Eur J Pediatr.* 1990;149:621–622.
355. Yagi H, Nagashima K, Miyake H, et al. Familial congenital hypopituitarism with central diabetes insipidus. *J Clin Endocrinol Metab.* 1994;78:884–889.
356. Djermane A, Elmaleh M, Simon D, Poidvin A, Carel JC, Leger J. Central diabetes insipidus in infancy with or without hypothalamic adipic hypernatremia syndrome: early identification and outcome. *J Clin Endocrinol Metab.* 2016;101:635–643.
357. Villadsen AB, Pedersen EB. Recumbent cranial diabetes insipidus. Studies in a patient with adipisia, hypernatremia, poikilothermia and polyphagia. *Acta Paediatr Scand.* 1987;76:179–1783.
358. Kilday JP, Laughlin S, Urbach S, Bouffet E, Bartels U. Diabetes insipidus in pediatric germinomas of the suprasellar region: characteristic features and significance of the pituitary bright spot. *J Neurooncol.* 2015;121:167–175.
359. Ono N, Kakegawa T, Zama A, et al. Suprasellar germinomas; relationship between tumour size and diabetes insipidus. *Acta Neurochirurg.* 1992;114:26–32.
360. Tarng DC, Huang TP. Diabetes insipidus as an early sign of pineal tumor. *Am J Nephrol.* 1995;15:161–164.
361. Appignani B, Landy H, Barnes P. MR in idiopathic central diabetes insipidus of childhood. *Am J Neuroradiol.* 1993;14.
362. Hung W, Fitz CR. The primary empty-sella syndrome and diabetes insipidus in a child. *Acta Paediatr.* 1992;81:459–461.
363. Eichhorn P, Rhyner K, Haller D, Hammerli R, Steuli R, Furrer J. [Diabetes insipidus in chronic myeloid leukemia. Remission of hypophyseal infiltration during busulfan treatment]. [German]. *Schweizerische Medizinische Wochenschrift, J Suisse Med.* 1988;275–279.
364. Puolakka K, Korhonen T, Lahtinen R. Diabetes insipidus in pre-leukaemic phase of acute myeloid leukaemia in 2 patients with empty sella turcica. A report of 2 cases. *Scand J Haematol.* 1984;32:364–366.
365. Foresti V, Casati O, Villa A, Lazzaro A, Confalonieri F. Central diabetes insipidus due to acute monocytic leukemia: case report and review of the literature. *J Endocrinol Invest.* 1992;15:127–130.
366. de la Chapelle A, Lahtinen R. Monosomy 7 predisposes to diabetes insipidus in leukaemia and myelodysplastic syndrome. *Eur J Haematol.* 1987;39:404–411.
367. Kanabar DJ, Betts DR, Gibbons B, Kingston JE, Eden OB. Monosomy 7, diabetes insipidus and acute myeloid leukemia in childhood. *Pediatr Hematol Oncol.* 1994;11:111–114.
368. La Starza R, Falzetti D, Fania C, Tabilio A, Martelli MF, Mecucci C. 3q aberration and monosomy 7 in ANLL presenting with high platelet count and diabetes insipidus. *Haematologica.* 1994;79:356–359.
369. Ra'anani P, Shpilberg O, Berezin M, Ben-Bassat I. Acute leukemia relapse presenting as central diabetes insipidus. *Cancer.* 1994;73:2312–2316.
370. Lavabre-Bertrand T, Bourquard P, Chiesa J, et al. Diabetes insipidus revealing acute myelogenous leukaemia with a high platelet count, monosomy 7 and abnormalities of chromosome 3: a new entity? *Eur J Haematol.* 2001;66:66–69.
371. Breccia M, Petti MC, Ottaviani E, et al. Diabetes insipidus as first manifestation of acute myeloid leukaemia with EVI-1-positive, 3q21q26 syndrome and T cell-line antigen expression: what is the EVI-1 gene role? *Br J Haematol.* 2002;118:438–441.
372. Curley C, Kennedy G, Haughton A, Love A, McCarthy C, Boyd A. Acute myeloid leukemia, the 3q21q26 syndrome and diabetes insipidus: a case presentation. *Asia Pac J Clin Oncol.* 2010;6:77–79.
373. Cull EH, Watts JM, Tallman MS, et al. Acute myeloid leukemia presenting with panhypopituitarism or diabetes insipidus: a case series with molecular genetic analysis and review of the literature. *Leuk Lymphoma.* 2013;55(9):2125–2129.
374. Dunger DB, Broadbent V, Yeoman E, Seckl JR, Lightman SL, Grant DB. The frequency and natural history of diabetes insipidus in children with Langerhans-cell histiocytosis. *N Engl J Med.* 1989;321:1157–1162.
375. Grois N, Flucher-Wolfram B, Heitger A, Mostbeck GH, Hofmann J, Gadner H. Diabetes insipidus in Langerhans cell histiocytosis: results from the DAL-HX 83 study. *Med Pediatr Oncol.* 1995;24:248–256.
376. Broadbent V, Dunger DB, Yeomans E, Kendall B. Anterior pituitary function and computed tomography/magnetic resonance imaging in patients with Langerhans cell histiocytosis and diabetes insipidus. *Med Pediatr Oncol.* 1993;21:649–654.
377. Tien RD, Newton TH, McDermott MW, Dillon WP, Kucharczyk J. Thickened pituitary stalk on MR images in patients with diabetes insipidus and Langerhans cell histiocytosis. *Am J Neuroradiol.* 1990;11.
378. Minehan KJ, Chen MG, Zimmerman D, Su JQ, Colby TV, Shaw EG. Radiation therapy for diabetes insipidus caused by Langerhans cell histiocytosis [see comments]. *Int J Radiat Oncol Biol Phys.* 1992;23:519–524.
379. Imura H, Nakao K, Shimatsu A, et al. Lymphocytic infundibuloneurohypophysitis as a cause of central diabetes insipidus. *N Engl J Med.* 1993;329:683–689.
380. Johnston PC, Chew LS, Hamrahian AH, Kennedy L. Lymphocytic infundibulo-neurohypophysitis: a clinical overview. *Endocrine.* 2015;50:531–536.
381. Paja M, Estrada J, Ojeda A, Ramon Garcia-Urria J, Lucas T. Lymphocytic hypophysitis causing hypopituitarism and diabetes insipidus, and associated with autoimmune thyroiditis, in a non-pregnant woman. *Postgrad Med J.* 1994;70:220–224.
382. Koshiyama H, Sato H, Yorita S, et al. Lymphocytic hypophysitis presenting with diabetes insipidus: case report and literature review. *Endocr J.* 1994;41:93–97.
383. Kojima H, Nojima T, Nagashima K, Ono Y, Kudo M, Ishikura M. Diabetes insipidus caused by lymphocytic infundibuloneurohypophysitis. *Arch Pathol Lab Med.* 1989;113:1399–1401.
384. Ahmed SR, Aiello DP, Page R, Hopper K, Towfighi J, Santen RJ. Necrotizing infundibulo-hypophysitis: a unique syndrome of diabetes insipidus and hypopituitarism. *J Clin Endocrinol Metab.* 1993;76:1499–1504.
385. Rossi GP, Pavan E, Chiesura-Corona M, Rea F, Poletti A, Pessina AC. Bronchocentric granulomatosis and central diabetes insipidus successfully treated with corticosteroids. *Eur Respir J.* 1994;7:1893–1898.
386. Lewis R, Wilson J, Smith FW. Diabetes insipidus secondary to intracranial sarcoidosis confirmed by low-field magnetic resonance imaging. *Magn Reson Med.* 1987;5:466–470.
387. Zhao C, Tella SH, Del Rivero J, et al. Anti-PD-L1 treatment induced central diabetes insipidus. *J Clin Endocrinol Metab.* 2018;103:365–369.
388. Gunawan F, George E, Roberts A. Combination immune checkpoint inhibitor therapy nivolumab and ipilimumab associated

- with multiple endocrinopathies. *Endocrinol Diabetes Metabol Case Rep.* 2018;2018. pii: 17-0146.
389. Scherbaum WA, Wass JA, Besser GM, Bottazzo GF, Doniach D. Autoimmune cranial diabetes insipidus: its association with other endocrine diseases and with histiocytosis X. *Clin Endocrinol (Oxf).* 1986;25:411-420.
 390. De Bellis A, Bizzarro A, Amoresano Paglionico V, et al. Detection of vasopressin cell antibodies in some patients with autoimmune endocrine diseases without overt diabetes insipidus. *Clin Endocrinol (Oxf).* 1994;40:173-177.
 391. Vokes TJ, Gaskill MB, Robertson GL. Antibodies to vasopressin in patients with diabetes insipidus. Implications for diagnosis and therapy. *Ann Int Med.* 1988;108:190-195.
 392. Christensen C, Bank A. Meningococcal meningitis and diabetes insipidus. *Scand J Infect Dis.* 1988;20:341-343.
 393. Statz H, Hsu BS. Permanent central diabetes insipidus as a complication of *S. pneumoniae* meningitis in the pediatric population. *S D Med.* 2016;69:203-207.
 394. Sloane AE. Transient diabetes insipidus following listeria meningitis. *Irish Med J.* 1989;82:132-134.
 395. Brandle M, Vernazza PL, Oesterle M, Galeazzi RL. [Cerebral toxoplasmosis with central diabetes insipidus and panhypopituitarism in a patient with AIDS]. [German]. *Schweizerische Medizinische Wochenschrift J Suisse Med.* 1995;684-687.
 396. Mena W, Royal S, Pass RF, Whitley RJ, Phillips JB. Diabetes insipidus associated with symptomatic congenital cytomegalovirus infection. *J Pediatr.* 1993;122:911-913.
 397. Nolt D, Saad R, Kouatli A, Moritz ML, Menon RK, Michaels MG. Survival with hypopituitarism from congenital syphilis. *Pediatrics.* 2002;109:e63.
 398. Watanabe A, Ishii R, Hirano K, et al. Central diabetes insipidus caused by nonspecific chronic inflammation of the hypothalamus: case report. *Surg Neurol.* 1994;2:70-73.
 399. Arisaka O, Arisaka M, Ikebe A, Nijijima S, Shimura N, Hosaka A. Central diabetes insipidus in hypoxic brain damage. *Childs Nerv Syst.* 1992;8:81-82.
 400. Outwater KM, Rockoff MA. Diabetes insipidus accompanying brain death in children. *Neurology.* 1984;34:1243-1246.
 401. Fiser DH, Jimenez JF, Wrape V, Woody R. Diabetes insipidus in children with brain death. *Crit Care Med.* 1987;15:551-553.
 402. Barzilay Z, Somekh E. Diabetes insipidus in severely brain damaged children. *J Med.* 1988;19:47-64.
 403. Hohenegger M, Vermes M, Mauritz W, Redl G, Spom P, Eiselsberg P. Serum vasopressin (AVP) levels in polyuric brain-dead organ donors. *Eur Arch Psychiatry Neurol Sci.* 1990;239:267-269.
 404. Tack LJW, T'Sjoen G, Lapauw B. Exacerbation of pre-existing diabetes insipidus during pregnancy, mechanisms and management. *Acta Clin Belg.* 2017;72:213-216.
 405. Durr JA. Diabetes insipidus in pregnancy. *Am J Kidney Dis.* 1987;19:276-283.
 406. Hadi HA, Mashini IS, Devoe LD. Diabetes insipidus during pregnancy complicated by preeclampsia. *A case report J Reprod Med.* 1985;30:206-208.
 407. Harper M, Hatjis CG, Appel RG, Austin WE. Vasopressin-resistant diabetes insipidus, liver dysfunction, hyperuricemia and decreased renal function. *A case report J Reprod Med.* 1987;32:862-865.
 408. Frenzer A, Gyr T, Schaer HM, Herren H, Krahenbuhl S, Schaer M. [Triplet pregnancy with HELLP syndrome and transient diabetes insipidus]. [German]. *Schweizerische Medizinische Wochenschrift, J Suisse Med.* 1994;687-691.
 409. Kleeman CR, Rubini ME, Lamdin E, et al. Studies on alcohol diuresis. II. The evaluation of ethyl alcohol as an inhibitor of the neurohypophysis. *J Clin Invest.* 1955;34:448-455.
 410. Tata PS, Buzalkov R. Vasopressin studies in the rat. III. Inability of ethanol anesthesia to prevent ADH secretion due to pain and hemorrhage. *Pfluegers Arch.* 1966;290:294-297.
 411. Miller M, Moses AM. Clinical states due to alteration of ADH release and action. In: Moses AM, Share L, eds. *Neurohypophysis.* Basel: Karger; 1977:153-166.
 412. Sklar AH, Schrier RW. Central nervous system mediators of vasopressin release. *Physiol Rev.* 1983;63:1243-1280.
 413. Hatab SZ, Singh A, Felner EI, Kamat P. Transient central diabetes insipidus induced by ketamine infusion. *Ann Pharmacother.* 2014;48:1642-1645.
 414. Rittig S, Knudsen UB, Norgaard JP, Pedersen EB, Djurhuus JC. Abnormal diurnal rhythm of plasma vasopressin and urinary output in patients with enuresis. *Am J Physiol.* 1989;256:F664-F671.
 415. Wille S, Aili M, Harris A, Aronson S. Plasma and urinary levels of vasopressin in enuretic and non-enuretic children. *Scand J Urol Nephrol.* 1994;28:119-122.
 416. Terho P, Kekomaki M. Management of nocturnal enuresis with a vasopressin analogue. *J Urol.* 1984;131:925-927.
 417. Evans JH, Meadow SR. Desmopressin for bed wetting: length of treatment, vasopressin secretion, and response [see comments]. *Arch Dis Child.* 1992;67:184-188.
 418. Steffens J, Netzer M, Isenberg E, Alloussi S, Ziegler M. Vasopressin deficiency in primary nocturnal enuresis. Results of a controlled prospective study. *Eur Urol.* 1993;24:366-370.
 419. Onol FF, Guzel R, Tahra A, Kaya C, Boylu U. Comparison of long-term efficacy of desmopressin lyophilisate and enuretic alarm for monosymptomatic enuresis and assessment of predictive factors for success: a randomized prospective trial. *J Urol.* 2015;193:655-661.
 420. Uribarri J, Kaskas M. Hereditary nephrogenic diabetes insipidus and bilateral nonobstructive hydronephrosis. *Nephron.* 1993;65:346-349.
 421. Seow WK, Thomsett MJ. Dental fluorosis as a complication of hereditary diabetes insipidus: studies of six affected patients [see comments]. *Pediatr Dent.* 1994;16:128-132.
 422. Colliver D, Storey R, Dickens H, Subramaniam R. Nonobstructive urinary tract dilatation in children with diabetes insipidus. *J Pediatr Surg.* 2012;47:752-755.
 423. Jakobsson B, Berg U. Effect of hydrochlorothiazide and indomethacin treatment on renal function in nephrogenic diabetes insipidus. *Acta Paediatr.* 1994;83:522-525.
 424. Blanco EJ, Lane AH, Aijaz N, Blumberg D, Wilson TA. Use of subcutaneous DDAVP in infants with central diabetes insipidus. *J Pediatr Endocrinol Metab.* 2006;19:919-125.
 425. Hameed S, Mendoza-Cruz AC, Neville KA, Woodhead HJ, Walker JL, Verge CF. Home blood sodium monitoring, sliding-scale fluid prescription and subcutaneous DDAVP for infantile diabetes insipidus with impaired thirst mechanism. *Int J Pediatr Endocrinol.* 2012;2012:18.
 426. Smego AR, Backeljauw P, Gutmark-Little I. Buccally administered intranasal desmopressin acetate for the treatment of neurogenic diabetes insipidus in infancy. *J Clin Endocrinol Metab.* 2016;101:2084-2088.
 427. Mavinkurve M, McGrath N, Johnston N, Moloney S, Murphy NP, Hawkes CP. Oral administration of diluted nasal desmopressin in managing neonatal central diabetes insipidus. *J Pediatr Endocrinol Metab.* 2017;30:623-628.
 428. McDonald JA, Martha Jr PM, Kerrigan J, Clarke WL, Rogol AD, Blizzard RM. Treatment of the young child with postoperative central diabetes insipidus. *Am J Dis Child.* 1989;143:201-204.
 429. Ralston C, Butt W. Continuous vasopressin replacement in diabetes insipidus. *Arch Dis Child.* 1990;65:896-897.
 430. Wise-Faberowski L, Soriano SG, Ferrari L, et al. Perioperative management of diabetes insipidus in children [corrected]. *J Neurosurg Anesthesiol.* 2004;16:14-19.
 431. Muglia LJ, Majzoub JA. Diabetes Insipidus. In: Burg F, Ingelfinger J, Wald E, eds. *Gellis and Kagan's Current Pediatric Therapy* 14. 1sted. Philadelphia: WB Saunders Co; 1993:318-319.
 432. Aylward PE, Floras JS, Leimbach Jr WN, Abboud FM. Effects of vasopressin on the circulation and its baroreflex control in healthy men. *Circulation.* 1986;73:1145-1154.
 433. Andersen LJ, Andersen JL, Schutten HJ, Warberg J, Bie P. Antidiuretic effect of subnormal levels of arginine vasopressin in normal humans. *Am J Physiol.* 1990;259:R53-R60.
 434. Seckl JR, Dunger DB, Bevan JS, Nakasu Y, Chowdrey C, Burke CW. Vasopressin antagonist in early postoperative diabetes insipidus. *Lancet.* 1990;335:1353-1356.
 435. Moreno-Sanchez D, Casis B, Martin A, et al. Rhabdomyolysis and cutaneous necrosis following intravenous vasopressin infusion. *Gastroenterology.* 1991;101:529-532.
 436. Pierce ST, Nickl N. (1993). Rhabdomyolysis associated with the use of intravenous vasopressin. *Am J Gastroenterol.* 1993;88:424-427.

437. Mauro VF, Bingle JF, Ginn SM, Jafri FM. Torsade de pointes in a patient receiving intravenous vasopressin. *Critic Care Med*. 1988;16:200–201.
438. Stoof SC, Cnossen MH, de Maat MP, Leebeek FW, Kruij MJ. Side effects of desmopressin in patients with bleeding disorders. *Haemophilia*. 2016;22:39–45.
439. Sharma R, Stein D. Hyponatremia after desmopressin (DDAVP) use in pediatric patients with bleeding disorders undergoing surgeries. *J Pediatr Hematol Oncol*. 2014;36. e371–375.
440. Mason JA, Robertson JD, McCosker J, Williams BA, Brown SA. Assessment and validation of a defined fluid restriction protocol in the use of subcutaneous desmopressin for children with inherited bleeding disorders. *Haemophilia*. 2016;22:700–705.
441. Cortina G, Hansford JR, Duke T. Central diabetes insipidus and cisplatin-induced renal salt wasting syndrome: a challenging combination. *Pediatr Blood Cancer*. 2016;63:925–927.
442. Bryant WP, O'Marcaigh AS, Ledger GA, Zimmerman D. Aqueous vasopressin infusion during chemotherapy in patients with diabetes insipidus. *Cancer*. 1994;74:2589–2592.
443. Polycarpe E, Arnould L, Schmitt E, et al. Low urine osmolarity as a determinant of cisplatin-induced nephrotoxicity. *Int J Cancer*. 2004;111:131–137.
444. Kataoka Y, Nishida S, Hirakawa A, Oiso Y, Arima H. Comparison of incidence of hyponatremia between intranasal and oral desmopressin in patients with central diabetes insipidus. *Endocr J*. 2015;62:195–200.
445. Williams TD, Dunger DB, Lyon CC, Lewis RJ, Taylor F, Lightman SL. Antidiuretic effect and pharmacokinetics of oral 1-desamino-8-D-arginine vasopressin. 1. Studies in adults and children. *J Clin Endocrinol Metab*. 1986;63:129–132.
446. Cunnah D, Ross G, Besser GM. Management of cranial diabetes insipidus with oral desmopressin (DDAVP). *Clin Endocrinol (Oxf)*. 1986;24:253–257.
447. Stick SM, Betts PR. Oral desmopressin in neonatal diabetes insipidus. *Arch Dis Child*. 1987;62:1177–1178.
448. Puri M, Azam A, Loechner KJ. Unmasking of partial diabetes insipidus during stress but not maintenance dosing of glucocorticoids in an infant with septo-optic dysplasia. *Int J Pediatr Endocrinol*. 2011;2011:817954.
449. Pivonello R, Colao A, Di Somma C, et al. Impairment of bone status in patients with central diabetes insipidus. *J Clin Endocrinol Metab*. 1998;83:2275–2280.
450. Hiyama TY, Utsunomiya AN, Matsumoto M, et al. Adipsic hypernatremia without hypothalamic lesions accompanied by autoantibodies to subfornical organ. *Brain Pathol (Zurich, Switzerland)*. 2017;27:323–331.
451. Nakamura-Utsunomiya, A., Hiyama, T.Y., Okada, S., Noda, M., Kobayashi, M. Characteristic clinical features of adipsic hypernatremia patients with subfornical organ-targeting antibody. *Clin Pediatr Endocrinol*, 26, 197–205.
452. Moses AM, Sangani G, Miller JL. Proposed cause of marked vasopressin resistance in a female with an X-linked recessive V2 receptor abnormality. *J Clin Endocrinol Metab*. 1995;80:1184–1186.
453. Giri D, Hart R, Jones C, Ellis I, Ramakrishnan R. An unusual case of hereditary nephrogenic diabetes insipidus (HNDI) affecting mother and daughter. *J Pediatr Endocrinol Metab*. 2016;29:93–96.
454. Kobrinsky NL, Doyle JJ, Israels ED, Winter JS, Cheang MS, Walker RD. Absent factor VIII response to synthetic vasopressin analogue (DDAVP) in nephrogenic diabetes insipidus. *Lancet*. 1985;1:1293–1294.
455. Bichet DG, Razi M, Lonergan M, Arthus MF, Papukna V, Kortas C. Hemodynamic and coagulation responses to 1-desamino[8-D-arginine] vasopressin in patients with congenital nephrogenic diabetes insipidus. *N Engl J Med*. 1988;318:881–887.
456. Knoers VV, Janssens PM, Goertz J, Monnens LA. Evidence for intact V1-vasopressin receptors in congenital nephrogenic diabetes insipidus. *Eur J Pediatr*. 1992;151:381–383.
457. Brink HS, Derkx FH, Boomsma F, Brommer EJ, Schalekamp MA. 1-Desamino-8-D-arginine vasopressin (DDAVP) in patients with congenital nephrogenic diabetes insipidus. *Netherl J Med*. 1993;43:5–12.
458. Ohzeki T. Urinary adenosine 3',5'-monophosphate (cAMP) response to antidiuretic hormone in diabetes insipidus (DI): comparison between congenital nephrogenic DI type 1 and 2, and vasopressin sensitive DI. *Acta Endocrinol*. 1985;108:485–490.
459. Bichet DG, Razi M, Arthus MF, et al. Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus. Evidence for a pre-cyclic AMP V2 receptor defective mechanism. *Kidney Int*. 1989;36:859–866.
460. Moses AM, Weinstock RS, Levine MA, Breslau NA. Evidence for normal antidiuretic responses to endogenous and exogenous arginine vasopressin in patients with guanine nucleotide-binding stimulatory protein-deficient pseudohypoparathyroidism. *J Clin Endocrinol Metab*. 1986;62:221–224.
461. Knoers N, Monnens LA. Nephrogenic diabetes insipidus: clinical symptoms, pathogenesis, genetics and treatment. [Review]. *Pediatr Nephrol*. 1992;6:476–482.
462. Kavanagh C, Uy NS. Nephrogenic diabetes insipidus. *Pediatr Clin North Am*. 2019;66:227–234.
463. Vest M, Talbot NB, Crawford JD. Hypocaloric dwarfism and hydronephrosis in diabetes insipidus. *Am J Dis Child*. 1963;105:175–181.
464. Macaulay D, Watson M. Hypermnatremia in infants as a cause of brain damage. *Arch Dis Child*. 1967;42:485–491.
465. Freycon MT, Lavocat MP, Freycon F. [Familial nephrogenic diabetes insipidus with chronic hypernatremia and cerebral calcifications]. [French]. *Pediatr*. 1988;43:409–413.
466. Nozue T, Uemasu F, Endoh H, Sako A, Takagi Y, Kobayashi A. Intracranial calcifications associated with nephrogenic diabetes insipidus [see comments]. *Pediatr Nephrol*. 1993;7:74–76.
467. Schofer O, Beetz R, Kruse K, Rascher C, Schutz C, Bohl J. Nephrogenic diabetes insipidus and intracerebral calcification. *Arch Dis Child*. 1990;65:885–887.
468. Tohyama J, Inagaki M, Koeda T, Ohno K, Takeshita K. Intracranial calcification in sibs with nephrogenic diabetes insipidus: CT and MRI. *Neuroradiology*. 1993;35:553–555.
469. Bode HH, Crawford JD. Nephrogenic diabetes insipidus in North America. The Hopewell hypothesis. *N Engl J Med*. 1969;280:750–754.
470. Rosenthal W, Seibold A, Antaramian A, et al. Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. *Nature*. 1992;359:233–235.
471. Bichet DG, Birnbaumer M, Lonergan M, et al. Nature and recurrence of AVPR2 mutations in X-linked nephrogenic diabetes insipidus. *Am J Hum Genet*. 1994;55:278–286.
472. Bichet DG, Hendy GN, Lonergan M, et al. X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. *Am J Hum Genet*. 1992;51:1089–1102.
473. Birnbaumer M, Gilbert S, Rosenthal W. An extracellular congenital nephrogenic diabetes insipidus mutation of the vasopressin receptor reduces cell surface expression, affinity for ligand, and coupling to the Gs/adenylyl cyclase system. *Mol Endocrinol*. 1994;8:886–894.
474. Holtzman EJ, Kolakowski Jr LF, Geifman-Holtzman O, O'Brien DG, Guillot AP, Ausiello DA. Mutations in the vasopressin V2 receptor gene in two families with nephrogenic diabetes insipidus. *J Am Soc Nephrol*. 1994;5:169–176.
475. Holtzman EJ, Kolakowski Jr LF, O'Brien D, Crawford JD, Ausiello DA. A Null mutation in the vasopressin V2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus in the Hopewell kindred. *Hum Mol Genet*. 1993;2:1201–1204.
476. Knoers NV, van den Ouweland AM, Verdijk M, Monnens LA, van Oost BA. Inheritance of mutations in the V2 receptor gene in thirteen families with nephrogenic diabetes insipidus. *Kidney Int*. 1994;46:170–176.
477. Yuasa H, Ito M, Oiso Y, et al. Novel mutations in the V2 vasopressin receptor gene in two pedigrees with congenital nephrogenic diabetes insipidus. *J Clin Endocrinol Metab*. 1994;79:361–365.
478. Oksche A, Dickson J, Schulein R, et al. Two novel mutations in the vasopressin V2 receptor gene in patients with congenital nephrogenic diabetes insipidus [published erratum appears in *Biochem Biophys Res Commun*, 207(3), 1059]. *Biochem Biophys Res Commun*. 1995;205:552–557.
479. Pan Y, Metzzenberg A, Das S, Jing B, Gitschier J. Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. *Nat Genet*. 1992;(2):103–106.
480. Tsukaguchi H, Matsubara H, Aritaki S, Kimura T, Abe S, Inada M. Two novel mutations in the vasopressin V2 receptor gene in unrelated Japanese kindreds with nephrogenic diabetes insipidus. *Biochem Biophys Res Commun*. 1993;197:1000–1010.

481. van den Ouweland AM, Dreesen JC, Verdijk M, Knoers NV, Monnens LA, van Oost BA. Mutations in the vasopressin type 2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus. *Nat Genet.* 1992;2:99–102.
482. Wenkert D, Merendino Jr JJ, Shenker A, et al. Novel mutations in the V2 vasopressin receptor gene of patients with X-linked nephrogenic diabetes insipidus. *Hum Mol Genet.* 1994;3:1429–1430.
483. Wildin RS, Antush MJ, Bennett RL, Schoof JM, Scott CR. Heterogeneous AVPR2 gene mutations in congenital nephrogenic diabetes insipidus. *Am J Hum Genet.* 1994;55:266–277.
484. Saglar E, Deniz F, Erdem B, et al. A large deletion of the AVPR2 gene causing severe nephrogenic diabetes insipidus in a Turkish family. *Endocrine.* 2014;46:148–153.
485. Scherthaler-Reiter MH, Adams D, Trivellini G, et al. A novel AVPR2 splice site mutation leads to partial X-linked nephrogenic diabetes insipidus in two brothers. *Eur J Pediatr.* 2016;175:727–733.
486. Makita N, Sato T, Yajima-Shoji Y, et al. Analysis of the V2 vasopressin receptor (V2R) mutations causing partial nephrogenic diabetes insipidus highlights a sustainable signaling by a non-peptide V2R agonist. *J Biol Chem.* 2016;291:22460–22471.
487. Friedman E, Bale AE, Carson E, et al. Nephrogenic diabetes insipidus: an X chromosome-linked dominant inheritance pattern with a vasopressin type 2 receptor gene that is structurally normal. *Proc Natl Acad Sci U S A.* 1994;91:8457–8461.
488. Oksche A, Rosenthal W. The molecular basis of nephrogenic diabetes insipidus. *J Mol Med.* 1998;76:326–337.
489. Pan Y, Wilson P, Gitschier J. The effect of eight V2 vasopressin receptor mutations on stimulation of adenylyl cyclase and binding to vasopressin. *J Biol Chem.* 1994;269:31933–31937.
490. Rosenthal W, Antaramian A, Gilbert S, Birnbaumer M. Nephrogenic diabetes insipidus. A V2 vasopressin receptor unable to stimulate adenylyl cyclase. *J Biol Chem.* 1993;268:13030–13033.
491. Rosenthal W, Seibold A, Antaramian A, et al. Mutations in the vasopressin V2 receptor gene in families with nephrogenic diabetes insipidus and functional expression of the Q-2 mutant. *Cell Mol Biol.* 1994;40:429–436.
492. Bichet DG. Molecular and cellular biology of vasopressin and oxytocin receptors and action in the kidney. [Review]. *Curr Opin Nephrol Hypertens.* 1994;3:46–53.
493. Waring AJ, Kajdi L, Tappan V. A congenital defect of water metabolism. *Am J Dis Child.* 1945;69:323.
494. Langley JM, Balfe JW, Selander T, Ray PN, Clarke JT. Autosomal recessive inheritance of vasopressin-resistant diabetes insipidus. *Am J Med Genet.* 1991;38:90–94.
495. Knoers N, Monnens LA. A variant of nephrogenic diabetes insipidus: V2 receptor abnormality restricted to the kidney. *Eur J Pediatr.* 1991;150:370–373.
496. Mulders SM, Bichet DG, Rijss JP, et al. An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J Clin Invest.* 1998;102:57–66.
497. Botton R, Gaviria M, Battle DC. Prevalence, pathogenesis, and treatment of renal dysfunction associated with chronic lithium therapy. *Am J Kidney Dis.* 1987;10:329–345.
498. Bendz H, Aurell M, Balldin J, Mathe AA, Sjodin I. Kidney damage in long-term lithium patients: a cross-sectional study of patients with 15 years or more on lithium. *Nephrol Dialysis Transplant.* 1994;9:1250–1254.
499. Yamaki M, Kusano E, Tetsuka T, et al. Cellular mechanism of lithium-induced nephrogenic diabetes insipidus in rats. *Am J Physiol.* 1991;261:F505–F511.
500. Marples D, Christensen S, Christensen EI, Ottosen PD, Nielsen S. Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest.* 1995;95:1838–1845.
501. Hirji MR, Mucklow JC. Transepithelial water movement in response to carbamazepine, chlorpropamide and demeclocycline in toad urinary bladder. *Br J Pharmacol.* 1991;104:550–553.
502. Navarro JF, Quereda C, Quereda C, et al. Nephrogenic diabetes insipidus and renal tubular acidosis secondary to foscarnet therapy. *Am J Kidney Dis.* 1996;27:431–434.
503. Bendz H, Aurell M. Drug-induced diabetes insipidus: incidence, prevention and management. *Drug Saf.* 1999;21:449–456.
504. Hohler T, Teuber G, Wanitschke R, Meyer zum Buschenfeld KH. Indomethacin treatment in amphotericin B induced nephrogenic diabetes insipidus. *Clin Investigat.* 1994;72:769–771.
505. Vigerat P, Kanfer A, Kenouch S, Blanchet F, Mougnot B, Mery JP. Nephrogenic diabetes insipidus and distal tubular acidosis in methicillin-induced interstitial nephritis. *Adv Exp Med Biol.* 1987;212:129–134.
506. Quinn BP, Wall BM. Nephrogenic diabetes insipidus and tubulointerstitial nephritis during continuous therapy with rifampin. *Am J Kidney Dis.* 1989;14:217–220.
507. Kato A, Hishida A, Ishibashi R, et al. Nephrogenic diabetes insipidus associated with bilateral ureteral obstruction. *Intern Med.* 1994;33:231–233.
508. Nagayama Y, Shigeno M, Nakagawa Y, et al. Acquired nephrogenic diabetes insipidus secondary to distal renal tubular acidosis and nephrocalcinosis associated with Sjogren's syndrome. *J Endocrinol Invest.* 1994;17:659–663.
509. Wolf RB, Kassim AA, Goodpaster RL, DeBaun MR. Nocturnal enuresis in sickle cell disease. *Exp Rev Hematol.* 2014;7:245–254.
510. Hartenberg MA, Cory M, Chan JC. Nephrogenic diabetes insipidus. Radiological and clinical features. *Int J Pediatr Nephrol.* 1985;6:281–286.
511. Al Nofal A, Lteif A. Thiazide diuretics in the management of young children with central diabetes insipidus. *J Pediatr.* 2015;167:658–661.
512. Alon U, Chan JC. Hydrochlorothiazide-amiloride in the treatment of congenital nephrogenic diabetes insipidus. *Am J Nephrol.* 1985;5:9–13.
513. Libber S, Harrison H, Spector D. Treatment of nephrogenic diabetes insipidus with prostaglandin synthesis inhibitors. *J Pediatr.* 1986;108:305–311.
514. Rascher W, Rosendahl W, Henrichs IA, Maier R, Seyberth HW. Congenital nephrogenic diabetes insipidus-vasopressin and prostaglandins in response to treatment with hydrochlorothiazide and indomethacin. *Pediatr Nephrol.* 1987;1:485–490.
515. Vierhapper H, Jorg J, Favre L, Vallotton MB, Waldhausl W. Comparative therapeutic benefit of indomethacin, hydrochlorothiazide, and acetyl-salicylic acid in a patient with nephrogenic diabetes insipidus. *Acta Endocrinol.* 1984;106:311–316.
516. Uyeki TM, Barry FL, Rosenthal SM, Mathias RS. Successful treatment with hydrochlorothiazide and amiloride in an infant with congenital nephrogenic diabetes insipidus. *Pediatr Nephrol.* 1993;7:554–556.
517. Battle DC, von Riotte AB, Gaviria M, Grupp M. Amelioration of polyuria by amiloride in patients receiving long-term lithium therapy. *N Engl J Med.* 1985;312:408–414.
518. Weinstock RS, Moses AM. Desmopressin and indomethacin therapy for nephrogenic diabetes insipidus in patients receiving lithium carbonate. *Southern Med J.* 1990;83:1475–1477.
519. Welch EM, Barton ER, Zhuo J, et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature.* 2007;447:87–91.

13

Thyroid Disorders in Children and Adolescents

Scott Rivkees, Andrew J. Bauer

CHAPTER OUTLINE

INTRODUCTION	395
Thyroid Hormones and Their Action	395
Regulation of Thyroid Function	397
CLINICAL AND BIOCHEMICAL ASSESSMENT OF THYROID STATUS	397
Clinical Evaluation of the Thyroid	397
Biochemical Evaluation of Thyroid Function	398
HYPOTHYROIDISM	400
Hashimoto or Autoimmune Thyroiditis	400
Hashitoxicosis	400
Subclinical Hypothyroidism	401
Juvenile Acquired Hypothyroidism	401
Iodine-Induced Hypothyroidism	401
Hypothalamic-Pituitary Dysfunction	402
Giant Hemangiomas	402
Hypothyroidism in Cancer Survivors	402
THYROID HORMONE RESISTANCE	402
HYPERTHYROIDISM	403
Clinical Evaluation of the Hyperthyroid State	403
Graves Disease	403
Antithyroid Drug Therapy	403
Antithyroid Drug Therapy Duration and Prediction of Remission	404
Strategies for the Child With an Adverse Event to Methimazole	404
Radioactive Iodine Therapy	405
Risks of Radioactive Iodine in Children Treated for Graves Disease	405
Thyroidectomy and Risks	406
Somatic Complications of Graves Disease	406
NEONATAL THYROTOXICOSIS	406
OTHER CAUSES OF HYPERTHYROIDISM	407
Activating Mutations of the Thyroid-Stimulating Hormone Receptor	407
McCune–Albright Syndrome	407
Thyroid-Stimulating Hormone Producing Adenomas	407
Infectious Bacterial Thyroiditis	407
Subacute Thyroiditis	407
Hyperfunctioning Nodules	408
Toxic Multinodular Goiters	408
Amiodarone	408
Excessive Iodine Intake	408
THYROID NODULES AND THYROID CANCER	408
Background	408
Evaluation of a Thyroid Nodule	408
Differentiated Thyroid Cancer	409
Molecular Landscape	411
Surgical Management	411
Radioactive Iodine Therapy	413
Levothyroxine Therapy	414
Follow-Up	414
Treatment of Persistent or Recurrent Disease	415
Systemic Therapy for Children With Progressive Disease	415
Medullary Thyroid Cancer	415
References	416

INTRODUCTION

Thyroid disease can present with overt symptoms, insidiously, or with isolated thyromegaly. Thyroid disease in children can encompass isolated biochemical abnormalities that have little or no physiological consequence, or with overt clinical symptoms. Clinically, hypothyroidism occurs more commonly than hyperthyroidism. Thyroid nodules and masses occur much less commonly than functional disorders but can portend the presence of thyroid cancer. [Box 13.1](#) provides a classification of thyroid disorders in children. This chapter focuses on the most common conditions that affect the thyroid gland of children and adolescents.

Thyroid Hormones and Their Action

Few hormones exert as profound and essential role in human physiology as thyroid hormones. The major hormones released by the thyroid gland include tetraiodothyronine, or thyroxine (T₄), and triiodothyronine (T₃).¹ The production of these hormones involves several discrete biochemical steps that are shown in [Fig. 13.1](#). Of these hormones, T₃ plays the pivotal role in affecting physiology, being the molecule that principally

binds to the thyroid hormone receptor (TRs).¹ The thyroid hormone nuclear receptor belongs to the steroid hormone–retinoic acid receptor superfamily and is a regulator of deoxyribonucleic acid (DNA) transcription.

Two genes encode the TR; one on chromosome 17 designated alpha (*TRa*) and one on chromosome 3 designated beta (*TRb*). The TRs can exist as monomers or homodimers, and they can dimerize with other members of the family of nuclear receptors.¹ After T₃ binding to the TR, gene transcription is regulated in many tissues.

T₄ is the predominant hormone released from thyroid follicular cells. After release, it circulates in protein-bound and free states at a ratio of about 1000 to 1. Thyroid hormone-binding proteins in the blood include thyroxine-binding globulin (TBG), prealbumin or transthyretin, and albumin.² TBG is the predominant carrier protein for T₄; TBG and albumin also carry T₃. In the euthyroid study, the circulating concentration of free T₄ (FT₄) and free T₃ are about 0.03% and 0.30%, respectively, of total hormone concentrations.

It is important to recognize that circulating levels of thyroid hormones and carrier proteins change with age ([Tables 13.1](#) and [13.2](#)). Absolute mean free T₄ and free T₃ concentrations

BOX 13.1 Thyroid Disorders in Childhood and Adolescence**AUTOIMMUNE THYROID DISEASE**

- Hashimoto thyroiditis, juvenile acquired hypothyroidism
- Stimulating antibody, Graves disease
- Blocking antibody, hypothyroidism

INFECTIOUS THYROIDITIS

- Suppurative thyroiditis
- Subacute thyroiditis

BINDING PROTEIN ABNORMALITIES

- Complete TBG deficiency
- Partial TBG deficiency
- TBG excess
- Transthyretin variants

TSH RECEPTOR MUTATIONS

- Loss-of-function hypothyroidism
- Gain-of-function hyperthyroidism

THYROID HORMONE RESISTANCE SYNDROMES

- Thyroid hormone beta receptor (TR β) mutations
- Peripheral tissue resistance syndrome

- Pituitary resistance syndrome
- Thyroid hormone membrane transport defects

IODINE DEFICIENCY SYNDROMES

- Goiter
- Mental impairment
- Cretinism

DIFFUSE NONTOXIC GOITER

- Nonthyroidal illness
- Thyroid neoplasia
- Adenoma
- Nonfunctional
- Functional
- Papillary-follicular carcinoma
- Medullary carcinoma
- MEN2A, 2B, Ret mutations
- Sporadic
- Undifferentiated
- Metastatic

MEN, Multiple endocrine neoplasia; TBG, thyroxine-binding globulin; TSH, thyroid-stimulating hormone.

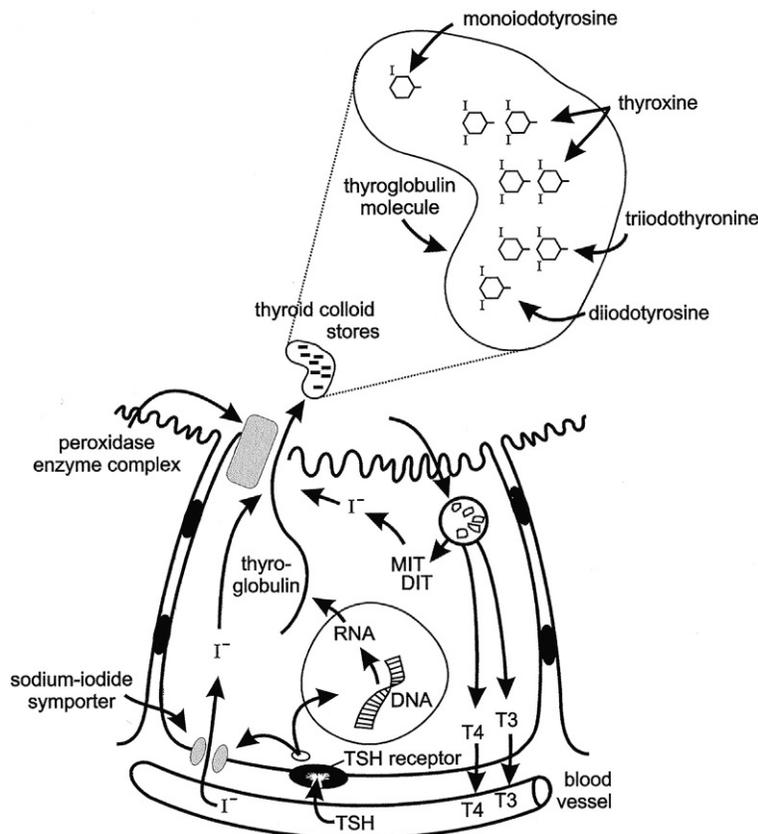


Fig. 13.1 Illustration of thyroid hormone synthesis and secretion. Thyroid-stimulating hormone (TSH) regulates the process via the G protein–linked plasma membrane TSH receptor. TSH binding stimulates thyroglobulin synthesis and sodium-iodide symporter (iodide transporter) uptake of circulating iodide. Iodide diffuses in the cytosol to the apical membrane and is transported to the apical lumen by Pendrin, an anion-bicarbonate family exchanger, making iodide available to the enzyme organification complex (Pendrin; thyroid peroxidase, TPO, THOX). The tyrosine residues of thyroglobulin are iodinated at the apical cell membrane and are catalyzed by thyroid peroxidase, the organification enzyme. The resulting monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues couple to form the iodothyronines thyroxine (T₄) and triiodothyronine (T₃) within the stored thyroglobulin molecule. TSH stimulates micropinocytosis of colloid droplets and progressive thyroglobulin proteolysis within the resulting phagolysosomes. T₄ and T₃ are secreted into the circulation. The uncoupled MIT and DIT are deiodinated by iodotyrosine deiodinase to release iodide, which is largely recycled within the follicular cell. (From Fisher and Greuters [2008] *Thyroid disorders in childhood and adolescence*. In: *Pediatric Endocrinology*, M. Sperling, ed. 3rd ed., Saunders, Philadelphia, pp. 227–253).

TABLE 13.1 Changes With Age in Serum Concentrations of T4, TSH, TBG, and Thyroglobulin (Tg)

Age	TSH ^a (μU/mL)	T4 ^b (μg/dL)	TBG ^b (mg/dL)	Tg ^b (ng/mL)
Cord blood	1–20	6.6–15	0.8–5.2	15–101
1–7 days	1–39	11–22	0.8–5.2	1–110
1–4 weeks	0.5–6.5	8.2–17	0.6–5	11–92
1–12 months	0.5–6.5	5.9–16	1.6–3.6	12–113
1–5 years	0.6–8	7.3–15	1.4–2.8	5–72
6–10 years	0.6–8	6.4–13	1.4–2.8	3–40
11–15 years	0.6–8	5.5–12	1.4–2.8	3–40
16–20 years	0.5–6	4.2–12	1.4–2.8	2–36
21–50 years	0.5–6	4.3–12	1.2–2.6	2–35

^aMean and 95 % range.^bMean and two standard deviations range.

T4, Thyroxine; TBG, thyroxine-binding globulin; TSH, thyroid-stimulating hormone.

(From Fisher, D.A., & Vanderschueren-Lodeweycky, M. (1985). Laboratory tests for thyroid diagnosis in infants and children. In: Delange, F., Fisher, D.A. (Eds.). *Pediatric Thyroidology* (pp. 127–142). Basel: Karger; Walfish, P.G., & Tseng, K.H. (1989). Thyroid physiology and pathology. In: Collu, R., Ducharme, J.R., Guyda H. (Eds.), *Pediatric Endocrinology* (pp. 367–448). New York: Raven; Delange, F., Dahlem, A., Bourdoux, P., et al (1984). Increased risk of primary hypothyroidism in preterm infants. *Pediatrics*, 105, 462; Pazzino, V., Filetti, S., Belfiore, A., et al. (1981). Serum thyroglobulin levels in the newborn. *J Clin Endocrinol Metab*, 52, 3634; Delange, F. (1993). Thyroid hormones: biochemistry and physiology. In: J. Bertrang, J., R. Rappaport, R., P. C. Sizonenko P.C., (Eds.), *Pediatric Endocrinology* (pp. 242–251). Baltimore: Williams and Wilkins; Lazar, L., Frumkin, R. B., Battat, E., et al. (2009). *J Clin Endocrinol Metab*, 94, 1678–1682.)

TABLE 13.2 Changes With Age in Serum Concentrations of T3, rT3, Free T4, and Free T3

	T3 ^a (ng/dL)	rT3 ^a (ng/dL)	Free T4 ^b (ng/dL)	Free T3 ^b (pg/mL)
Cord blood	14–86	100–501	1.2–2.2	—
4–7 days	36–316	34–258	2.2–5.3	1.3–6.1
1–4 weeks	105–345	26–290	0.9–2.3	2.2–8
1–12 months	105–245	11–129	0.8–2.1	2.5–7
1–5 years	105–269	15–71	0.8–2	2.8–5.2
6–10 years	94–241	17–79	0.8–2	2.8–5.2
11–15 years	83–213	19–88	0.8–2	2.9–5.6
16–20 years	80–210	25–80	0.8–2	2.4–5
21–50 years	70–204	30–80	0.9–2.5	2.4–4.4

T3, Triiodothyronine; rT3 reverse T3; T4, thyroxine.

^aGeometric mean and range.^bTwo standard deviations range, by tracer dialysis.

(From Delange, F. (1993). Thyroid hormones: biochemistry and physiology. In: Bertrang, J., Rappaport, R., Sizonenko, P.C. (Eds.), *Pediatric endocrinology* (pp. 242–251). Baltimore: Williams and Wilkins; Lucas, C., Carayan, P., Bellhileh, J., & Giraud, F. (1980). Changes in levels of free thyroid hormones in children from 1 to 16 years: comparison with other thyroid indices. *Pediatric*, 35, 197; Nelson, J. C., Clark, S. J., Borut, D. L., et al. (1993). Age related changes in serum free thyroxine during childhood adolescence. *J Pediatr*, 123, 899.)

are about 10 and 4 pg/mL, respectively, and differ according to age. In adolescents and adults, the plasma concentrations of the several binding proteins are 1 to 3 mg/dL for TBG, 20 to 30 mg/dL for thyroxine-binding prealbumin, and 2 to 5 g/dL for albumin.² TBG concentrations are greater in children than in adults, and they decline to adult levels during adolescence.² Because the thyroid hormone-binding proteins are produced in the liver, they are acute phase reactants, with concentrations increasing during acute illness, they also increase in response to estrogen exposure.

Conversion of T4 to T3 involves the deiodination of T4 (Fig. 13.2). Monodeiodination of the beta or outer ring by monodeiodinase (MD) type II produces T3. Monodeiodination of the alpha or inner ring produces reverse T3 (rT3), which is inactive metabolically. Under normal circumstances, T3 and rT3 are produced at similar rates. About 70% to 90% of circulating T3 is derived from peripheral conversion of T4, and 10% to 30% of circulating T3 is from the thyroid gland. Reflecting

the age-related changes in the hormones that regulate T4 stability, the clearance of T4 generally decreases from infancy to adulthood (Table 13.3).

Regulation of Thyroid Function

The production of T4 and T3 within the thyroid gland is regulated by the thyroid-stimulating hormone ([TSH]; also called *thyrotropin*), which is released from the anterior pituitary gland (Fig. 13.3).³ TSH receptors are present on thyroid follicular cells and are G protein-coupled receptors with a large extracellular amino terminus.³ Mutations of the TSH receptor can result in constitutive activation of the receptor with severe hyperthyroidism, whereas inactivating mutations result in TSH unresponsiveness and hence hypothyroidism.

TSH receptor activation stimulates adenylate cyclase accumulation within follicular cells, which in turn causes accumulation of cyclic adenosine monophosphate (cAMP). Increased cellular concentrations of cAMP promote iodide trapping, iodotyrosine synthesis, thyroglobulin (TG) synthesis, and hormone release.

TSH release is regulated by the hypothalamic hormone thyrotropin-releasing hormone (TRH) (see Fig. 13.3). This peptide hormone is produced in medial neurons of the paraventricular nucleus of the hypothalamus and is released into the portal circulation of the pituitary gland. Several different neurotransmitters have been observed to influence TRH release.

In addition to normal regulation of TSH receptor activity by TSH, thyroid function can be adversely affected by antibodies that can either stimulate or block TSH action. TSH receptor-stimulating immunoglobulin antibodies (TSIs), or thyroid receptor antibodies (TRAbs), are present in the circulation of individuals with Graves disease (GD) and activate TSH receptors. Conversely, TSH receptor-blocking immunoglobulins (TBIs) antagonize TSH action and can lead to hypothyroidism.

CLINICAL AND BIOCHEMICAL ASSESSMENT OF THYROID STATUS

Clinical Evaluation of the Thyroid

Thyroid disease can present with overt symptoms, insidiously, or with isolated thyromegaly. Thus evaluation of the thyroid gland should be included in the routine examination of children. The thyroid gland can be visualized by having the patient

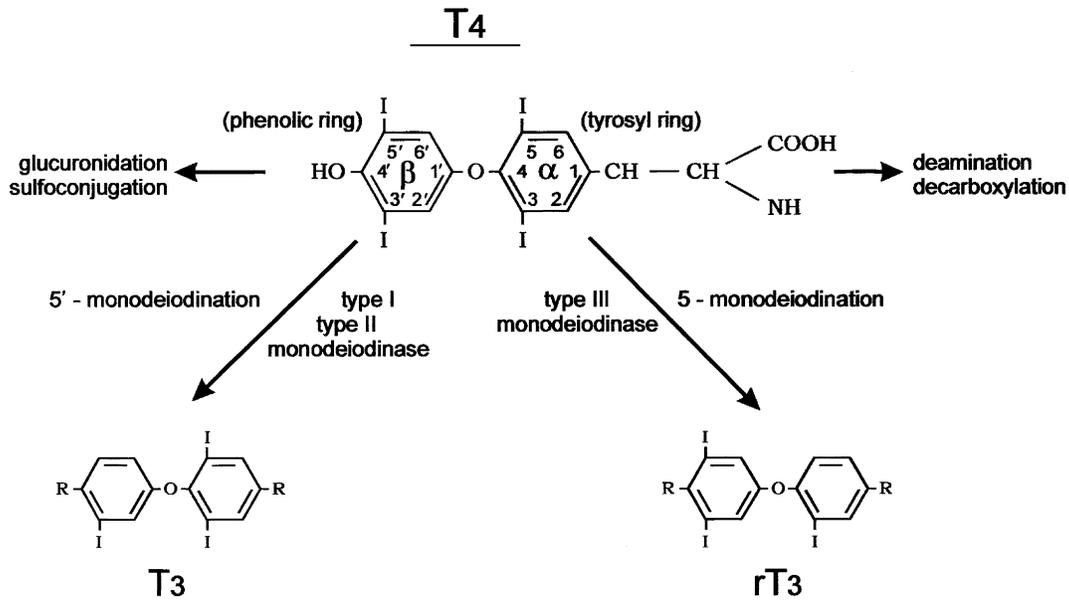


Fig. 13.2 The metabolism of thyroxine (tetraiodothyronine). The major metabolic pathway is progressive monodeiodination mediated by the three iodothyronine monodeiodinase enzymes type I, type II, and type III. Outer (phenolic) ring 5' monodeiodination produces active 3,5,3' triiodothyronine. Inner (tyrosyl) ring 5' monodeiodination produces inactive reverse 3,3,5' triiodothyronine. Type I deiodinase is also capable of inner-ring monodeiodination. The alanine side chain of the tyrosyl ring is also subject to degradative reactions, including deamination and decarboxylation. Sulfoconjugation and glucuronide conjugation reactions at the 4' phenolic ring site occur largely in liver tissue. (From Fisher and Greuters [2008] Thyroid disorders in childhood and adolescence. In: *Pediatric Endocrinology*, M. Sperling, ed. 3rd ed., Saunders, Philadelphia, pp. 227–253).

TABLE 13.3 Variation in Peripheral Thyroxine Metabolism With Age^a

Thyroxine Kinetic Parameter	Children (3–9 years)	Adolescents (10–16 years)	Adults (23–26 years)
Half-life (d)	5 (0.13)	6 (0.35)	6.7 (0.30)
Fractional clearance ^b	0.14 (0.005)	0.12 (0.008)	0.11 (0.004)
Distribution volume (L/kg)	0.16 (0.008)	0.16 (0.014)	0.12 (0.005)
Thyroxine turnover (μg/kg/day)	1.9 (0.09)	1.5 (0.07)	1.1 (0.06)

^aData are means and SE.

^bFraction of extrathyroid pool per day.

(From Beckers, C., Malvaux, C., & De Visscher, M. (1966). Quantitative aspects of the secretion and degradation of thyroid hormones during adolescence. *J Clin Endocrinol Metab*, 26, 202–306; Sterling, K., & Chodos, R. (1956). Radiothyroxine turnover studies in myxedema, thyrotoxicosis and hypermetabolism without endocrine disease. *J Clin Invest*, 35, 806–813.)

look to the ceiling and swallow.⁴ As the thyroid moves, the margins of the gland are viewed to estimate size and symmetry. The thyroid should be palpated to assess size, consistency, and symmetry. This can be performed with the clinician standing behind the patient and palpating the neck with the fingertips. The texture of the thyroid can be assessed to determine if it is smooth or irregular and if nodules are present, which may feel firm or soft. If any asymmetry or abnormal thyroid fullness is noted, ultrasonographic evaluation is recommended because pathological thyroid nodules may feel like normal tissue.

To assess gland size, one may estimate the size of each thyroid lobe relative to that of a teaspoon (5 g) or a tablespoon (15 g).⁴ In general, until the end of puberty, gland size (in grams) approximates the patient's age in years times 0.5 to 0.7.⁵ Thus

each thyroid lobe of a 10-year-old child is approximately one-half of a teaspoon for a total gland size of 5 to 7 g.⁵ For teens and adults, each lobe of the thyroid may reach one teaspoon in size for an approximate total gland size of about 10 g.⁵

In newborns and young infants, the thyroid can be examined by placing the infant supine on the parent's lap, with the head toward the parent's knees. The head can then be gently lowered backward to expose the neck, which facilitates thyroid palpation. If the examiner can palpate each ring of the trachea from the sternal notch to above the larynx, then the absence of pretracheal thyroid tissue is suggested, which is observed. Such an absence occurs in cases of failure of thyroid formation or migration. Failure to detect pretracheal thyroid tissue in older children warrants visual examination of the base of the tongue for ectopic thyroid tissue.

When a sublingual thyroid gland is discovered late in childhood or in adolescence, the tissue should be palpated with a gloved finger, during regular office visits, because nodules and malignancies may develop in ectopic thyroid glands.⁶ In contrast, when an ectopic thyroid is detected in infancy and replacement therapy is started, the residual thyroid tissue becomes atrophic and does not present long-term problems.

Biochemical Evaluation of Thyroid Function

Approximately 97% of the thyroid hormone released from the thyroid gland is T4.⁷ After its release, less than 1% of T4 remains free. The rest of the thyroid hormone circulates bound to the proteins thyroglobulin (TGB; 70%), prealbumin (transthyretin; 10%), and albumin (15% to 20%).⁷ T3 is also released from the thyroid and is generated peripherally. Although T4 constitutes the bulk of circulating thyroid hormone, T3 has far greater affinity for nuclear TRs and exerts most of the potent cellular effects of thyroid hormone action.^{8,9}

Thyroid function can be assessed by measurement of total T4 and total T3 levels, along with indices that reflect thyroid hormone-binding proteins (T3 or T4 resin uptake).⁷ The levels

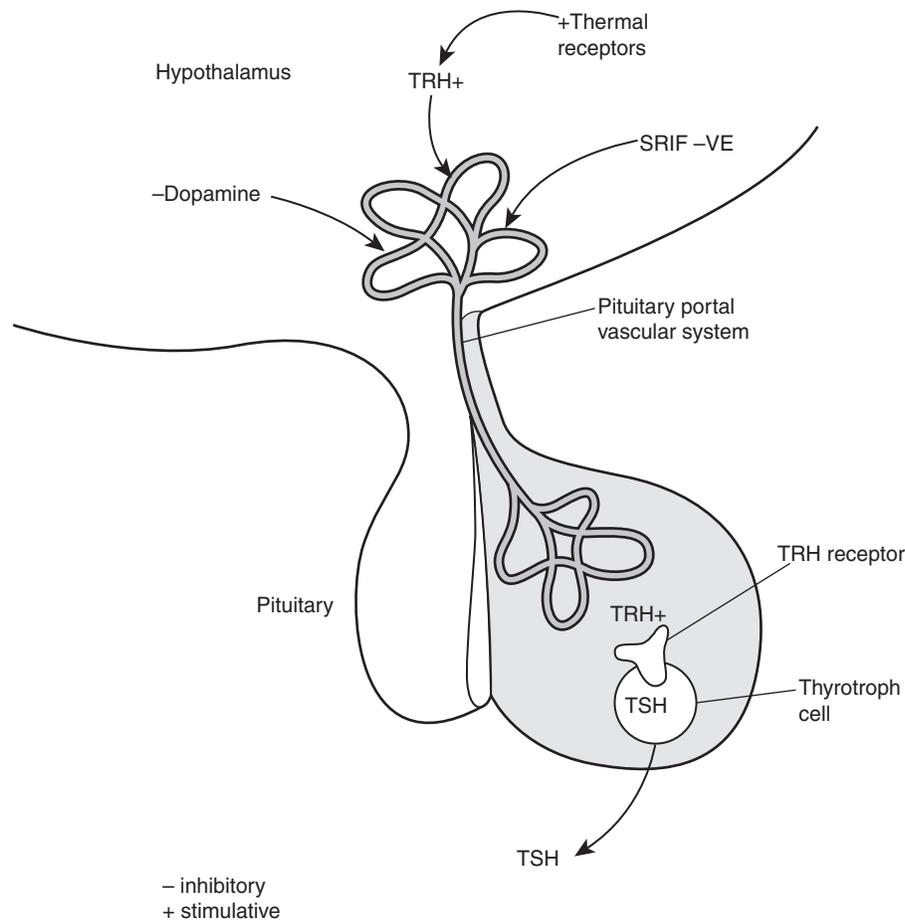


Fig. 13.3 The hypothalamic-pituitary thyroid-stimulating (TSH) axis. Thyrotropin-releasing hormone (TRH) secreted into the pituitary portal vascular system stimulates TSH synthesis and secretion from the pituitary thyrotroph cell. TRH secretion is modulated by central and peripheral thermal sensors. Dopamine or somatostatin (SRIF) can inhibit TSH release. (From Fisher and Greuters [2008] *Thyroid disorders in childhood and adolescence*. In: *Pediatric Endocrinology*, M. Sperling, ed. 3rd ed., Saunders, Philadelphia, pp. 227–253).

of estimated free (unbound) T₄ (FT₄) are measured to assess thyroid hormone status, without the confounding influences of carrier proteins.

Several conditions are seen in which thyroid hormone levels are abnormal, yet the individual is euthyroid. Because of their confusing nature, these conditions may result in the patient being erroneously diagnosed or treated for hypothyroidism or hyperthyroidism.

When FT₄ values are normal, yet total T₄ values are high, familial dysalbuminemic hyperthyroxinemia needs to be considered.^{10,11} This autosomal dominant disorder is most commonly seen in Hispanic individuals and can be diagnosed by thyroid hormone-binding protein electrophoresis. If FT₄ values are normal but total T₄ values are low, the possibility of TBG deficiency must be entertained. TBG deficiency is an X-linked disorder that may be associated with color blindness.¹² In these and other conditions affecting thyroid hormone binding, treatment is not needed and the patient should be educated about the condition to avoid treatment by unsuspecting practitioners.

T₄ is much more abundant in the circulation and T₃ is the more metabolically active thyroid hormone. T₃ is produced peripherally from T₄ and is also secreted by the thyroid. A metabolically inactive form of T₃, reverse T₃, is also produced, and its level is elevated in conditions, such as euthyroid sick syndrome, or nonthyroidal illness syndrome (see Fig. 13.2).^{13,14}

It is important to consider that estimated FT₄ values may not be accurate in infants because of the fact that circulating

levels of TBG are elevated,^{15–17} confounding biochemical assessment in this age group. FT₄ levels determined by equilibrium dialysis assay, though, are accurate.^{15,16}

Ultrasensitive thyrotropin or TSH assays have been developed, and assessment of TSH has greatly improved the evaluation of thyroid status.¹⁸ TSH levels help to distinguish many thyroid disorders that present with either low or high T₄ levels in most cases. TSH values within the normal range for the assay are indicative of a euthyroid state if the hypothalamic-pituitary axis is intact. Elevations of TSH generally indicate primary thyroid dysfunction; suppressed TSH values indicate hyperthyroidism. When both FT₄ and TSH levels are elevated, TSH-producing pituitary adenomas and thyroid hormone resistance need to be considered.

Hyperthyroidism is distinguished from subclinical hyperthyroidism, a condition in which levels of T₄, FT₄, and T₃ are normal, but TSH levels are suppressed in the absence of thyroid overt disease.¹⁹ The causes of subclinical hyperthyroidism are similar to those of overt hyperthyroidism. Thus it is important to reevaluate individuals with isolated suppression of TSH levels every 3 to 6 months, until the clinical situation declares itself.¹⁹

Critical in the interpretation of TSH levels in children is recognition that the normative range differs from that of adults (see Box 13.1), which is defined by an upper limit values of about 4 μU/mL or less.^{20–23} In comprehensive studies of this issue, the upper limit of TSH values in healthy children and

adolescents without thyroid disease is about 7 $\mu\text{U/mL}$.^{24–26} The application of adult reference range to children thus results in the erroneous diagnosis of subclinical hypothyroidism and results in the unnecessary referral of children for subspecialty care by primary care providers.

HYPOTHYROIDISM

Disorders of the thyroid lead to hypothyroidism much more commonly than to hyperthyroidism. Hypothyroidism may be present at birth, acquired during childhood or adolescence, present with or without symptoms, or present gradually or acutely.^{4,14,27–29}

It is commonly believed by the public and many practitioners that hypothyroidism is associated with and is a cause of obesity, yet there is little support for the notion that the hypothyroid state contributes to obesity.^{30,31} It is also important to note that TSH levels are slightly higher in obese individuals than nonobese individuals.^{32–35} With weight loss, TSH levels normalize in these children.^{32–34,36} Thus slight elevations in TSH in obese individuals are physiological and do not warrant therapy.

Hypothyroidism may be elusive, with symptoms elicited only in retrospect. In the extreme, hypothyroidism can be associated with cold intolerance, bradycardia, carotenemia, coarse and brittle hair, dry skin, pallor, and myxedema. These symptoms may not be distressing, which allows prolonged hypothyroidism to escape detection.

The most common causes of hypothyroidism in children are autoimmune processes resulting in Hashimoto thyroiditis.^{37,38} Autoimmune thyroiditis also leads to juvenile acquired hypothyroidism that can present with growth failure when chronically present.³⁹ Hypothyroidism in children can be caused by iodine exposure or hypothalamic pituitary dysfunction. Other causes of hypothyroidism include exogenous goitrogens, cystinosis, acute thyroiditis, and thyroid irradiation during cancer treatment. Hypothyroidism in the newborn is a serious health concern and is detected by newborn screening programs.

Hashimoto or Autoimmune Thyroiditis

Autoimmune thyroiditis with thyroid enlargement is one of the most common presentations of childhood thyroid disease.^{38,40} It is associated with antibodies against thyroglobulin and thyroperoxidase and is characterized by lymphocytic infiltration of the thyroid gland, which results in thyromegaly.^{38,40} Depending on the nature of the antithyroid antibodies, Hashimoto disease may be associated with a euthyroid state, hypothyroidism, or transient hyperthyroidism.^{38,40}

Hashimoto thyroiditis may rarely occur in very young infants,⁴¹ and typically presents in adolescents, affecting females more than males.³⁸ The thyroid gland is usually diffusely enlarged and may have an irregular, cobblestone feel. Asymmetric thyroid enlargement, mimicking a thyroid nodule, may be noted. The presence of antithyroid antibodies and the absence of nodules on ultrasonography can distinguish inflammation from other pathologic processes.

Importantly, the presence of antithyroid antibodies does not portend the development of complete or partial thyroid failure that will warrant therapy. In the healthy adult population, up to 5% of individuals had circulating antithyroid antibodies that are present.⁴² Less than 10% of these individuals will develop hypothyroidism, with those having elevated antithyroperoxidase (anti-TPO) antibodies being much more at risk than those having anti-TG antibodies.⁴²

In children, the incidence of antithyroid antibodies at a population level is not generally known. Of those children with antithyroid antibodies, about 20% are reported to develop

hypothyroidism needing therapy.^{43,44} These children often have very high antithyroid antibody titers. Thus if a child is found to have low levels of antithyroid antibodies, it is reasonable to assess thyroid indices every 6 to 12 months and initiate therapy when the TSH rises above the upper limit of normal for children. However, if high titers are present at presentation, it is reasonable to initiate therapy at that point.

Untreated in some children, Hashimoto thyroiditis can result in progressive thyromegaly and hypothyroidism.^{38,43} Treatment with levothyroxine prevents hypothyroidism and the TSH elevations that stimulate gland enlargement. When T4 levels are modestly depressed ($>5 \mu\text{dL}$) or normal, treatment can be initiated with 1 to 2 mcg/kg/day of levothyroxine. If profound hypothyroidism is present, pseudotumor cerebri may develop when children are treated with conventional doses.⁴⁵ Thus treatment is often initiated with one-third to one-half of the usual dose of levothyroxine. After 2 to 4 weeks, the patient can be advanced to conventional doses. However, children with profound hypothyroidism can develop pseudotumor cerebri, even if treatment is initiated with low doses of levothyroxine.^{46–48}

Interestingly, it has been recently reported that severe hypothyroidism in children with TSH elevations $>500 \mu\text{U/mL}$, experience complete resolution of the hypothyroid state.⁴⁹ Based on the experience of others, this happenstance is very uncommon.

Although it has been reported that there are some differences in the oral bioavailability of different levothyroxine preparations,^{50–52} from a practical vantage, these differences are small.^{51–53} Thus the routine use of less expensive generic compounds versus more expensive brand-name products is justified.

The timing of levothyroxine ingestion has been the subject of study. Taking the medication at bedtime is associated with higher T4 levels and lower TSH levels over the course of the day.^{54,55} This is believed to be related to better gastrointestinal absorption in the evening than day.^{54,55}

The suggestion has also been made that hypothyroidism in adolescents can be treated with a single dose given weekly.⁵⁶ This approach is not recommended, however, because thyroid hormone levels are high, shortly after the dose is administered, and are low by the week's end.⁵⁶ Treatment of congenital hypothyroidism with weekly doses of levothyroxine also can result in mental retardation.⁵⁷ It is also recognized that excessive soy intake can interfere with the absorption of levothyroxine.^{58–60}

Of note, other potential therapies that may theoretically alter the autoimmune process have been tested. No proven benefit has been observed in patients who have taken selenium.⁶¹

Hashitoxicosis

Uncommonly, patients may present with Hashitoxicosis, in which immunologic destruction of thyroid tissue results in the release of preformed thyroid hormone, which leads to elevated T4 levels.⁶² In contrast to GD, hyperthyroidism is transient, eye findings are absent, radionuclide uptake is low, and elevated levels of thyroid-stimulating immunoglobulins are not present.⁶²

Hashimoto thyroiditis may be associated with other autoimmune diseases, including diabetes mellitus, adrenal insufficiency, vitiligo, and hypoparathyroidism.⁶³ Autoimmune thyroiditis is also seen in patients with inflammatory bowel disease and juvenile arthritis.^{63,64} Annual surveillance of thyroid gland size and TSH levels should thus be considered for children with other autoimmune problems, and clinicians should be vigilant for signs of hyperthyroidism or hypothyroidism. Conversely, children with autoimmune thyroiditis should be observed for signs of diabetes mellitus and Addison disease.

The incidence of celiac disease in the setting of Hashimoto thyroiditis is about 1%.⁶⁵ If patients manifest abdominal discomfort, weight loss, or gastrointestinal symptoms, Celiac disease screening should be performed, but it does not need to be done routinely in children with thyroid disease. We have found a 1% incidence of autoimmune liver disease in children with autoimmune thyroid disease. Because such liver disease can be occult, we annually assess circulating transaminase levels, and if levels are elevated, evaluation of possible liver disease is initiated.

Several groups of children are at risk for autoimmune thyroiditis. Because girls with Turner syndrome are predisposed to autoimmune thyroiditis,⁶⁶ TSH levels should be assessed annually. Turner syndrome should also be considered in girls with hypothyroidism, especially if the child is prepubertal at presentations.^{67,68} Children with Down syndrome also warrant annual screening for hypothyroidism.^{69,70}

Subclinical Hypothyroidism

Subclinical hypothyroidism refers to a condition in which circulating T4 and T3 levels are normal, but TSH levels are elevated.^{31,71} As noted earlier, many children are erroneously diagnosed with this condition when TSH levels are found to be elevated relative to adult reference range values.²⁵ However, if proper pediatric-based TSH levels are applied, the vast majority of children so diagnosed will not have hypothyroidism. Thus some experts have questioned if subclinical hypothyroidism is a real entity in children.^{31,71}

Studies of children with mild TSH elevations (5–10 $\mu\text{U/mL}$) reveal that only a small fraction will progress to TSH elevations over 10 $\mu\text{U/mL}$.^{31,71} Data also show that treatment of children with TSH levels 5 to 10 $\mu\text{U/mL}$ does not exhibit somatic or other benefits when treated with levothyroxine.^{31,71} Thus treatment of children with TSH levels lower than 10 $\mu\text{U/mL}$ is not needed. For children with TSH levels over 10 $\mu\text{U/mL}$, treatment with low doses of levothyroxine is indicated.

Juvenile Acquired Hypothyroidism

When autoimmune thyroiditis occurs during childhood, it is referred to as juvenile acquired hypothyroidism. In children, severe hypothyroidism can be well tolerated. Thus prolonged hypothyroidism may not be detected until growth failure occurs.^{39,72}

Because untreated infantile hypothyroidism is associated with mental retardation, the assumption is often made that juvenile hypothyroidism is associated with learning problems and poor academic performance. This notion is not correct, as children with juvenile hypothyroidism can be successful academically and do not manifest overt learning problems related to the hypothyroid state, nor cognitive impairment.

Children with severe hypothyroidism may manifest cold intolerance, decreased frequency of bowel movements, and decreased physical activity.^{39,72} Bradycardia, facial puffiness, delayed reflexes, and carotenemia may be present. In comparison with Hashimoto thyroiditis, the thyroid gland is either small or only modestly enlarged.^{39,72} Antithyroid antibodies are usually present.^{39,72} These patients are generally not obese, and body mass index values are similar before and after treatment.^{30,73} The development of slipped capital femoral epiphyses may antedate the detection of hypothyroidism.⁷⁴

Some children with juvenile hypothyroidism may present with signs of puberty without pubic hair.^{75–77} Boys may present with testicular enlargement and girls may present with menarche, with or without breast development.⁷⁸ With treatment of the hypothyroid state, these characteristics may regress.^{75–77} Available evidence suggests that the hypothyroid state leads

to increased gonadotropin secretion, which triggers gonadal activity.^{75,78} In some children, puberty may develop within a year or two of treatment onset, which may limit catch-up growth.³⁹

Juvenile hypothyroidism may not be recognized until a sizable statural deficit is present, and the lost height is usually not completely recovered.³⁹ Children with juvenile hypothyroidism who present with growth failure manifest very low T4 values that are often less than 2 mcg/dL, and profoundly elevated TSH levels that are higher than 250 $\mu\text{U/mL}$.³⁹ Hypercholesterolemia and anemia may be present.³⁹

The magnitude of the height deficit is proportional to the duration of hypothyroidism, which can be estimated as the difference between the chronologic and bone ages.³⁹ When the individual is treated with conventional doses of levothyroxine, accelerated skeletal maturation is observed, with the skeletal age advancing disproportionately faster than gains in height.³⁹ Thus predicted heights fall, and generic growth potential is not achieved.

Because of the poor outcomes of patients with hypothyroidism, we have treated these patients with low doses of levothyroxine (0.25–0.5 mcg/kg/day; e.g., 50 mcg for a 10-year-old). Interestingly, we find that, with low-dose levothyroxine therapy, T4 values normalize (6–7 mcg/dL) within 2 months, and TSH levels normalize or remain only modestly elevated. When serial bone age determinations have been made, we have not observed the disproportionate advancement of skeletal age seen with conventional therapy. However, we do not know if this approach leads to more favorable height outcomes.⁷⁹ Some have also suggested that treating these children with gonadotropin-releasing hormone analogs will lead to improved long-term growth.^{80–84} Yet, we have found that catch-up growth slows markedly in some hypothyroid children receiving gonadotropin-releasing hormone analog therapy and predicted adult heights fall and others have not observed added benefit.⁸⁵ Because the loss in adult height is proportional to the duration of hypothyroidism,³⁹ early detection of this disorder is the best intervention for preventing statural deficits.

Iodine-Induced Hypothyroidism

Iodine is a trace element that is essential for thyroid hormone formation (see Fig. 13.1). Recommended dietary iodine intake is approximately 8 pg/kg, or 100 to 150 pg/day, for adolescents and adults.^{86,87} Although modest iodine intake is essential for thyroid function, high-level iodine exposure results in an acute block in the release of preformed thyroid hormone and impaired thyroid hormone synthesis, a phenomenon referred to as the Wolff-Chaikoff effect.⁸⁸ When iodine-induced hypothyroidism is suspected, it can be diagnosed by the detection of high iodine levels in urine samples.⁸⁹

In children, iodine can be absorbed through the skin, and iodine-induced hypothyroidism has been observed after cutaneous iodine or betadine use.^{89–91} We have also observed iodine-induced suppression of thyroid hormone production in children with central intravenous lines, when regular cleansing of the insertion site with iodine was included in central line care. Neonatal hypothyroidism has also been associated with maternal povidone iodine exposure at the time of delivery.⁹⁰

In preterm infants, iodine-induced hypothyroidism warrants special attention, because the suggestion has been made that cutaneous iodine exposure is a major cause of hypothyroidism in premature infants.⁹² Fortunately, studies show that iodine-induced hypothyroidism is uncommon in the United States.⁹³

Significant iodine exposure also occurs from amiodarone, an antiarrhythmic drug that contains 37% iodine.⁹⁴

Hypothyroidism occurs in 10% of individuals treated with this compound.^{95,96} Amiodarone can also reach the fetus by transplacental passage and induce fetal hypothyroidism.⁹⁴

In addition to iodine excess, iodine deficiency also leads to hypothyroidism. Estimates are that more than 1 billion people worldwide are at risk for iodine deficiency.⁹⁷ Clinically, iodine deficiency is associated with goiter, hypothyroidism,⁹⁷ and endemic cretinism.⁹⁸

In the United States, geographic areas of iodine deficiency exist.^{99,100} With the prevalent use of iodized salt, however, the incidence of iodine deficiency has been markedly reduced, and hypothyroidism and goiter caused by iodine deficiency are rare.^{99,100} Of note, the iodine intake in the United States has declined over the past decade, an issue that may have future clinical implications.^{99,100} In Australia, reduction in iodine intake has been recently observed, with potential implications for pregnant and lactating women.¹⁰¹ The exclusive use of deiodized salt, which includes sea salt, is thus not recommended.

Hypothalamic-Pituitary Dysfunction

Central hypothyroidism should be considered in children with a history of head trauma, brain tumors, meningitis, central nervous system irradiation, or congenital nervous system malformations. Central hypothyroidism has also been associated with the use of retinoid X receptor-selective ligands in the treatment of lymphomas.¹⁰²

In contrast to primary hypothyroidism, the diagnosis of hypothyroidism, secondary to hypothalamic-pituitary dysfunction, may be difficult to establish. Often, circulating levels of T4 are in the low-normal range, and TSH levels may be low, normal, or elevated.^{103,104} FT4 values, however, are usually low.

Whereas congenital central hypothyroidism will be diagnosed in states that perform T4 screening of newborns, neonatal screening programs that rely on TSH determinations will not detect this condition. Central hypothyroidism should therefore be suspected in infants with cholestasis, poor growth, hypoglycemia, structural nervous system problems, or pituitary insufficiency.¹⁰⁵ When neonatal T4 values are interpreted, care should also be taken to use infant thyroid hormone values for comparison, because infantile T4 levels are higher than those seen in adults (see Tables 13.1 and 13.2).¹⁰⁶

Importantly, up to 30% of children who will develop central hypothyroidism may have normal T4 and TSH levels at birth.¹⁰⁷ Thus all children with evidence of hypopituitarism should be regularly monitored for the onset of central hypothyroidism.

When central hypothyroidism is suspected, the TRH test helps distinguish pituitary (secondary) and hypothalamic (tertiary) hypothyroidism.^{104,106,108} Central nervous system imaging should also be performed to look for congenital malformations or hypothalamic-pituitary lesions. Care should be taken to search for other pituitary hormone deficiencies, especially abnormalities of the hypothalamic-pituitary adrenal and growth hormone axes.

Treatment consists of replacement therapy with levothyroxine. Interestingly, some children with central hypothyroidism require doses lower than those used to treat primary hypothyroidism.¹⁰⁹ Because TSH values are not helpful in guiding treatment, measurement of FT4 levels is recommended.¹⁰⁹ One recommendation is to use a dose 1.6 mcg/kg body weight. This dose was found to be associated with improved markers commonly associated with central hypothyroidism and suggests that levothyroxine dosage, based on body weight, and aiming for a FT4 level in the upper reference range, is superior to titration of levothyroxine dose, aiming at middle normal FT4 concentrations in these patients.¹¹⁰

It is important to consider that children with central hypothyroidism may also have other endocrine deficiencies. These

individuals have been shown to be at increased risk for sudden death.¹¹¹

Giant Hemangiomas

Hypothyroidism has been associated with giant hemangiomas.¹¹² In some infantile hemangiomas, the endothelium of these vascular structures produces type 3 iodothyronine deiodinase, which degrades circulating T4 (see Fig. 13.2). Treatment of hypothyroidism (consumptive) in this setting requires high doses of levothyroxine.¹¹²

Hypothyroidism in Cancer Survivors

It is well recognized that children who are cancer survivors, who have had head and neck irradiation, are at increased risk for differentiated thyroid cancer.^{113,114} More common, though, is the development of mild hypothyroidism.^{115–117} Up to 30% of children who have had head and neck irradiation will develop primary hypothyroidism.¹¹⁷ Thus annual TSH screening is suggested. In addition, ultrasound studies are recommended, beginning 5 years after radiation exposure. Practitioners who argue that palpation alone is sufficient for the follow-up of individuals who have had head and neck irradiation need to recognize that ultrasonography will detect thyroid nodules well before palpation.^{114,118}

THYROID HORMONE RESISTANCE

Thyroid hormones exert their effects by binding to specific nuclear receptors to regulate cellular gene expression.¹¹⁹ When the TR is mutated, impaired tissue responsiveness results, leading to thyroid enlargement, elevated levels of T4 and T3, tachycardia, and behavioral problems.^{120–123} Unlike GD, TSH levels are normal or slightly elevated.

The most common forms of thyroid hormone resistance are caused by mutations of the TR beta gene.^{121–123} More than 100 mutations have been identified that result in impaired affinity for T3.¹²¹ Mutant TRs also block the function of normal TRs.¹²¹ Thus thyroid hormone resistance is a dominant-negative mutation, and inheritance is autosomal dominant.¹²² Detection of thyroid hormone resistance in the index case may therefore lead to diagnosis of the condition in other family members. In up to 50% of children with thyroid hormone resistance, the mutations are spontaneous.

Most individuals with resistance to thyroid hormone have generalized thyroid hormone resistance.¹²¹ These individuals are eumetabolic and asymptomatic, with TSH levels in the normal range.

In contrast, some individuals have isolated pituitary thyroid hormone resistance. These individuals have symptoms of hyperthyroidism, because they are sensitive to the effects of increased thyroid hormone levels.¹²⁴ Resistance to thyroid hormone can also be associated with central nervous system problems. Approximately 50% of individuals with resistance to thyroid hormone have attention deficit hyperactivity disorder, and a minority have mental retardation.¹²⁵

Because individuals compensate for thyroid hormone resistance by secreting more thyroid hormone, treatment is generally not necessary.^{121,126} However, patients with thyroid hormone resistance may be improperly diagnosed as having GD and undergo ablation of the thyroid. In this situation, replacement therapy with large doses of exogenous thyroid hormone is needed. With the earlier recognition of resistance to thyroid hormone because of newborn screening, the issue of whether children with resistance to thyroid hormone should be treated prenatally or during infancy has been raised.^{121,126} Treatment is generally reserved for infants who show elevated TSH levels, growth failure, seizures, and developmental delay.^{121,126}

In some cases, TSH secretion may be profound, leading to massive thyromegaly, which may adversely impact upper airway function.^{127,128} These cases are generally associated with severe loss of function mutations. Treatment with high doses of T3 every other day has been shown to be somewhat effective in this setting.^{127,128} In other cases,^{127,128} thyroidectomy is needed to prevent airway compromise.

HYPERTHYROIDISM

Hyperthyroidism has profound influences on the fetus, neonate, growing child, and adolescent, including physical and behavioral effects.^{28,29,129–132} In children, thyrotoxicosis is often present for extended periods before recognition, contributing to significant health problems. A number of conditions result in hyperthyroidism in childhood (see Table 13.1), yet GD is the most common cause,^{131,132} and thus will be the major focus of this chapter with treatment approaches stratified (Fig. 13.4).

Other causes of hyperthyroidism in children include autonomously functioning thyroid nodules, Hashitoxicosis, neonatal thyrotoxicosis, and infections of the thyroid. Hyperthyroidism also results from thyroid hormone ingestion, McCune–Albright syndrome, struma ovarii, and TSH-producing pituitary adenomas. Epidemic hyperthyroidism has also been seen when thyroid tissue has been inadvertently included in meat products.¹³³ Several conditions can masquerade as hyperthyroidism, including thyroid hormone resistance and thyroid hormone-binding protein disorders, yet are best left untreated.

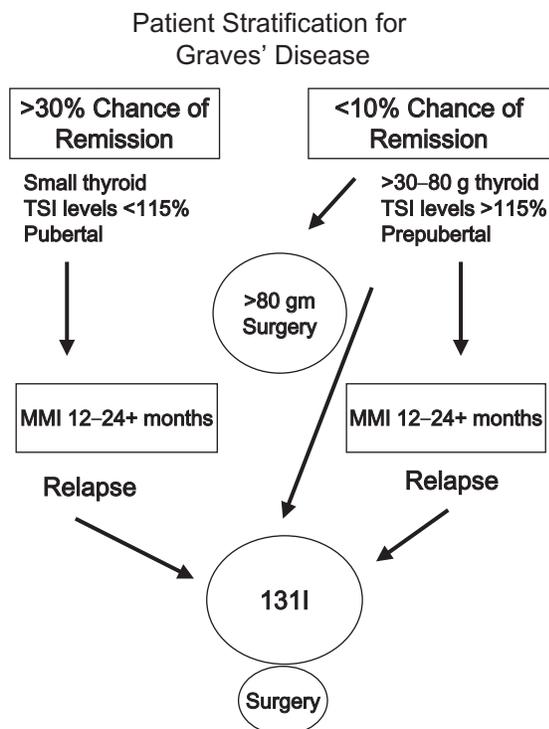


Fig. 13.4 Graves disease therapy in children: stratification by clinical characteristics. Patients can be grouped into those with a better or worse chance of remission based on age, TSI levels, and thyroid size. For very large glands (>80 g), surgery is the treatment of choice. Patients can be treated with antithyroid drugs for 12 to 24 months and the drug withdrawn to see if remission is achieved. If there is no remission, either surgery or radioactive iodine can be administered. Alternatively, the patient can be restarted on antithyroid medication. Only methimazole or carbimazole should be used.

Clinical Evaluation of the Hyperthyroid State

Pediatric thyroid disease can present with overt symptoms, silently, or with isolated thyromegaly.^{28,29,129–132} When hyperthyroidism is suspected during childhood, the practitioner should look for somatic signs (see Table 13.2). One of the universal features of hyperthyroidism is tachycardia.^{28,29} In the absence of such, it is unlikely that hyperthyroidism is present. Other clinical features can include a prominent stare and proptosis, although eye findings occur less commonly in children and adults.^{132,134} It is the common perception that children presenting with hyperthyroidism present thin with weight loss, yet this is often not the situation.¹³⁵

Graves Disease

GD is the most common cause of hyperthyroidism in children and affects 1 in 10,000 children.⁵ Interestingly, the incidence of GD in children may be increasing.¹³⁶ An autoimmune disorder, GD is caused by thyroid gland stimulation by TRAbs or TSI, and involves genetic factors.⁵

Hyperthyroidism can exert profound adverse effects on children, including excessive physical activity, tremor, tachycardia, flushing, palpitations, weight loss, accelerated linear growth, reduced bone mineralization, and poor school performance.⁶ In comparison with adults,^{7,8} eye disease occurs in the minority of pediatric patients with GD, and when it occurs, is usually mild.⁹

Over the past several years, additional outcome data have become available^{137–141} to complement older studies looking at spontaneous remission rates of children with GD.^{7,10–13,18,20–23} Collectively, these studies show that the majority of pediatric patients with GD will not undergo spontaneous remission, even after many years of antithyroid drug (ATD) therapy (see Table 13.3). Thus most pediatric patients will require either radioactive iodine (RAI,¹³¹I) or surgery.

Antithyroid Drug Therapy

ATDs act by inhibiting oxidation and organic binding of thyroid iodide to impair thyroid hormone production and include methimazole (MMI) and propylthiouracil (PTU).²⁹ MMI is 10- to 20-fold more potent than PTU and has a longer half-life.¹²⁹ Importantly, these medications do not cure the hyperthyroid state, rather they palliate the condition, until spontaneous remission occurs or definitive therapy is rendered.

Each of these medications is associated with adverse events that must be considered when prescribed. Before the initiation of drug therapy, a back-up plan that takes into account the patient's age and treatment risks must be developed at therapy onset in the event that a toxic reaction occurs. Failure to initially consider alternative treatments can result in a crisis when adverse effects occur.

Because it takes 1 or 2 months until biochemical hyperthyroidism resolves on drug therapy,¹³⁰ treatment with beta-blockers (propranolol, atenolol, or metoprolol), can be used to control GD symptoms. Focusing on symptom control with beta-blockers also alleviates the false perceived need for initial high-dose ATD therapy. Because atenolol does not cross the blood barrier as much as propranolol, patients will not feel as tired on this medication and thus is preferred.¹⁴² For individuals who have reactive airway disease,¹⁴³ the cardiac selective beta-blocker metoprolol may be used.

In 2008 Rivkees brought to public attention a number of serious complications associated with PTU therapy in children.^{40,130,133} PTU-induced liver injury occurs in up to 1 in 2000 children, occurs rapidly, and is often irreversible.^{144,145} Serial monitoring of transaminase levels in a child on PTU is not viewed as useful in reducing the hepatotoxicity risk.¹⁴⁶ Thus the only way to reduce the risks of PTU-related hepatotoxicity is to avoid the use of this medication.

PTU, though, may be needed in special circumstances.¹⁴⁷ These conditions include situations when neither prompt ¹³¹I or surgical treatment are options in a patient who has had a toxic reaction to MMI and ATD medication is necessary. In this setting, PTU use should be short term.²⁵

If PTU is prescribed, patients and guardians must be informed of the risk of liver failure and to be alert for signs and symptoms of liver abnormalities, including pruritus, jaundice, anorexia, light colored stools, dark urine, and abdominal pain. If these symptoms develop, the patient should immediately stop the medication, a physician contacted, and laboratory tests obtained to evaluate hepatic function and transaminase levels.²⁵

MMI is now the drug choice for hyperthyroidism. The MMI doses described in published reports range from 0.1 to 1.0 mg/kg/day.^{148–153} However, one does not need to use high doses at treatment onset, as MMI side effects are in part dose related.¹⁴⁵ The response to ATDs influencing circulating thyroid hormone levels is not instantaneous, and several months are needed for thyroid hormone levels to normalize.^{154,155} Thyroid function tests should be obtained monthly after therapy onset. After T4 levels become normal, in most cases the MMI dose can be cut by half to maintain euthyroidism.¹⁴⁵ Although MMI is often prescribed in divided doses over the day, once-a-day dosing is sufficient,¹⁵⁴ and is associated with better compliance than multiple daily doses.^{155–157}

MMI is available in 5, 10, and 20 mg tablets. When used in children, the following doses that are fractions of tablets can be used: infants, 1.25 mg/day; 1 to 5 years, 2.5 to 5.0 mg/day; 5 to 10 years, 5 to 10 mg/day; and 10 to 18 years, 10 to 20 mg/day. Because the hyperthyroid state can be associated with low white cell counts, and patients will be treated with a medication that can depress neutrophil levels, one should obtain a complete blood count at therapy onset.¹⁴⁵

MMI therapy is not without risks. Minor side effects may affect up to 20% of children, and major side effects may occur in 1% of children.^{158,159} The most common minor adverse side effects related to MMI are hives, arthralgia, and neutropenia.^{138,160} Children may also develop major side effects, including agranulocytosis, Stevens-Johnson syndrome, and vasculitis.¹³⁸ MMI adverse events most commonly occur within 6 months of therapy onset.^{159,161} Yet, children may develop adverse events more than 12 months after treatment onset.

Agranulocytosis is a potential serious ATD adverse event and occurs in 0.3% of adults taking PTU or MMI.^{138,160} The agranulocytosis risk is dose dependent and is rare.^{137,162} If an individual receiving MMI feels ill, becomes febrile, or develops pharyngitis, MMI should be stopped immediately, a practitioner contacted, and a complete blood cell count obtained.

Note that in the MMI-only era that we are now in, reports of MMI-associated agranulocytosis have recently been published.¹⁶³ Similar to the monitoring of adverse events associated with PTU, it is important that adverse events related to MMI be monitored and brought to the attention of the US Food and Drug Administration by practitioners via the MedWatch program (<https://www.fda.gov/Safety/MedWatch/>).

Antithyroid Drug Therapy Duration and Prediction of Remission

Based on considerable evidence,^{132,134,146,164–168} prolonged ATD therapy will not result in an increased chance of remission for most children, whereas in a minority of children it may. As such, with the initial evaluation of a child with GD, practitioners should attempt to stratify children into two groups: children with a 30% to 40% chance of remission with prolonged ATD therapy, and children with a slim chance of spontaneous remission.

Data shows that one can distinguish patients with a greater likelihood of remission from those with a much lower chance, following prolonged ATD therapy. The chance of remission after years of ATDs will be low if the thyroid gland is large (>2 times normal size for age), the child is young (<12 years), not Caucasian, serum TRAB/TSI levels are elevated, or the patient presents with profound hyperthyroidism at presentation ($FT_4 > 4$ ng/dL).^{113,169,170}

Prospective studies in adults show that if remission does not occur after 12 to 18 months of ATD therapy, it is unlikely remission will occur with prolonged therapy.¹⁶⁷ In the pediatric population, published data show that when ATDs are used for 1 to 2 years, remission rates are 15% to 30%, and possibly up to 40% in some children.^{113,146,151,166,167,170–172} Please note that remission is defined as being either euthyroid or hypothyroid for 1 year or more after cessation of therapy.

In adults, assessment of TRAB or TSI levels is useful in determining disease course and remission likelihood.^{169,173} This issue has been less studied in children. Consistent with the notion that GD will remit in only a small proportion of children, TRAB levels normalize after 24 months in only 18% of pediatric patients on ATDs.¹⁶⁹ TRAB levels thus persist longer in children than in adults.¹⁷⁴ There are no data showing normalization of TRAB levels in pediatric patients on ATDs for longer.

For children with unfavorable risk factors for spontaneous remission at treatment onset, it is reasonable to treat children for up to 2 years with MMI and see if spontaneous remission occurs. At that point, if there is no remission, it is appropriate to move on to definitive therapy if desired by the family. Alternatively, treatment for longer periods can be considered, as long as side effects to medication do not occur. This approach may be especially useful if the child is considered too young for surgery or RAI. For the child with favorable risk factors for remission, if spontaneous remission has not occurred after the 2 years of ATDs, continuation of antithyroid medication for prolonged periods is also acceptable, yet one needs to be attentive to adverse effects.

Strategies for the Child With an Adverse Event to Methimazole

With MMI being the drug of choice for treatment, and the risk of hepatotoxicity being present with the use of PTU, practitioners may face a clinical dilemma of what to do when a patient taking MMI develops an adverse reaction. There are few published reports addressing this issue in children.

For children who develop allergic symptomatology, antihistamine medications may be attempted.¹⁷⁵ However, this approach is generally not effective.

If an individual develops neutropenia, agranulocytosis, or hepatotoxicity, the antithyroid medication must be discontinued immediately. Because the hyperthyroid state will resume quickly, and it is best not to treat patients with RAI or surgery, while profoundly hyperthyroid, therapy to control the hyperthyroid state, until definitive therapy must be initiated within days. If a patient will be treated with RAI, beta blockade should be initiated and treatment rendered within the week the ATD is stopped to prevent recurrence of profound hyperthyroidism. This approach requires a coordinated effort with nuclear medicine experts, as soon as the decision is made to discontinue the ATD.

If the individual is viewed as being too hyperthyroid for treatment with RAI, practitioners may consider the short-term use of PTU to control the hyperthyroid state, until the patient is viewed as stable enough for treatment with RAI. Because PTU may also be associated with agranulocytosis,^{176–178} if PTU is used, white blood counts need to be very closely followed.

If the plan is surgical intervention, iodine drops may be started immediately after MMI is discontinued and continued

for at least 2 weeks. This period will allow the hyperthyroid state to resolve and allow adequate time for surgical planning.

Radioactive Iodine Therapy

The goal for ^{131}I therapy for GD is to induce hypothyroidism. There are several important caveats related to the use of RAI in children. RAI should not be given to cause euthyroidism in children, as this results in partially irradiated residual thyroid tissue that may be associated with a higher risk of thyroid neoplasm than the normal population.^{179,180}

It has been suggested that dosages delivering 30,000 to 40,000 centigray (cGy) (rad) to the thyroid are necessary to ablate the thyroid gland.^{181,182} But, dosages delivering 10,000 to 20,000 cGy to the thyroid are more often used and result in partial or complete destruction of the thyroid.^{181–183} Typically, administered thyroid doses of 150 $\mu\text{Ci/g}$ (5.5 mega-Becquerel [MBq]/g) generate radiation doses of 12,000 cGy to the thyroid.¹⁸⁰

Dosing is based on the Quimby-Marinelli equation: Dose ($\beta + \gamma$ radiation; in Gy) = $90 \times [\text{oral } ^{131}\text{I} \text{ dose (microcurie } [\mu\text{Ci}]) \times \text{oral 24-hour uptake (\%)/g} \times 100\%]$. For example, if the desired dosage is 300 $\mu\text{Ci/g}$, and the thyroid is 30 g with an uptake of 75% (0.75), the desired administered dosage will be 12 mCi. (Dosage in mCi = $300 \mu\text{Ci/g} \times 30 \text{ g}/0.75 \text{ uptake} = 12,000 \mu\text{Ci}$ or 12 mCi).

Some centers give a fixed administered dosage of 10 or 15 mCi ^{131}I to all children,¹⁸¹ rather than individually calculated administered activation. There are no studies comparing outcomes of fixed doses versus calculated doses in children. In adults, the two different approaches lead to similar outcomes;^{180,182} however, in children, a potential advantage of calculated versus fixed dosing, is that it is possible to use lower dosages of ^{131}I if the administered dose is calculated.

When children are to be treated with ^{131}I , ATDs should be stopped 3 to 5 days before treatment.¹⁸¹ Patients are placed on beta-blockers until T4 and/or FT4 levels normalize posttherapy. Whereas some clinicians restart ATDs after treatment with ^{131}I , this is rarely required in children.^{181–184} Thyroid hormone levels begin to decrease about 7 days after radioiodine therapy in children. Continued ATD use can make it difficult to assess if posttreatment hypothyroidism is the result of ^{131}I or the ATD.

Side effects of ^{131}I therapy are unusual. Less than 10% of children will complain of mild tenderness over the thyroid in the first week after ^{131}I therapy.^{184,185} This problem can be treated with either acetaminophen or nonsteroidal antiinflammatory agents for 24 to 48 hours.¹⁸⁶

There are rare reports of children with severe hyperthyroidism developing thyroid storm after ^{131}I .^{186,187} In general, these children were severely hyperthyroid when ^{131}I was rendered. Thus if T4 levels are over 20 mcg/dL or FT4 levels are greater than 5 ng/dL, children should be treated with MMI until T4 and/or free T4 levels normalize, before proceeding with ^{131}I therapy.^{183,188} Importantly, most children with GD have been hyperthyroid for months before diagnosis; there is no need to rush to ^{131}I therapy.

It usually takes 6 to 12 weeks after ^{131}I treatment for the patient to become biochemically euthyroid or hypothyroid. Until then, symptoms of hyperthyroidism can be controlled using beta-blockers.^{186,189,190} The use of potassium iodide or Lugol solution, 1 week after ^{131}I , will also quickly attenuate biochemical hyperthyroidism, without adversely affecting the outcome of radioiodine therapy.¹⁸⁵

Several studies have reported the details of ^{131}I therapy for childhood GD.^{191–194} Children as young as 1 years old have been treated with ^{131}I with excellent results.^{194,195} But, treatment of such young children is not common, nor is now recommended. ^{131}I dosages in children and teenagers have ranged

from 100 to 400 $\mu\text{Ci/g}$ of thyroid tissue.¹⁹⁶ Similar to that found in adults,^{197–199} responses to ^{131}I therapy are related to gland size and dose. Some 25% to 40% of children treated with 50 to 100 μCi of ^{131}I per gram of thyroid tissue are hyperthyroid several years after therapy.²⁰⁰ In children treated with 150 to 200 μCi of ^{131}I per gram of thyroid, hyperthyroidism remains in 5% to 20%, and 60% to 90% become hypothyroid.^{201–203}

The development of progression of ophthalmopathy following ^{131}I in adults has been reported.^{204–206} However, children rarely develop severe ophthalmopathy and proptosis is mild.^{146,207} Studies show that eye disease worsens in only a small percentage of children with GD, irrespective of therapy type.^{208,209}

In adults, it has been shown that progression of ophthalmopathy can be prevented by treatment with prednisone for 3 months following ^{131}I therapy.^{206,207} Adjunctive prednisone therapy is not routinely recommended for the majority of children, as most do not have significant eye disease. The prolonged administration of prednisone is also associated with growth failure, weight gain, and immune suppression. Nevertheless, prednisone (0.5 mg/kg \times 4–6 weeks) may be useful for the child who has moderate or severe eye disease and will be treated with ^{131}I .

Risks of Radioactive Iodine in Children Treated for Graves Disease

There is no evidence showing adverse effects to offspring of children treated with ^{131}I . Birth defects are not higher in offspring born to individuals treated with ^{131}I for hyperthyroidism during childhood or adolescence.¹⁴⁶ In addition, the rates of birth defects are not higher in children treated with 80 to 700 mCi of ^{131}I for thyroid cancer, which are dosages that are much higher than those used for GD.²¹⁰

The thyroid gland is unique in its developmental sensitivity to malignancy after low-level radiation exposure.^{113,169,170,174} There is an increased risk of thyroid cancer in individuals less than 20 years of age at the time of low-level thyroid irradiation.^{113,170,174} In contrast, individuals who are older than 20 years of age do not exhibit an increased risk of thyroid cancer when exposed to low-level thyroid irradiation.^{113,169,170,174}

The risk of thyroid neoplasms in children is greatest with exposure to low-level external radiation (0.1–25 Gy; \sim 0.09–30 $\mu\text{Ci/g}$),^{113,169,170,173,174} and not with the higher dosages used to treat GD. At present, we are not aware of any cases of thyroid cancer that developed in pediatric patients treated with more than 150 μCi of ^{131}I per gram of thyroid tissue for childhood GD that can be attributed to ^{131}I therapy.

Important in considering RAI use in children, is the potential influences of ^{131}I therapy on other cancers, as ^{131}I therapy results in low-level, whole-body radiation exposure. Several studies in adults have examined potential risks of ^{131}I therapy for GD on cancers. These studies have not revealed increased mortality or increased rates of cancer following ^{131}I for GD.^{211–217}

In comparison with studies in adults, few studies have focused on outcomes of ^{131}I therapy for childhood GD. The most extensive study of pediatric patients involved 36-year outcomes of patients who were less than 20 years old when treated with ^{131}I therapy for GD.¹⁶⁴ There was no evidence for increase cancer risk in this population. Yet, this sample size was modest.

The total-body radiation dose after ^{131}I varies with age, and the same absolute dose of ^{131}I will result in more radiation exposure in a young child than in an adolescent or adult.^{218,219} Currently, we do not have dosimetry data on ^{131}I use in pediatric patients with GD to assess total-body exposure in pediatric patients. Based on phantom modeling, it is estimated that at 0, 1, 5, 10, 15 years, and adulthood, respective total-body

radiation doses will be 11.1, 4.6, 2.4, 1.45, 0.90, and 0.85 Roentgen equivalent man (rem) (0.01 Sievert [Sv]) per millicurie of ^{131}I administered.^{218,219} Based on the Biologic Effects of Ionizing Radiation Committee V (BEIR VII) analysis of low-level, acute exposure to radiation,²²⁰ theoretical lifetime attributable risk of cancer mortality and all cancer incidence can be projected. Based on these theoretical calculations, we feel that it is prudent to avoid RAI therapy in children under 5 years of age and to avoid more than 10 mCi in patients less than 10 years old.

Thyroidectomy and Risks

Surgery is an effective form of therapy for GD if an expert surgeon can perform it, and in some situations it is preferable to RAI. When surgery is performed, near-total or total thyroidectomy is indicated, as subtotal thyroidectomy is associated with a higher relapse rate.²²¹ Hypothyroidism is nearly universal in children and adults who undergo total thyroidectomy.^{221–224} In comparison, after subtotal thyroidectomy, hyperthyroidism recurs in 10% to 15% of patients.^{221–223}

Surgery is preferred in children younger than 5 years when definitive therapy is needed and can be performed by a skilled thyroid surgeon. In individuals who have large thyroid glands (>80 g), the response to ^{131}I is poor,^{225,226} and surgery is recommended for these patients.

In preparation for surgery, the patient should be rendered euthyroid. Typically, this is done by continuing MMI until T4 levels normalize. A week before surgery, iodine drops are started (1–3 drops, three times per day), which inhibits thyroid hormone production and causes the gland to become firm and less vascular.²²⁷

Postoperatively, younger pediatric patients are at a higher risk for transient hypoparathyroidism than adolescents or adults.²²⁸ To mitigate postoperative hypocalcemia, we treat children with 0.5 mcg of calcitriol, twice a day, for 3 days before surgery. Postoperatively, the calcitriol is weaned over 15 days (0.5 mcg twice a day \times 5 days; 0.5 mcg every day \times 5 days; 0.5 mcg every other day \times 5 days).²²⁹ Using this approach, only 5% of patients require postoperative calcium infusions versus 40% of patients without preoperative treatment.²²⁹

Acute complications that follow thyroidectomy include hemorrhage, hypocalcemia, and recurrent laryngeal nerve paresis.^{228,230–233} In children, rates from 0 to 6 years were 22%; from 7 to 12 years, 11%; and from 13 to 17 years, 11%.²²⁸ These rates are higher than those observed in adults.

Complication rates are related to the expertise of the surgeon.²²⁹ Considering these data, if local pediatric thyroid surgery expertise is unavailable, referral of a child with GD to a high-volume, thyroid surgery center with pediatric experience should be considered.^{234,235} Very low complication rates for children undergoing the thyroidectomies for GD have been reported with this type of multidisciplinary model.^{229,234}

Following our model,²²⁹ there is the recent trend in the development of pediatric thyroid centers across the United States. These centers often have experts in pediatric thyroid surgery.

Somatic Complications of Graves Disease

In addition to focusing on the thyroid-related aspects of GD, somatic considerations are important as well. Individuals with GD have an increased basal metabolic rate and increased caloric needs. This may result in altered dietary patterns, resulting in increased caloric intake. After correction of the hyperthyroid state, it is recognized that body mass index will increase, which can be dramatic in some individuals.^{135,236} As such, anticipatory guidance and monitoring of body weight after implementation of antithyroid therapy is important.

GD is also associated with increased bone turnover, which can lead to osteopenia in affected individuals.^{237,238} GD has thus been associated with an increased fracture risk.

Thyrotoxicosis can trigger thyrotoxic periodic paralysis,²³⁹ which is a life-threatening condition associated with profound hypokalemia. This condition is most frequently observed in Asian men and is associated with hypokalemia, which affects the lower extremities and is secondary to thyrotoxicosis. The condition results from an intracellular shift of potassium induced by the thyroid hormone sensitization of Na^+/K^+ -adenosine triphosphatase and is associated with mutations of the inwardly rectifying potassium channel Kir2.6.^{239–241}

Another potential concern related to the resolution of GD is the development of acute myopathy.²⁴² This has been sporadically reported in children following correction of the hyperthyroid state, resulting in large increases in circulating muscle enzymes as hyperthyroid state transitions to hypothyroidism.²⁴² Following treatment, individuals presenting with muscle cramps should be evaluated for this possibility through the monitoring of creatine phosphokinase levels.

Autoimmune thyroiditis may be associated with other autoimmune diseases, including diabetes mellitus, adrenal insufficiency, vitiligo, and hypoparathyroidism.²⁴³ Autoimmune thyroiditis is also seen in patients with inflammatory bowel disease and juvenile arthritis.^{63,64} Annual surveillance of thyroid gland size and TSH levels should thus be considered for children with other autoimmune problems, and clinicians should be vigilant for signs of hyperthyroidism or hypothyroidism. Conversely, children with autoimmune thyroiditis should be observed for signs of diabetes mellitus and Addison disease.

The incidence of celiac disease in the setting of autoimmune thyroiditis is about 1%.⁶⁵ If patients manifest abdominal discomfort, weight loss, or gastrointestinal symptoms, Celiac disease screening should be performed, but it does not need to be done routinely in children with thyroid disease. We have found a 1% incidence of autoimmune liver disease in children with autoimmune thyroid disease. Because such liver disease can be occult, we annually assess circulating transaminase levels, and if levels are elevated, evaluation of possible liver disease is initiated.

Several groups of children are at risk for autoimmune thyroiditis and GD. Because girls with Turner syndrome are predisposed to autoimmune thyroiditis,⁶⁶ TSH levels should be assessed annually. Turner syndrome should also be considered in girls with hypothyroidism, especially if the child is prepubertal at presentation.^{67,68} Children with Down syndrome also warrant annual screening for thyroid dysfunction.^{69,70}

NEONATAL THYROTOXICOSIS

Thyrotoxicosis in the neonate is a severe and life-threatening condition that can be associated with lasting neurologic problems.^{186,244,245} Neonatal thyrotoxicosis most commonly occurs in the setting of active or past maternal GD. If a mother has GD, the chance is 1 in 80 that TRAbs will be transferred to the fetus, resulting in intrauterine or neonatal hyperthyroidism.¹⁸⁴ Rarely neonatal thyrotoxicosis will persist, like the GD disease seen in older children.¹⁸⁶ In other rare cases, persistent neonatal thyrotoxicosis is caused by activation of the TSH receptor.^{246,247}

The fetal thyroid gland is responsive to maternal TRAbs, which, if present at elevated levels, may result in hyperthyroidism.^{180–182,207} Fetal hyperthyroidism manifests during the second half of gestation, as transfer of TRAbs from the mother to the fetus increases with progression of pregnancy.^{180–182}

The risk of fetal hyperthyroidism and neonatal GD is proportional to the magnitude of elevation of TRAb levels.^{180–182,245} Fetal hyperthyroidism is generally associated with levels of

TRABs more than 2- to 4-fold greater than the upper limit of normal for assay.^{180–182} Because the fetus is at risk for hyperthyroidism when there is active or past maternal GD, fetal growth and heart rate should be regularly assessed from mid-pregnancy onward.^{180–182} Excessive fetal heart rate (>160 beats per minute after 20 weeks) and the presence of a fetal goiter suggest hyperthyroidism in the fetus. In addition, accelerated maturation of the femoral ossification center is seen with fetal hyperthyroidism.¹⁸¹

If a mother with GD is taking antithyroid medications during pregnancy, fetal thyroid hormone synthesis will be inhibited, which will prevent the development of intrauterine hyperthyroidism.¹⁸³ However, the infant may be born with a goiter and hypothyroidism.¹⁶⁵ At birth, circulating levels of T4 may be low and TSH levels elevated. In most cases, the effects of ATDs wane, and thyroid function normalizes within a week.¹⁸⁴ If significant transplacental passage of TRABs has occurred, however, thyrotoxicosis will develop.^{184,185}

If a mother with a history of GD is not taking ATDs during pregnancy, the fetus may develop intrauterine hyperthyroidism.¹⁸⁶ If the condition is not recognized, it may result in profound intrauterine thyrotoxicosis and growth retardation.¹⁸⁶ Such infants have prematurely fused cranial sutures, advanced skeletal age, long-term learning problems, and mental retardation.^{186,188} If fetal hyperthyroidism is recognized prenatally by the presence of fetal tachycardia (heart rate >160 beats per minute after 22 weeks), treatment of the mother with ATDs will reduce intrauterine thyrotoxicosis.^{183,189,190}

Treatment of thyrotoxic infants consists of administration of MMI (1.25 mg per day) and beta-blockers (propranolol 1 mg per kg per day). Lugol solution or saturated potassium iodide may be given (1–2 drops every 8 hours) for 7 to 10 days to more rapidly control biochemical hyperthyroidism. After approximately 2 weeks of ATD therapy, thyroid hormone levels will decline. When thyroid hormone levels fall below normal, supplementary levothyroxine (37.5 mcg/day for full-term infants) is added to prevent hypothyroidism. As TRABs are cleared from the infant's circulation, spontaneous recovery begins within 3 months and is usually complete by 6 months.^{185,186} Thus the infant can be weaned from treatment after 3 months. Monitoring of the infant's TRAB levels is also a useful predictor of when antithyroid medication can be tapered.^{191,192}

OTHER CAUSES OF HYPERTHYROIDISM

Activating Mutations of the Thyroid-Stimulating Hormone Receptor

Several mutations of the TSH receptor have been reported that resulted in ligand-independent receptor activation and hyperthyroidism.^{248,249} Affected individuals present with hyperthyroidism in the neonatal period with potential serious adverse effects on the child including advanced skeletal maturation and craniosynostosis, impaired growth, and neurologic impairment.^{248,249} This diagnosis should be considered in the young child presenting with hyperthyroidism and bone age advancement, in the absence of TSI elevation. The hyperthyroid state may be controlled with ATDs, yet, either RAI if surgery is needed. Because hyperthyroidism in the neonatal and early childhood can be associated with abnormal growth and maturation and neurologic abnormalities, it is essential that the hyperthyroid state be exquisitely controlled in young children.

McCune–Albright Syndrome

McCune–Albright syndrome is an inherited disorder characterized by spontaneous activation of the G protein alpha

subunit.^{250–252} Because the TSH receptor is G protein-coupled, somatic rotations of the G protein alpha subunit within the thyroid gland may result in hyperthyroidism. Children with McCune–Albright syndrome may be recognized with a distinctive pattern of café au lait spots with a ragged border, and/or history of polyostotic or monostotic fibrous dysplasia. Because there is clonal activation in the thyroid gland, which results in areas of increased thyroid proliferation and activity, and other regions that will not be affected, similar to the treatment of isolated hyperfunctioning thyroid nodules, definitive treatment by surgery is recommended.

Thyroid-Stimulating Hormone Producing Adenomas

Thyrotropinomas are rare in adults and exceedingly rare in children.²⁵³ Such tumors are typically macroadenomas presenting with the signs of hyperthyroidism and occasional central nervous system symptoms that may include visual field loss and headaches. Such individuals will have elevated levels of circulating thyroid hormones, but without a suppressed TSH.

Transsphenoidal surgery is the preferred treatment and adjunctive radiotherapy may be needed. In adults, successful treatment with octreotide has been reported.²⁵³ It is important to distinguish this condition from isolated central thyroid hormone resistance, which can present with similar biochemical features. Distinguishing features include the presence of a pituitary mass with TSH-producing tumors, the lack of response to TRH stimulation, and elevated levels of the α -glycoprotein subunit, in pituitary adenomas.²⁵³

Infectious Bacterial Thyroiditis

Occasionally, a child presents with hyperthyroidism, tenderness over the thyroid gland, and fever caused by bacterial infection of the thyroid, a condition called acute thyroiditis.¹⁹³ Acute thyroiditis can be associated with the presence of a fistula connecting the piriform sinus on the left side of the pharynx to the thyroid.¹⁹³ Fevers can be high, and erythrocyte sedimentation rates and white counts elevated. Ultrasonography may reveal a local abscess. In contrast to GD, uptake of technetium 99-pertechnetate or radioiodine is reduced when thyroid scanning is performed.

The offending bacteria include *Haemophilus influenzae* and group A streptococci.¹⁹³ Thus treatment with an antibiotic resistant to disruption by β -lactamase is recommended. In severe cases, hospitalization and intravenous antibiotic administration is indicated, because lymphatic drainage into the mediastinal region may occur. Surgical drainage is needed if a localized abscess develops and the response to antibiotics is poor.¹⁹³

Because the infectious process results in destruction of thyroid tissue, release of preformed thyroid hormone and hyperthyroidism may occur during infection. The hyperthyroid state is usually transient, and treatment with ATDs is not indicated.¹⁹³ If the patient becomes symptomatic, beta-blockers may be used.

After the child has recovered, pharyngography is indicated to test for a patent piriform sinus tract. Occasionally, the tract may close as the result of the infection. If the tract persists, however, and acute thyroiditis recurs, resection is needed.

Subacute Thyroiditis

Viral infections of the thyroid may occur and result in subacute thyroiditis.¹⁹⁴ In comparison with acute thyroiditis, subacute thyroiditis may be less severe.^{194,195} Fever, thyroid tenderness, and hyperthyroidism may be present and may last for several weeks.¹⁹⁶ Because clinically distinguishing between bacterial

and viral thyroid infections is difficult, antibiotic treatment is indicated when infectious thyroiditis is suspected.

Hyperfunctioning Nodules

Warm or hot nodules lead to excessive production of thyroid hormone and can be associated with clinical and biochemical hyperthyroidism.¹⁹⁷ Interestingly, activating mutations of the TSH receptor and G_s have been discovered in hyperfunctioning nodules.^{198,199} Although hyperfunctioning nodules may be ablated with radioiodine, surgical excision of hyperfunctioning nodules is recommended in children and adolescents, because radiation-exposed normal thyroid tissue will remain after the hyperfunctioning nodule is ablated. Although the risk of malignancy in hyperfunctioning nodules is low, thyroid cancers have been described in warm nodules.^{200,201}

Toxic Multinodular Goiters

Multinodular goiters are uncommon in children, but patients with this condition can develop thyrotoxicosis, which is usually related to the time the goiter has been present and goiter size. In this setting, hyperthyroidism develops when a single nodule in the thyroid becomes overly active, and functions autonomously.^{202,203} Some 46% of patients may have T3 thyrotoxicosis, and nodules are 3 cm or more in diameter.^{202,203}

In adults, ^{131}I is routinely used in the treatment of isolated toxic adenomas and toxic multinodular goiters.^{202,203} The use of radioiodine to treat these conditions in children is uncommon, and few follow-up data are available. Although strong justification exists for the use of radioiodine in the treatment of childhood GD, especially when appropriate doses are used, we recommend that radioiodine be avoided in children with toxic adenomas or multinodular goiters. When a toxic nodule is present, either as an isolated nodule or in the setting of a multinodular goiter, thyroid function is suppressed in the nontoxic regions. When radioiodine is given, uptake will be limited to the autonomously functioning tissue, and if large doses are administered, the remaining thyroid tissue will receive external irradiation. Because the risk of thyroid cancer following external radiation is very low after 20 years of age,^{254,255} the use of radioiodine for toxic nodule ablation in adults is not associated with increased thyroid cancer risks. In the child or adolescent treated with ^{131}I for toxic nodules, however, low-level irradiation to the remaining thyroid tissue will occur with an increased thyroid cancer risk.

Amiodarone

Amiodarone is a class III antiarrhythmic medication that is associated with both hyper- and hypothyroidism caused by amiodarone's high iodine content and direct toxic effect on the thyroid.^{95,96,256,257} Two types of amiodarone-induced thyrotoxicosis (AIT) have been described. In type I AIT, excess iodine results in enhanced thyroid hormone production.^{95,96,256,257} Type II AIT, is more common than type I and is a destructive thyroiditis that results in direct toxic effect of amiodarone on thyroid follicular epithelial cells in excess release of T4 and T3, without increased hormone synthesis. The treatment of this condition involves glucocorticoids.^{95,96,256,257}

Excessive Iodine Intake

Iodine is a micronutrient and typical intake is 150 mg/day for adults.⁸⁷ In general, excessive iodine intake does not result in thyroid disease. However, in some individuals with iodine deficiency who are predisposed to getting GD, excess iodine can trigger hyperthyroidism, a condition referred to as the *Jod-Basedow effect*.⁸⁷

THYROID NODULES AND THYROID CANCER

Background

Thyroid nodules in children and adolescents are less common when compared with adults; however, the risk of malignancy for a nodule diagnosed in a pediatric patient is approximately twofold higher.^{258,259} Over the past 2 decades, there has been a 2% to 4% per year increase in the number of patients diagnosed with thyroid nodules and differentiated thyroid cancer (DTC), with the largest increase noted in females between 15 and 19 years of age, where thyroid cancer accounts for 8% of all cancers.^{260,261} Similar to adults, over 85% of pediatric thyroid cancers are papillary thyroid cancer (PTC), with the remainder divided between follicular thyroid carcinoma (FTC) and medullary thyroid cancer (MTC), the latter most commonly associated with multiple endocrine neoplasia type 2 (MEN2).²⁶⁰

Thyroid nodules may be detected by physical examination or by incidental discovery during unrelated head and neck radiologic imaging. When a thyroid nodule is detected, serum TSH and a thyroid and neck ultrasound (US) should be obtained to determine the best plan for management.²⁶² If the TSH is suppressed, a radionuclide scan may be performed to identify a hyperfunctioning nodule, a lesion associated with a lower risk for malignancy.²⁶³ Serum calcitonin levels are not routinely assessed; however, calcitonin immunohistochemistry or a calcitonin washout from the fine needle aspiration (FNA) needle should be considered based on a family history of MEN2, physical examination concerns for MEN2B, or cytologic findings consistent with MTC.^{264,265}

Evaluation of a Thyroid Nodule

US is the most efficient and accurate method for determining if a thyroid nodule may be surveilled or should undergo further evaluation with FNA. US characteristics of nodules that should be assessed, included in the radiology report, and used to stratify nodules for FNA per the Thyroid Imaging and Reporting Data System (TI-RADS) include composition (cystic, spongiform, mixed or solid), echogenicity, (anechoic, hyperechoic, isoechoic, or hypoechoic), shape on transverse imaging (taller than wide or wider than tall), margin (smooth, ill-defined, lobulated, or with extrathyroidal extension), and echogenic foci (none, comet-tail, macrocalcifications, rim calcifications, or punctate calcifications) (Fig. 13.5).²⁶⁶ Cystic or mixed composition, with a greater than 75% cystic component, is the single, most reliable feature associated with a lower risk of malignancy.^{258,267} Features associated with a higher risk of malignancy include solid composition, hypoechogenicity, taller than wide shape on transverse imaging, lobulated or irregular margin, and punctate echogenic foci.²⁶⁸ US assessment of the lateral neck must be performed on all patients found to have an indeterminate or suspicious thyroid nodule to determine if there is lymphadenopathy concerning thyroid cancer metastasis. US features that are most predictive of an abnormal lymph node include rounded shape, punctate echogenic foci, and the presence of peripheral vascularity.²⁶⁹ Size is generally not a predictive feature for malignancy, and lymph nodes in the upper neck (level II) are typically larger than lymph nodes in the lower (levels III and IV) or posterior (level V) regions of the neck (see Fig. 13.6).

For many nodules, the US appearance alone cannot reliably distinguish between a benign and a malignant lesion.²⁷⁰ Thus FNA is indicated for children with indeterminate or suspicious thyroid nodules in an effort to determine if surgery is warranted, as well as to plan for the extent of surgery.²⁶² FNA is the most accurate means to evaluate if a thyroid nodule is malignant. Reports of FNAs performed in children describe

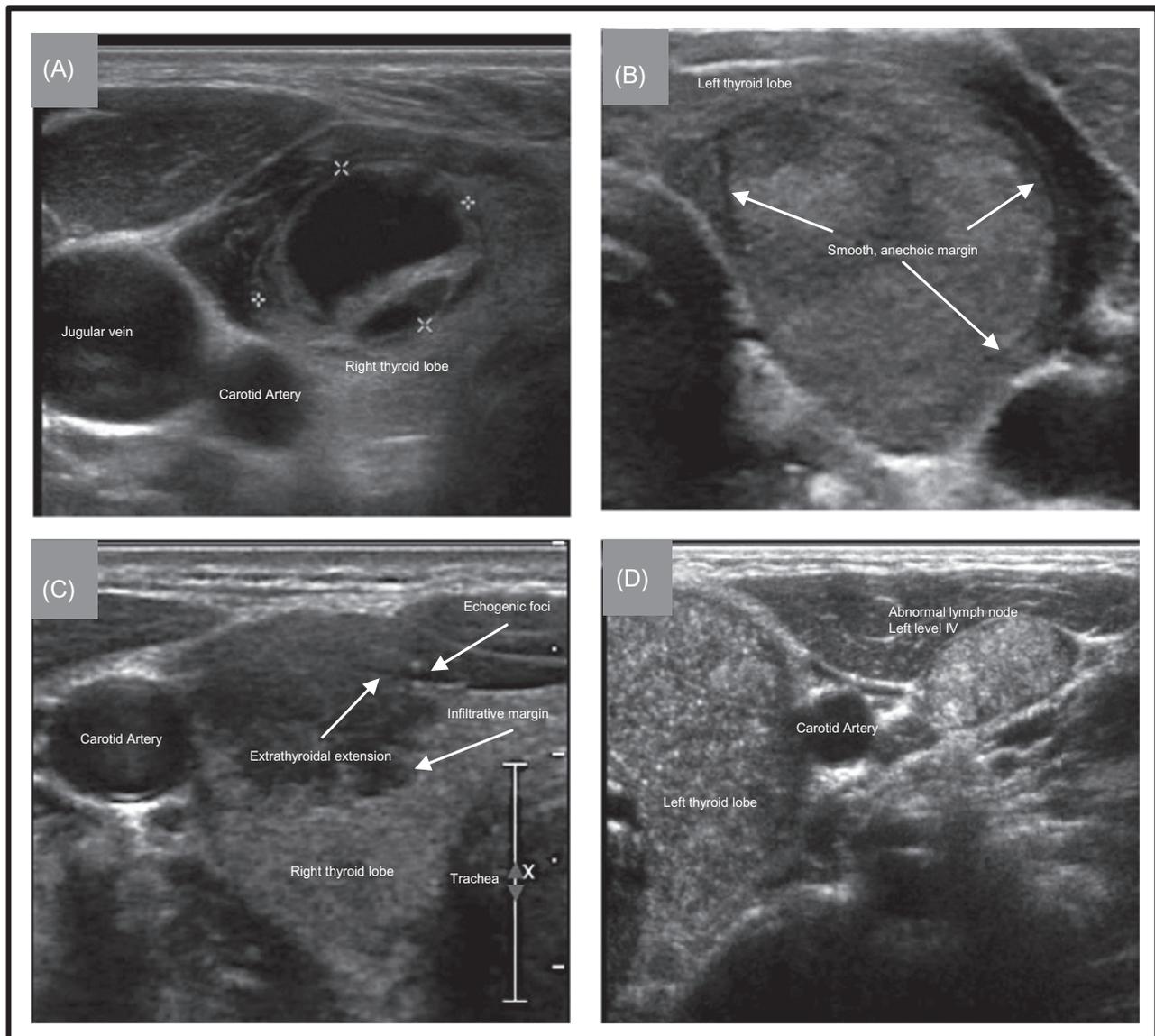


Fig. 13.5 Representative images of thyroid nodules depicting different features used in the Thyroid Imaging Reporting and Data System (TI-RADS). (A) Complex, mixed solid-cystic nodule (>80% cystic), solid portion is isoechoic with smooth margins, no echogenic foci, with small solid not taller than wide, smooth margins, and no echogenic foci (TI-RADS 2). (B) Solid, iso- (larger, posterior portion) and slightly hypoechoic (smaller, anterior area) nodule, not taller than wide, with smooth margins, and no echogenic foci (TI-RADS 3). (C) Solid, very hypoechoic, not taller than wide, lobulated, invasive margins with evidence of extrathyroidal extension (arrows), and several echogenic foci (TI-RADS 5). (D) Solid, hypoechoic, diffusely invasive lesion with too numerous to count echogenic foci (TI-RADS 5). An abnormal lymph node is noted in lateral neck with similar ultrasound features to the primary tumor.

similar specificity and sensitivity as adults, with a metaanalysis reporting sensitivity and specificity of 94% and 81%, respectively, and accuracy, positive, and negative predictive values of 84%, 55%, and 98%, respectively, assuming a 20% risk of malignancy.²⁷¹ Difficulty arises when the FNA cytology is “indeterminate,” as malignancy can be present up to 40% of nodules characterized by The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).^{272,273} The addition of oncogene testing may be of value with the presence of a known thyroid oncogene driver mutation associated with an increased risk of malignancy (see “Molecular Landscape” later).²⁷⁴ Alternative options for nodules with an indeterminate category III TBSRTC classification (“follicular lesion of undetermined significance” or “atypia of undetermined significance”) are to perform a diagnostic lobectomy or to consider repeat FNA within 3 to 6 months. For patients with a nodule having

indeterminate TBSRTC category IV classification (“follicular neoplasm” or “suspicious of a follicular neoplasm”), a diagnostic lobectomy should be performed (see Fig. 13.6). In adults, recent recommendations suggest that FNA be performed only when nodule diameter is or exceeds 1.5 cm.^{275,276} There are no data to stratify FNA based on size in pediatric patients. Clinical judgment based on predisposing risks of malignancy and US appearance of the nodule, and cervical lymph nodes should be considered before performing FNA on a nodule smaller than 1 cm in size.

Differentiated Thyroid Cancer

Over the last 2 decades, there has been an increasing number of children and adults diagnosed with thyroid cancer.²⁶¹ Within pediatrics, the greatest increase in the incidence of diagnosis

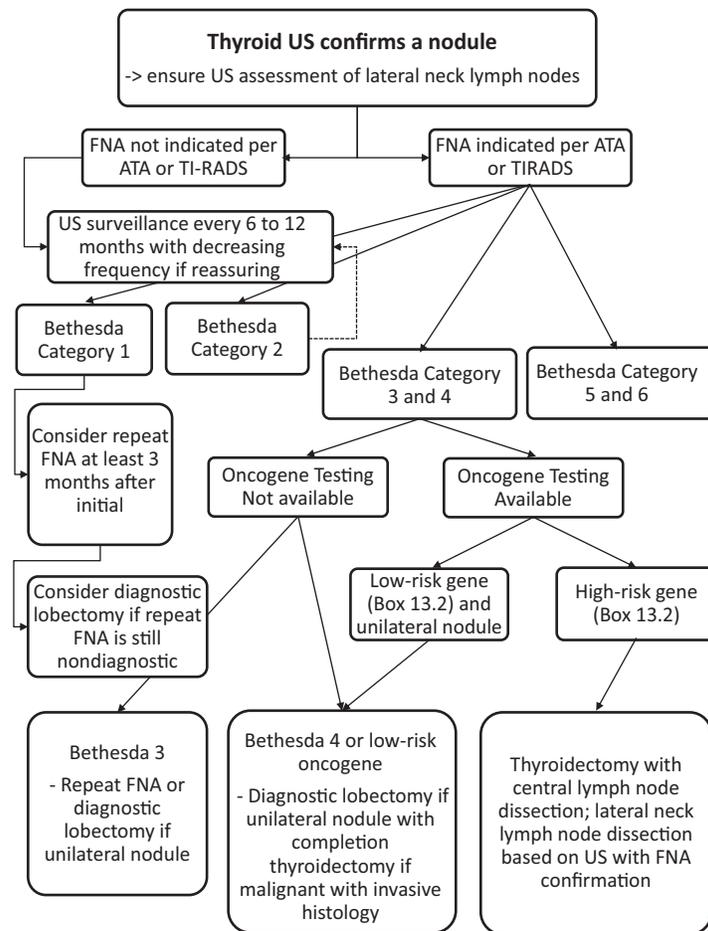


Fig. 13.6 Algorithm for the initial evaluation and management of sporadic thyroid nodules in children and adolescents. For patients with a thyroid nodule associated with autoimmune thyroid disease, postradiation exposure, or with a thyroid cancer predisposition syndrome, thyroidectomy may be preferable over lobectomy. ATA, American Thyroid Association; FNA, fine-needle aspiration; TI-RADS, Thyroid Imaging, Reporting and Data System; US, ultrasound.

is in adolescent girls and, similar to adults, the majority of tumors are PTC.^{260,261} In the most recent update from the National Cancer Institute Surveillance Epidemiology and End Results (SEER) database, PTC accounted for 58.8%, follicular-variant of PTC 23%, FTC 10.1%, and MTC 8.1%, with the predominance of MTC diagnosed in patients younger than 10 years of age associated with MEN2.²⁶⁰

Compared with adults, children with DTC present with more extensive disease at the time of diagnosis. Cervical neck lymph node involvement at diagnosis is seen in 50% to 90% of children,²⁷⁷ compared with 20% to 50% of adults.²⁷⁸ The prevalence of distant metastases, most commonly lung, is 6% to 8% in children versus 2% to 3% in adults, with an increased risk of 10% to 20% for pediatric patients with lateral neck (levels II, III, and IV) lymph node metastasis.^{262,279–281} Multifocal disease is more common in children than adults and is seen in about 40% of childhood PTC cases.²⁶² There are several reports that PTC in children less than 10 years of age at the time of diagnosis have an increased rate of regional and distant metastasis, a higher risk for recurrence, and increased disease-specific mortality when compared with adolescents and adults.^{282–284} However, other investigators report that DTC has similar clinical behavior when comparing younger children to adolescents.²⁸⁵

Fortunately, even in the presence of metastatic disease, long-term follow-up data show 30-year survival rates of 90% to 99% for children with DTC (see Fig. 13.2).^{261,286–290} Even with distant metastases, mortality rates are more favorable in children than adults,²⁹¹ and pulmonary metastases can remain stable over years to decades of time.^{292,293} The favorable prognosis reflects the fact that most young patients have well-differentiated tumors that maintain expression of the sodium-iodine symporter and an indolent pattern of growth.

Etiology of Differentiated Thyroid Cancer

For the majority of children, a specific risk factor for the development of DTC cannot be identified. However, several factors increase the risk of developing DTC. Exposure to low-level head and neck irradiation has been recognized for more than 6 decades as predisposing to thyroid nodules and DTC.^{294,295} Low-level radiation doses to the thyroid of less than 30 Gy (3000 cGy or rad) increase the risk for developing cancer, with the risk being higher at progressively younger ages.^{169,173,174} Above 20 years of age, the risk of thyroid cancer following low-level irradiation is either very low or undetectable.^{169,174} The latency period between the time of radiation

exposure and DTC onset in children is typically 10 to 20 years; however, thyroid cancer may develop within 5 to 10 years in patients exposed to radiation at a young age (<5–10 years of age).^{169,174,295,296} Ingestion of ionizing radiation also increases the risk for developing thyroid disease, including DTC. This may occur during medical treatment of radiosensitive tumors, such as neuroblastoma where ¹³¹I-labeled metaiodobenzylguanidine (MIBG) is commonly one of several agents used for therapy,²⁹⁷ or via accidental ingestion, as occurred after the 1981 Chernobyl nuclear accident.^{297–299} Recent guidelines suggest that either physical examination or US may be used to surveil patients at risk for developing radiation-induced thyroid cancer, with US having greater sensitivity for detection of earlier disease.^{300,301}

Thyroid cancer in children can also be observed in families, associated with an increased risk for developing DTC as a single tumor type or as one of several tumors associated with a tumor-predisposition syndrome. Familial nonmedullary differentiated thyroid cancer (FNMTc) is an inherited predisposition to developing DTC without an increased risk for developing other tumor types. Under the most strict criteria, FNMTc is diagnosed when three or more individuals in the family have DTC³⁰²; however, even in families with two or more first-degree relatives with DTC, there is a 8- to 10-fold increased risk of developing either PTC or FTC.³⁰³ Similar to sporadic DTC, 85% of FNMTc is comprised of patients with PTC. The transmission pattern is most consistent with an autosomal dominant mode of inheritance; however, to date, a single germline locus has not been identified.^{303,304} When FNMTc is present, it is recommended that thyroid ultrasonography be performed every year or 2, beginning at 8 years as part of tumor surveillance. DTC in children with FNMTc has been identified as early as 8 years of age and FNMTc displays “genetic anticipation,” with the age of cancer occurrence generally younger in the second and subsequent generations.^{304–307}

There are several familial tumor predisposition syndromes that have DTC as one of several different tumors that may develop in an affected person over their lifetime. These syndromes mostly follow an autosomal dominant pattern of inheritance, where there is a 50% risk of transmission of the germline mutation to offspring; however, de novo mutations may also occur in a child and be the index case for the family. Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome, previously known as Cowden syndrome, is caused by mutation in the *PTEN* gene and is associated with macrocephaly, hamartomas of mucosal surfaces (oral), gastrointestinal polyps, breast cancer (women only), endometrial cancer, colorectal cancer, renal cell carcinoma, and DTC.³⁰⁸ There is enrichment in the incidence of FTC in patients with PTEN hamartoma tumor syndrome (PHTS); however, patients may have nodules with mixed histological diagnosis and PTC is still more common than FTC.³⁰⁸ Thyroid nodules are ultimately diagnosed in more than 75%, with approximately 35% developing DTC as early as 8 years of age.^{308,309} Familial adenomatous polyposis, or Gardner syndrome, is caused by mutations of the adenomatous polyposis coli (*APC*) gene and is associated with hypertrophy of the retinal pigment epithelium (CHRPE), a propensity to develop hundreds to thousands of intestinal polyps with malignant transformation to small bowel and colorectal carcinoma, hepatoblastoma, dental and cutaneous abnormalities, and a 10% to 15% risk of developing DTC.³¹⁰ DICER1 syndrome is caused by a mutation in the *DICER1* gene and is associated with an increased risk of developing pleuropulmonary blastoma (birth to 5-years of age), Sertoli-Leydig cell tumors, cystic nephroma, botryoid embryonal rhabdomyosarcoma, several additional rare-tumors of the eye and nose, as well as multinodular goiter, and DTC.³¹¹ Carney complex is

one of the lentiginosis syndromes caused by mutations in the *PRKAR1A* gene.³¹² Carney complex is associated with an increased risk of developing nerve sheath tumors (schwannomas), myxomas, primary pigmented nodular adrenocortical disease (hypercortisolism), pituitary tumors (growth hormone secreting), and large-cell calcifying Sertoli-cell tumors of the testes, with about 50% developing thyroid nodules and less than 5% developing DTC.^{313,314} Updated reviews for familial tumor predisposition syndromes can be found at the National Institute of Health GeneReviews® website (<https://www.ncbi.nlm.nih.gov/books/NBK1116/>).

Molecular Landscape

Alterations in the oncogenes (mutations and fusions) that drive tumorigenesis in DTC and MTC are similar between pediatric and adult patients.²⁷⁴ However, because of the observed differences in clinical behavior of these tumors, most importantly, maintenance of cellular differentiation, there are likely differences in gene expression, as well as downstream signaling pathways between pediatric and adult DTC. The consequences of these differences are reflected by lower disease specific mortality for pediatric patients with regional and distant metastasis when compared with adults with similar disease burden.^{262,275}

In DTC, thyroid tumorigenesis and progression are associated with somatic point mutations in the *BRAF* and *RAS* genes, as well as fusions involving the rearranged during transfection (*RET*), *TRK*, and *ALK* tyrosine kinases with resultant constitutive activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signaling pathways.²⁷⁴ These oncogene mutations are most commonly mutually exclusive events and there is a predictable relationship between oncogenic genotype and histopathologic phenotype, with the fusion oncogenes (*RET*, *TRK*, and *ALK*) and *BRAF* mutations almost exclusively found in PTC. *RAS* is commonly associated with FTC; however, it may be found across the spectrum of thyroid tumors, from benign follicular adenomas to follicular variant PTC, FTC, and poorly differentiated thyroid carcinoma.²⁷⁴ In addition to the potential utility of oncogene testing in the diagnosis of thyroid nodules with indeterminate cytology (TBSRTC category III and IV), the presence of *RET/PTC* and *TRK*-fusion genes may be helpful in predicting PTC with increased metastatic potential, although there are no data to suggest that these alterations are associated with increased disease-specific mortality.²⁷⁴

Surgical Management

Confirmation of an accurate and complete preoperative evaluation of pediatric patients with thyroid nodules and thyroid cancer is the first step in deciding on surgical options and optimizing the surgical plan.³¹⁵ Cervical US using a high-frequency linear transducer (12.0 MHz) should be performed to examine the thyroid, as well as the central (levels VI and VII) and lateral (levels II, III, IV and V) levels of the neck. More than 70% of children with PTC will have metastatic cervical lymph node disease that follows a consistent pattern of metastasis based on the lymphatic drainage of the thyroid.^{262,316} Lowering the transducer frequency to 8 to 10 MHz allows for improved imaging of deeper fields of view, including the paratracheal region and tracheoesophageal groove. In addition, lower frequencies may also help improve imaging for patients with excess soft tissue.³¹⁷ FNA of at least one suspicious lymph node per cervical level before surgery should be performed to determine the extent of lymph node dissection. For patients with extensive, lymph node involvement or signs/symptoms of vocal cord

paresis/paralysis (hoarseness, cough), dysphagia, or respiratory symptoms (stridor, dyspnea, hemoptysis), cross-sectional imaging of the neck and upper mediastinum with computed tomography (CT) or magnetic resonance imaging (MRI) may provide supplemental information, including the location of suspicious lymph nodes in areas where US has a lower sensitivity for detection (parapharyngeal, retropharyngeal, subclavicular, or upper mediastinum regions), as well as assessment of the aerodigestive tract.³¹⁷ There is no contraindication for using iodinated contrast for CT imaging of the neck or mediastinum before surgery as the majority of patients will achieve an adequate, low-urine iodine level (defined as a spot urine iodine/creatinine <66.2 µg/gCr) 1 month after exposure to iodinated contrast on a 1- to 2-week low-iodine diet.^{317,318}

The goal of surgery is to maintain the low disease-specific mortality in pediatric patients with DTC, while reducing potential complications from therapy.^{228,319} Access to a high-volume thyroid surgeon, defined as a surgeon completing >25 total thyroidectomies annually, is associated with a lower rate of complications.^{228,262,319–322} Although the exact number of surgeries performed annually may not reflect the quality of the surgeon, it increases the likelihood that the surgeon understands the disease process in children and adolescents in an effort to balance complete resection, while minimizing the risk of incomplete surgical resection, permanent hypoparathyroidism, and recurrent laryngeal nerve damage.

For the majority of patients with PTC, a total thyroidectomy, with prophylactic central compartment lymph node dissection, is recommended secondary to the increased incidence of bilateral/multifocal disease in pediatric PTC,³²³ the high incidence of regional lymph node metastasis, and the increased risk for recurrence and subsequent second surgery when less than a near-total thyroidectomy or total thyroidectomy is performed.^{285,324–326} Routine prophylactic lateral neck dissection is not recommended. Lateral neck dissection should be based on preoperative imaging with confirmation of metastasis by preoperative FNA.²⁶² Any patient who is undergoing a lateral neck dissection should also undergo a therapeutic, ipsilateral central neck lymph node dissection based on lymphatic drainage anatomy. Lymph node dissections should be compartment-based lymph node dissection rather than individual lymph node dissection ('berry picking'), in an effort to achieve surgical remission.²⁶²

Similar to adults, a portion of pediatric patients with DTC will have noninvasive disease that is limited to the thyroid where lobectomy may be adequate to achieve surgical remission. On preoperative USS, nodules that are isoechoic to hyperechoic with round to oval shape, smooth margins, and no echogenic foci, are more likely to be associated with noninvasive behavior.³²⁷ Thus even in pediatrics, lobectomy may be considered for patients with an isolated, unilateral thyroid nodule, with noninvasive features on US to avoid lifelong thyroid hormone replacement therapy. On review of the anatomic pathology, lobectomy with surveillance may be considered adequate, even for a select group of patients with follicular variant PTC and FTC, based on the absence of histological evidence of multifocality, lymphovascular invasion, and extrathyroidal extension, features that are associated with an increased risk of regional metastasis.^{328–332} Risk factors where lobectomy would be contraindicated include a history of exposure to ionizing radiation, a personal history of a thyroid tumor predisposition syndrome, or a history of autoimmune thyroiditis where there is an increased risk of developing additional nodules and DTC in the remaining lobe if lobectomy were performed. For patients with unifocal papillary microcarcinoma (<1 cm), and no evidence of lymph node metastasis on preoperative imaging, lobectomy with surveillance may also be adequate; however, there are no data to predict the risk of persistent or recurrent disease.

BOX 13.2 Somatic Driver Mutations in Pediatric Thyroid Nodules and Differentiated Thyroid Cancer With Risk of Invasive Behavior and Potential Application in Stratifying Surgical Management

Somatic Driver Mutation	Increased Risk of Invasive Behavior	Considerations for Surgical Care
BRAF V600E	Yes – N1a common	Total thyroidectomy with strong consideration for prophylactic central neck lymph node dissection; lateral neck dissection based on US and FNA
RET-PTC fusion	Yes – N1b common	
NTRK-fusion	Yes – limited data	
ALK-fusion	Yes – limited data	
BRAF-fusion		
RAS	Lower risk for invasive disease	Consider lobectomy if unilateral disease in a patient without thyroid cancer predisposition syndrome and reassuring US features (smooth margins/encapsulated and no abnormal lymph nodes)
PTEN	Rare cases of	
DICER1	DICER1 associated	
APC	with poorly differentiated thyroid cancer	
PAX8-PPARgamma		
TSHR	Commonly associated with benign lesions	Surveillance or definitive treatment if associated with suppressed TSH
THADA		
GNAS		
AKT1	Unknown	No specific recommendation
CTNNB1		
EIF1AX		
TERT		
Others		

FNA, Fine needle aspiration; TSH, thyroid-stimulating hormone; US, ultrasound.

All patients undergoing lobectomy for DTC need to be compliant with surveillance and be counseled for the potential need for additional surgery if persistent or recurrent disease is found.^{333,334} Knowledge of the somatic driver mutation driver may be helpful in guiding the surgical approach; however, additional studies are needed to confirm the utility to this approach to stratification of care (Box 13.2).²⁷⁴

Unlike PTC, FTC is often unifocal and does not have a propensity toward lymph node invasion, but may exhibit early hematogenous metastasis. As the diagnosis of FTC is based on evidence of anatomic invasion, not on nuclear or cytoplasmic alterations, the majority of patients ultimately diagnosed with FTC will have indeterminate cytology on FNA and undergo a lobectomy for their initial surgery. Similar to PTC, completion thyroidectomy should be considered for patients with underlying thyroid disease, bilateral nodules, or a known diagnosis of a thyroid tumor predisposition syndrome. In contrast to PTC, there is no advantage in performing a prophylactic central neck lymph node dissection for patients with FTC, as regional lymph node metastasis is uncommon. Minimally invasive FTC is defined as FTC with microscopic or no capsular invasion and/or limited vascular invasion, defined as less than 4 vessels

in or adjacent to the tumor capsule. Widely invasive FTC is defined as FTC with widespread capsular invasion, widespread vascular invasion, or extension into surrounding thyroid tissue. Invasion of 4 or more vessels is associated with more aggressive disease, an increased risk of distant metastases, and a poorer prognosis. A completion thyroidectomy should be performed for patients diagnosed with widely invasive FTC so that patients can be assessed for evidence of distant metastasis on postoperative RAI whole-body scan.²⁶²

Surgical complication rates from pediatric thyroidectomy range from 0% to 32% for permanent hypoparathyroidism and 0% to 40% for recurrent laryngeal nerve injury,^{277,333} with higher complication rates in children aged 0 to 6 years (22%) when compared with older children (15% for 7–12 years and 11% for 13–17 years).²²⁸ Patients treated in low-volume hospitals and by low-volume surgeons (≤ 30 thyroidectomies/year) are more likely to undergo care that is discordant to national practice guidelines, have an increased rate of complications (14.3% vs. 35.9%, odds ratio [OR], 0.16; 95% confidence interval [CI] 0.05–0.51; $P = .02$) and an increased rate of readmission within 30 days of surgery compared with high-volume centers.^{279,335} The use of intraoperative parathyroid hormone levels helps identify patients at risk of postoperative hypoparathyroidism affording an opportunity for early administration of calcium and calcitriol.³³⁶ Early identification of hypoparathyroidism, with subsequent initiation of calcitriol and calcium decreases the risk of symptomatic hypocalcemia, as well as shortens the duration of postoperative hospitalization.³³⁷ The perioperative calcium and phosphorus must be monitored to ensure stable values before discharge from the inpatient setting.^{338,339}

The risk of vocal fold paralysis from recurrent laryngeal nerve (RLN) injury ranges from 1% to 8.6%, with rates of bilateral nerve injury at the lower end of the range, a particular challenge, because of respiratory distress and potential need for tracheotomy.^{279,340} Flexible fiberoptic laryngoscopy³⁴¹ or transcutaneous laryngeal US^{342,343} should be performed before surgery for patients with a history of preoperative voice abnormalities, extensive cervical and/or upper mediastinal lymph node metastasis. Because of the significant morbidity associated with bilateral RLN paresis, ipsilateral RLN function should be assessed midway during total thyroidectomy, after completing the dissection of the first thyroid lobe. RLN function can be monitored either actively with neuromonitoring, or manually. If the RLN is intact but not responsive to stimulation, the surgeon should consider altering the surgical plan to avoid the risk of bilateral RLN injury.³⁴⁴ Thus although the use of active RLN monitoring may not decrease the rate of unilateral RLN injury, it is very effective at reducing the potential need for a surgical airway secondary to bilateral RLN injury.

Radioactive Iodine Therapy

RAI, ¹³¹I, also referred to as *radioiodine*, was observed to kill thyroid tumor cells more than 70 years ago.^{345,346} To this date, RAI remains the most effective and safe targeted therapeutic agent to destroy persistent DTC that is not amenable to surgical resection. There are several considerations that should be kept in mind when considering using RAI in pediatrics: (1) RAI benefit is clearly demonstrated in patients with metastasis, but not with disease limited to the thyroid, and remnant ablation is not associated with improved outcome; (2) different approaches have been used to select delivered therapeutic activity including empiric dosing, blood-based upper-limit dosimetry, and tumor dosimetry, without one approach showing clear efficacy over the others; (3) RAI efficacy is dependent on factors that include tumor biology and the radiation dose to the tumor; (4) relatively high cumulative activities of RAI

(>300 mCi; 11 GBq) may be associated with an increased risk of second primary malignancies (SPMs); and (5) patients may be considered RAI-refractory in the following situations: the foci never concentrate RAI, foci that previously concentrated RAI lose avidity, there is anatomic progression within 1-year of RAI therapy, or the cumulative RAI dose exceeds 600 mCi.²⁷⁵

There are similar considerations and concerns with using RAI in children and adolescents amplified by 3-fold increased use of RAI treatment in pediatric patients with DTC over the last four decades, the length of time that pediatric patients bear the burden of short-term RAI-induced complications, and the length of time over which long-term RAI-induced complications have time to develop. Short-term complications of RAI therapy in treating DTC include acute sialoadenitis, xerostomia, bone-marrow suppression, and pneumonitis. Long-term complications include xerostomia, dental caries, pulmonary fibrosis, gonadal toxicity, and development of SPM.²⁶² Initiation of sialogogues, 2 hours after administration of a therapeutic dose of RAI, with continuous use over the next 1 to 2 days (every 15–30 minutes, even overnight) can help reduce salivary gland exposure to RAI.^{347,348} In regard to the risk of RAI-induced SPM, the most recent retrospective review of the National Cancer Institute SEER cancer registry reported that approximately 1 in 227 RAI-treated patients will develop an SPM (a 0.125% increased risk over patients that did not receive RAI) with 1 in 588 developing an RAI-induced salivary gland cancer.³⁴⁹ Thus the overall risk of RAI-associated complications is very low and must be taken within the context of the benefit of RAI, where multiple studies have shown a reduced risk of recurrence associated with the administration of RAI to patients found to have regional and distant metastasis at the time of diagnosis.^{350–353}

In contrast with adults with thyroid cancer, there is no staging system for children and adolescents with PTC, secondary to the extremely low disease-specific mortality, even for patients with pulmonary metastasis.²⁶¹ However, the American Joint Committee on Cancer Tumor, Nodes, Metastases (TNM) classification system³⁵⁴ is used to describe the extent of disease and stratify an approach to further evaluation and management, based on the risk of persistent postsurgical disease.²⁶² The three groups from 2015 American Thyroid Association (ATA) Pediatric guidelines²⁶² are as follows, adjusted based on more recent data^{330–332}:

1. **ATA Pediatric Low-Risk:** Disease grossly confined to the thyroid with N0 (no lymph node metastasis) or NX (no lymph nodes assessed) disease or patients with incidental ≤ 5 metastatic lymph nodes in the central neck (N1a).
2. **ATA Pediatric Intermediate-Risk:** Presence of extrathyroidal extension or >6 metastatic lymph nodes (N1a) or lateral neck lymph node metastasis (N1b).
3. **ATA Pediatric High-Risk:** Presence of more than 10 metastatic lymph nodes or distant metastasis.

Irrespective of risk level, all patients undergo surveillance. For ATA Low-Risk patients, patients may be placed on TSH-suppressive (TSH <0.5 $\mu\text{U/L}$) levothyroxine therapy and initiate laboratory surveillance with 3- to 6-month interval TG and anti-TG levels and neck US on a 6- to 12-month interval rather than pursuing a stimulated TG level, with diagnostic whole-body scan (DxWBS) in the immediate postoperative time frame. The TSH-stimulated TG and DxWBS can be performed at a later time, if there is any concern over persistent or recurrent disease that cannot be located on nonradioisotope imaging.^{262,321,322} For intermediate- and high-risk patients, a TSH-stimulated TG and a ¹²³I DxWBS are recommended to assess for evidence of residual disease. Some 2 to 3 weeks before the DxWBS, liothyronine or levothyroxine is withheld with a goal of achieving a TSH above 30 $\mu\text{U/L}$. The ability to

achieve adequate TSH elevation using recombinant-human TSH (rhTSH) has been studied in children using the typical adult dose (0.9 mg given 24 hours apart); however, pediatric data on the efficacy of rhTSH on treatment outcomes are limited.^{355,356} During this time frame, patients are also placed on a low-iodine diet to optimize absorption of radioiodine. For children who received iodinated contrast agents, it is recommended to wait 1 to 2 months and to confirm iodine deficiency with a spot or 24-hour urine iodine level before administration of RAI.³¹⁸ For the DxWBS, either ¹³¹I or ¹²³I may be used, however, ¹²³I is favored secondary to superior imaging quality, decreased radiation exposure, and prevention of stunning (reduced ¹³¹I absorption of the therapeutic dose).³⁵⁷ The addition of single-photon emission computed tomography (SPECT) should be considered to confirm disease that is discovered in unexpected or unusual anatomic locations.³⁵⁸

For patients with minimal thyroid bed uptake on DxWBS after initial surgery, ¹³¹I therapy can be considered based on the TSH-stimulated TG level, on a case-by-case basis. A TSH-stimulated TG lower than 2 ng/mL has a 94.9% predictive value for being disease-free.³⁵⁹ If TSH-stimulated TG is 2 to 10 ng/mL, ¹³¹I therapy can be considered for patients with thyroid bed uptake and should be considered for DTC with invasive histology (diffuse sclerosing variant PTC and widely invasive follicular variant PTC), and for tumors found to have invasive features on anatomic pathology, including extra-thyroidal extension, extensive regional metastasis (>6 lymph node), or evidence of extranodal extension.^{262,330} If the TSH-stimulated TG is greater than 10 ng/mL, ¹³¹I therapy is indicated. Repeat surgery before administration of ¹³¹I should be pursued if there is evidence of “bulky,” macroscopic, persistent disease noted during the initial postoperative evaluation.²⁶²

Options for selection of the therapeutic ¹³¹I dose is either empiric or determined based on bone marrow dose limited dosimetry. Empiric dosing is given as a fraction (child’s weight in kilograms divided by 70 kg) of a typical adult dose used to treat similar disease extent or based on weight (1.0–1.5 mCi/kg).²⁶² Bone marrow dose-limited dosimetry is targeted to limit lung retention to less than 80 mCi at 48 hours and blood/bone marrow exposure to less than 200 cGy. Dosimetry should be considered in younger children (<10 years), those with diffuse pulmonary metastases, and those who may have reduced bone marrow reserve, including a history of radiation therapy for other malignancies.^{360,361} A posttreatment WBS (RxWBS) should be obtained 5 to 7 days after all ¹³¹I therapy as up to 15% of patients may have newly identified foci not seen on the DxWBS that may impact the subsequent surveillance and treatment plan.³⁶²

Levothyroxine Therapy

After completion of initial therapy for DTC, patients are placed on supraphysiologic doses of levothyroxine to suppress the TSH below the lower limit of detection, as it is well recognized that TSH suppression can reduce rates of recurrence.^{363,364} The optimal degree of TSH suppression is debated in low-risk patients, as it is not clear if complete suppression of TSH secretion confers benefit. In adults, the long-term impact of supraphysiologic doses of thyroid hormone on bone mineral density and cardiovascular risks is well recognized.^{365,366} In children, high levels of thyroid hormones can have effects on growth and profoundly impact on behavior and learning ability.³⁶⁷

The TSH goals proposed in the ATA pediatric guidelines recommend maintaining TSH levels in the low normal range

(0.5–1.0 µU/mL) for low-risk patients, between 0.1 to 0.5 µU/L for intermediate-risk patients, and less than 0.1 µU/L for high-risk patients.²⁶² Once a patient enters remission TSH, suppression may be relaxed into the low-risk range, between 0.5 to 1.0 µU/L.

Follow-Up

Follow-up care of the child with DTC involves the regular assessment of circulating thyroid hormone levels, measurement of TG and anti-TG, and radiological imaging. TG levels must be interpreted in relation to simultaneous TSH and every effort must be made to use the same laboratory and assay method to accurately determine the trend in the TG level.³⁶⁸ Laboratory assessment is typically obtained every 3 to 6 months, decreasing to 6 to 12 months for patients in remission, and then to once annually once linear growth has been completed.

A very pertinent issue is the criteria used to assess if a patient is disease free. With highly sensitive TG assays, one can aim for an undetectable TG level (<0.2 mcg/L) as indicative of a disease-free state. However, with these highly sensitive TG assays, there will be patients that have a minimally detectable TG or anti-TG that may not have any evidence of anatomic disease. For many of these patients, continued surveillance may be the best plan in an attempt to balance the risks and benefits of additional therapy.

Up to 25% of patients with DTC have detectable anti-TG levels that can interfere with TG assays. For these patients, the trend in TGAb should be followed as a marker of disease status.³⁶⁹ Many laboratories will reflexively run the TG by radioimmunoassay or liquid chromatography/tandem mass spectrometry, if TGAb is detected. For patients that did not receive postsurgical RAI therapy, the TSH-suppressed TG should decrease to less than 0.5 mcg/L 6 to 12 months after total thyroidectomy.³⁷⁰ If the TG remains mildly elevated, between 2 to 10 mcg/L, continued monitoring may be pursued depending on the trend in TG over time, as well as evidence of persistent or recurrent disease based on radiological imaging. Increasing or frankly elevated levels of TSH-suppressed TG (>10 mcg/L) warrant further evaluation to localize disease and to decide whether additional surgery and/or ¹³¹I therapy would be beneficial.²⁶² A TSH-stimulated TG can be obtained with a repeat DxWBS for patients being evaluated for additional therapy, as well as to confirm remission for patients with a history of invasive disease.

Neck US is the most effective and efficient radiologic tool to monitor for persistent or recurrent anatomic disease. The first US should be performed approximately 6 months after the initial surgery and then at 6- to 12-month intervals for ATA pediatric intermediate- and high-risk patients. Serial chest CT at 6- to 12-month intervals should be used to monitor patients with known pulmonary metastasis. A TSH-stimulated TG and a DxWBS may be obtained in ATA pediatric intermediate- and high-risk patients, previously treated with ¹³¹I, with known iodine-avid metastatic disease, if the TG level has plateaued or is increasing, and anatomic disease is not found on neck US or cross-sectional imaging (chest CT or MRI).²⁶² This is particularly important for patients with diffuse, micronodular pulmonary metastasis because up to 50% of the time the WBS will detect small 1- to 2-mm lesions that may be missed even with high-resolution CT scanning.²⁸³ The use of 18(F)-fluorodeoxyglucose-positron emission tomography (FDG-PET)/CT should be limited to patients with increasing TG levels suspected to have persistent anatomic disease that is non-RAI avid, based on previous RAI treatment and WBS imaging.³⁷¹

Treatment of Persistent or Recurrent Disease

Cervical lymph nodes are the most common location for residual and recurrent PTC.³⁷² The decision to treat or to observe identifiable cervical disease should be individualized according to the size, anatomic location, prior treatment history, the iodine avidity of cervical disease, and the presence of distant metastasis. For macroscopic cervical disease (≥ 1 cm in size) confirmed by FNA, surgery is preferable to ¹³¹I therapy.²⁶² For children with iodine-avid small volume cervical disease, therapeutic ¹³¹I therapy can be considered or the patient may be observed with continued TSH suppression, depending on the individual risk to benefit ratio, as well as the absence or presence of distant metastasis.³⁷³

The majority of children with pulmonary metastasis have micronodular disease demonstrating excellent iodine avidity. Thus in contrast to adults, children and adolescents with pulmonary metastasis have low disease-specific mortality.^{293,374} However, although many pediatric patients achieve remission from pulmonary disease,^{293,350,374} at least one-third of children will have stable, persistent disease that will not respond to repeat doses of ¹³¹I.²⁹³ Re-treatment of ¹³¹I iodine-avid pulmonary metastases should be considered in children who have demonstrated previous improvement but continue to have persistent disease based on a plateau or increasing trend in TG or TGA, as well as data from cross-sectional imaging.^{262,353} The timing of additional ¹³¹I should be at least 12 or more months from the previous treatment, with several studies demonstrating a continuous decline in serum TG levels for 18 to 24 months, or longer, following the previous RAI therapy.^{374,375}

Systemic Therapy for Children With Progressive Disease

RAI-refractory (RAIR) disease is more common in patients over 45 years of age; however, a small proportion of children and adolescents also develop progressive DTC that is refractory to ¹³¹I treatment. Over the last decade, there have been an increasing number of oral systemic therapies that have been incorporated into clinical practice. These agents target constitutively activated protein kinases in the MAPK and PI3K signaling pathways.³⁷⁶ Knowledge of the somatic oncogene driver mutation in the refractory tumor is critical in selection of the drug that is likely to have the greatest clinical effect. The multitargeted tyrosine kinase inhibitors (TKIs), including sorafenib, lenvatinib, and others, target the vascular endothelial growth factor receptors, as well as a combination of the epithelial growth factor receptors, fibroblast growth factor receptors, platelet-derived growth factor receptor, and RET. These agents have been shown to slow progression and to decrease tumor burden for many patients, including tumors with BRAF v600E, as well as in tumors with unknown oncogene status. Unfortunately, the effect is often transient and many patients experience side effects, including hypertension, diarrhea, anorexia with weight loss, and fatigue.³⁷⁷ The newer selective inhibitors have increased efficacy and less toxicity. Resensitization of tumors to RAI has been achieved for patients with tumors harboring either BRAF or RAS mutations after administration of selective BRAF, as well as MEK inhibitors.³⁷⁸ Equally effective selective inhibitors are also in clinical trial and practice, with several approved down to 12 years of age for patients with tumors harboring BRAF, RET, NTRK, ALK, or ROS1 fusion genes.³⁷⁷ Although these agents are providing clinical benefit, the majority are cytostatic, not cytotoxic. Tumors eventually develop resistance and there is limited knowledge on the best choice for a follow-on drug for salvage therapy if or when a tumor

becomes refractory to the initial agent. With the potential for significant side effects, pediatric patients that may benefit from therapy should be referred to centers that have experience with these drugs in the treatment of RAI thyroid cancer, so that the timing, selection, monitoring, and adjustment of therapy can be optimized, and the risk of adverse reactions minimized.^{262,377,379}

Medullary Thyroid Cancer

MTC is a neuroendocrine cancer that derives from the neural crest and originated parafollicular C-cells of the thyroid gland.²⁶⁵ Thus in contrast to PTC and FTC, follicular-cell-derived thyroid tumors, MTC cells are not responsive to TSH, do not express the sodium-iodine symporter, and do not produce TG. Rather, MTC secretes calcitonin and carcinoembryonic antigen (CEA), both of which serve as the tumor markers of MTC. In children, MTC is most commonly a monogenic disorder caused by a dominantly inherited or de novo gain-of-function mutation in the RET protooncogene, associated as one component of MEN2, either MEN2A or 2B, depending on the specific mutation.^{380,381} Sporadic MTC is not associated with a germline mutation; rather it develops secondary to somatic mutations in the RET protooncogene, RAS, or several others genes, including ALK fusions.³⁸²

There is a strong genotype and phenotype correlation in the RET protooncogene allowing for prediction of the rapidity with which an individual may develop MTC, as well as the risk and timing for development of the other MEN2-related characteristics and tumors, including pheochromocytoma and hyperparathyroidism.^{265,380,381,383} The ATA divides the most common RET mutations into three risk categories: (1) highest risk, (2) high risk, and (3) moderate risk, and bases the recommended age for initial screening, and the timing of prophylactic thyroidectomy to coincide with the goal of achieving surgical remission from disease.²⁶⁵ There are multiple codon mutations associated with MEN2A with the highest risk of developing MTC associated with mutations in C634 and A883F. The remaining mutations carry an increased risk of developing MTC, although for individual patients, the course from C-cell hyperplasia to MTC may be indolent with MTC not developing until the third, fourth, or later decades of life. In addition to MTC, in MEN2A there is a 10% to 50% risk of developing hyperparathyroidism and/or pheochromocytoma, defined by the individual codon mutation. Two additional features associated with MEN2A are cutaneous lichen amyloidosis, a pruritic, plaque-like rash typically on the upper back associated with mutations in C634 and V804M, and Hirschsprung disease, associated with mutations in C609, C611, C618, and C620.²⁶⁵

MEN2B is associated with a mutation at codon 918 in the RET protooncogene. Because mutations in codon 918 are more often de novo compared with other RET protooncogene codon mutations, recognition of the early clinical signs and symptoms is critically important to diagnose the syndrome before MTC metastasis, which typically occurs before 4 years of age.³⁸⁴ These signs and symptoms include alacrima (the inability or decreased ability, to make tears), constipation (associated with ganglioneuromatosis), and hypotonia (feeding difficulties with failure to thrive, club feet, hip dislocation). The more classically defining symptoms, including oral and lip mucosal neuromas and elongated Marfanoid facies, are not clinically evident until school age, around 5 years of age.^{384,385} Some 50% of patients will develop a pheochromocytoma; however, unlike patients with MEN2A, there does not appear to be an increased risk for developing hyperparathyroidism.²⁶⁵

Based on these observations, the ATA recommends the timing of total thyroidectomy as follows; within the first year of life for carriers of the highest risk mutation (codon 918), at or before age 5 years for those with a high risk mutation (codons 634 and 883), and for all other moderate risk mutations when the serum calcitonin level shows an increasing upward trend, a nodule is found on surveillance thyroid US, or if the parents do not wish to continue to embark on a long period of laboratory and radiological surveillance. For patients with MEN2B,²⁶⁵ if thyroidectomy is not completed by 4 years of age, there is reduced likelihood for surgical remission.³⁸⁶

For patients undergoing thyroidectomy, a central lymph node dissection is recommended in children whose basal calcitonin is greater than 40 pg/mL or with any evidence of lymph node metastasis.^{265,387} After initial surgery, levothyroxine medication is given to normalize, not suppress, the TSH. In contrast to the follicular cell in nonmedullary DTC, parafollicular C-cells do not accumulate iodine because they do not express the sodium-iodide symporter. Thus postoperative ¹³¹I therapy is not indicated following thyroidectomy for MTC.³⁸⁸ Calcitonin and CEA levels should be monitored as tumor markers every 6 to 12 months, with decreasing frequency once remission is confirmed. Neck US should be followed for patients with persistently detectable tumor markers or initial lymph node metastasis. If the tumor markers remain significantly elevated, or show rapid doubling time, additional imaging, such as chest CT, contrast-enhanced liver MRI or CT, bone scan, MRI of the axial skeleton, 18F-FDG PET/CT or gallium-68 dotatate PET/CT should be performed.^{265,389}

Patients with hereditary MTC should receive continued and life long follow-up, including genetic counseling, psychosocial support, and prospective screening of pheochromocytoma and primary hyperparathyroidism. Annual screening for pheochromocytoma with a urine or serum fractionated metanephrine panel is initiated at age 11 years for ATA highest risk patients⁹¹⁸ and at 16 years for ATA high and moderate risk patients, with the addition of annual screening for hyperparathyroidism for ATA high- and moderate-risk patients at the same time.²⁶⁵

Metastatic MTC is generally incurable, but may show an indolent clinical course with stable disease over decades. A rapid calcitonin doubling time and a disparate low calcitonin, associated with a high CEA level, is associated with aggressive behavior and poorer prognosis.^{265,390} For MTC patients with symptomatic or progressive metastatic disease, the treatment using multi-targeted TKI, or, the newer and more effective selective targeted therapies that inhibit *RET*, should be considered.^{376,391} Similar to the use of these drugs in patients with DTC, pediatric patients that may benefit from these oral chemotherapeutic agents should be referred to centers that have experience with these drugs in the treatment of progressive MTC, so that the timing, selection, monitoring, and adjustment of therapy can be optimized and the risk of adverse reactions minimized.^{262,377,392}

REFERENCES

- Rousset B, Dupuy C, Miot F, Dumont J. Thyroid Hormone Synthesis And Secretion. In: *Thyroid Disease Manager*; 2015.
- Refetoff S. Thyroid Hormone Serum Transport Proteins. In: *Thyroid Disease Manager*; 2015.
- Mariotti S, Beck-Peccoz P. Physiology of the Hypothalamic-Pituitary-Thyroid Axis. In: *Thyroid Disease Manager*; 2016.
- Rivkees SA. Thyroid disorders in children and adolescents. In: Sperling MA, ed. *Pediatric Endocrinology*. 4th ed. Philadelphia: Saunders; 2014:444–470.
- Ueda D. Normal volume of the thyroid gland in children. *J Clin Ultrasound*. 1990;18:455–462.
- Hopwood NJ, Carroll RG, Kenny FM, Foley Jr TP. Functioning thyroid masses in childhood and adolescence. Clinical, surgical, and pathologic correlations. *J Pediatr*. 1976;89:710–718.
- Kaplan MM. Clinical perspectives in the diagnosis of thyroid disease. *Clin Chem*. 1999;145:1377–1383.
- Ribeiro RC, Apriletti JW, West BL, et al. The molecular biology of thyroid hormone action. *Ann N Y Acad Sci*. 1995;758:366–389.
- Lazar MA. Steroid and thyroid hormone receptors. *Endocrinol Metab Clin North Am*. 1991;20:681–695.
- Faror C, Wellby ML, Beng C. Familial dysalbuminaemic hyperthyroxinaemia and other causes of euthyroid hyperthyroxinaemia. *J R Soc Med*. 1987;80:750–752.
- Ruiz M, Rajatanavin R, Young RA, et al. Familial dysalbuminemic hyperthyroxinemia: a syndrome that can be confused with thyrotoxicosis. *N Engl J Med*. 1982;306:635–639.
- Nikolai TF, Seal US. X-chromosome linked inheritance of thyroxine-binding globulin deficiency. *J Clin Endocrinol Metab*. 1967;27:1515–1520.
- De Groot LJ. Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinol Metab*. 1999;84:151–164.
- Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. *Thyroid*. 2014;24:1456–1465.
- Nelson JC, Weiss RM, Wilcox RB. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. *J Clin Endocrinol Metab*. 1994;79:76–79.
- Nelson JC, Nayak SS, Wilcox RB. Variable underestimates by serum free thyroxine (T4) immunoassays of free T4 concentrations in simple solutions. *J Clin Endocrinol Metab*. 1994;79:1373–1375.
- Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. *Best Pract Res Clin Endocrinol Metab*. 2013;27:745–762.
- Ross DS. Serum thyroid-stimulating hormone measurement for assessment of thyroid function and disease. *Endocrinol Metab Clin North Am*. 2001;30:245–264. vii.
- Biondi B, Cooper DS. Subclinical Hyperthyroidism. *N Engl J Med*. 2018;378:2411–2419.
- Spencer CA, Hollowell JG, Kazarosyan M, Braverman LE. National Health and Nutrition Examination Survey III thyroid-stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. *J Clin Endocrinol Metab*. 2007;92:4236–4240.
- Zarkovic M, Ciric J, Beleslin B, et al. Further studies on delineating thyroid-stimulating hormone (TSH) reference range. *Horm Metab Res*. 2011;43:970–976.
- Volzke H, Schmidt CO, John U, Wallaschofski H, Dorr M, Nauck M. Reference levels for serum thyroid function tests of diagnostic and prognostic significance. *Horm Metab Res*. 2010;42:809–814.
- Surks MJ, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab*. 2005;90:5489–5496.
- Verburg FA, Kirchgassner C, Hebestreit H, et al. Reference ranges for analytes of thyroid function in children. *Horm Metab Res*. 2011;43:422–426.
- Lazar L, Frumkin RB, Battat E, Lebenthal Y, Phillip M, Meyerovitch J. Natural history of thyroid function tests over 5 years in a large pediatric cohort. *J Clin Endocrinol Metab*. 2009;94:1678–1682.
- Brabant G, Beck-Peccoz P, Jarzab B, et al. Is there a need to redefine the upper normal limit of TSH? *Eur J Endocrinol*. 2006;154:633–637. 2006.
- Hanley P, Lord K, Bauer AJ. Thyroid disorders in children and adolescents: a review. *JAMA Pediatr*. 2016;170:1008–1019.
- Talbot NBSE, McArthur JW, Crawford JD. *Functional Endocrinology: From Birth to Adolescence*. Cambridge: Harvard University Press; 1952:1–51.
- Wilkins L. *The Diagnosis and Treatment of Endocrine Disorders in Children and Adolescence*. Springfield: Charles Thomas; 1965: 141–150. Hypothyroidism.
- Kaplowitz PB. Subclinical hypothyroidism in children: normal variation or sign of a failing thyroid gland? *Int J Pediatr Endocrinol*. 2010;2010. 281453.
- Rapa A, Monzani A, Moia S, et al. Subclinical hypothyroidism in children and adolescents: a wide range of clinical, biochemical, and genetic factors involved. *J Clin Endocrinol Metab*. 2009;94: 2414–2420.
- Reinehr T. Thyroid function in the nutritionally obese child and adolescent. *Curr Opin Pediatr*. 2011;23:415–420.

33. Dekelbab BH, Abou Ouf HA, Jain I. Prevalence of elevated thyroid-stimulating hormone levels in obese children and adolescents. *Endocr Pract.* 2010;16:187–190. 2010.
34. Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W. Thyroid hormones and their relation to weight status. *Horm Res.* 2008;70:51–57.
35. Radetti G, Kleon W, Buzi F, et al. Thyroid function and structure are affected in childhood obesity. *J Clin Endocrinol Metab.* 2008;93:4749–4754.
36. Reinehr T, de Sousa G, Andler W. Hyperthyrotropinemia in obese children is reversible after weight loss and is not related to lipids. *J Clin Endocrinol Metab.* 2006;91:3088–3091.
37. Lafranchi S. Thyroiditis and acquired hypothyroidism. *Pediatr Ann.* 1992;21(29):32–39.
38. Rapoport B. Pathophysiology of Hashimoto's thyroiditis and hypothyroidism. *Annu Rev Med.* 1991;42:91–96.
39. Rivkees SA, Bode HH, Crawford JD. Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. *N Engl J Med.* 1988;318:599–602.
40. LeFranchi S, Mandel SH. *Graves' Disease in the Neonatal Period and Childhood.* Philadelphia: Clinical Text JB Lippincott; 1995:1237–1246.
41. Foley Jr TP, Abbassi V, Copeland KC, Draznin MB. Brief report: hypothyroidism caused by chronic autoimmune thyroiditis in very young infants. *N Engl J Med.* 1994;330:466–468.
42. Wells BJ, Hueston WJ. Are thyroid peroxidase antibodies associated with cardiovascular disease risk in patients with subclinical hypothyroidism? *Clin Endocrinol.* 2005;62:580–584.
43. Radetti G, Maselli M, Buzi F, et al. The natural history of the normal/mild elevated TSH serum levels in children and adolescents with Hashimoto's thyroiditis and isolated hyperthyrotropinaemia: a 3-year follow-up. *Clin Endocrinol.* 2012;76:394–398.
44. Radetti G, Gottardi E, Bona G, Corrias A, Salardi S, Loche S. The natural history of euthyroid Hashimoto's thyroiditis in children. *J Pediatr.* 2006;149:827–832.
45. Van Dop C, Conte FA, Koch TK, Clark SJ, Wilson-Davis SL, Grumbach MM. Pseudotumor cerebri associated with initiation of levothyroxine therapy for juvenile hypothyroidism. *N Engl J Med.* 1983;308:1076–1080.
46. Raghavan S, DiMartino-Nardi J, Saenger P, Linder B. Pseudotumor cerebri in an infant after L-thyroxine therapy for transient neonatal hypothyroidism. *J Pediatr.* 1997;130:478–480.
47. Campos SP, Olitsky S. Idiopathic intracranial hypertension after L-thyroxine therapy for acquired primary hypothyroidism. *Clin Pediatr.* 1995;34:334–337.
48. Rohn R. Pseudotumor cerebri following treatment of hypothyroidism. *Am J Dis Child.* 1985;139:752.
49. Kaplowitz PB. Case report: rapid spontaneous recovery from severe hypothyroidism in 2 teenage girls. *Int J Pediatr Endocrinol.* 2012;2012:9.
50. Dong BJ, Brown CH. Hypothyroidism resulting from generic levothyroxine failure. *J Am Board Fam Pract.* 1991;4:167–170.
51. Bolton S. Bioequivalence studies for levothyroxine. *AAPS J.* 2005;7 E47–53.
52. Fischer MA, Avorn J. Economic consequences of underuse of generic drugs: evidence from Medicaid and implications for prescription drug benefit plans. *Health Serv Res.* 2003;38:1051–1063.
53. Dong BJ, Hauck WW, Gambertoglio JG, et al. Bioequivalence of generic and brand-name levothyroxine products in the treatment of hypothyroidism. *JAMA.* 1997;277:1205–1213.
54. Bolk N, Visser TJ, Nijman J, Jongste IJ, Tijssen JG, Berghout A. Effects of evening vs morning levothyroxine intake: a randomized double-blind crossover trial. *Arch Intern Med.* 2010;170:1996–2003.
55. Bolk N, Visser TJ, Kalsbeek A, van Domburg RT, Berghout A. Effects of evening vs morning thyroxine ingestion on serum thyroid hormone profiles in hypothyroid patients. *Clin Endocrinol.* 2007;66:43–48.
56. Sekadde CB, Slaunwhite Jr WR, Aceto Jr T, Murray K. Administration of thyroxin once a week. *J Clin Endocrinol Metab.* 1974;39:759–764.
57. Rivkees SA, Hardin DS. Cretinism after weekly dosing with levothyroxine for treatment of congenital hypothyroidism. *J Pediatr.* 1994;125:147–149.
58. Bell DS, Ovalle F. Use of soy protein supplement and resultant need for increased dose of levothyroxine. *Endocr Pract.* 2001;7:193–194.
59. Jabbar MA, Larrea J, Shaw RA. Abnormal thyroid function tests in infants with congenital hypothyroidism: the influence of soy-based formula. *J Am Coll Nutr.* 1997;16:280–282.
60. Chorazy PA, Himelhoch S, Hopwood NJ, Greger NG, Postellon DC. Persistent hypothyroidism in an infant receiving a soy formula: case report and review of the literature. *Pediatrics.* 1995;96:148–150.
61. Karanikas G, Schuetz M, Kontur S, et al. No immunological benefit of selenium in consecutive patients with autoimmune thyroiditis. *Thyroid.* 2008;18:7–12.
62. Fisher DA, Pandian MR, Carlton E. Autoimmune thyroid disease: an expanding spectrum. *Pediatr Clin North Am.* 1987;34:907–918.
63. Mihailova D, Grigороva R, Vassileva B, et al. Autoimmune thyroid disorders in juvenile chronic arthritis and systemic lupus erythematosus. *Adv Exp Med Biol.* 1999;455:55–60.
64. Jarnerot G, Azad Khan AK, Truelove SC. The thyroid in ulcerative colitis and Crohn's disease. II. Thyroid enlargement and hyperthyroidism in ulcerative colitis. *Acta Med Scand.* 1975;197:83–87.
65. Sattar N, Lazare F, Kacer M, et al. Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease. *J Pediatr.* 2011;158:272–275.e1.
66. Ranke MB, Saenger P. Turner's syndrome. *Lancet.* 2001;358:309–314.
67. Gawlik A, Gawlik T, Januszek-Trzciakowska A, Patel H, Malecka-Tendera E. Incidence and dynamics of thyroid dysfunction and thyroid autoimmunity in girls with Turner's syndrome: a long-term follow-up study. *Horm Res Paediatr.* 2011;76:314–320.
68. Livadas S, Xekouki P, Fouka F, et al. Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. *Thyroid.* 2005;15:1061–1066.
69. Murphy J, Philip M, Macken S, et al. Thyroid dysfunction in Down's syndrome and screening for hypothyroidism in children and adolescents using capillary TSH measurement. *J Pediatr Endocrinol Metabol.* 2008;21:155–163. 2008.
70. Fort P, Lifshitz F, Bellisario R, et al. Abnormalities of thyroid function in infants with Down syndrome. *J Pediatr.* 1984;104:545–549.
71. Wasniewska M, Salerno M, Cassio A, et al. Prospective evaluation of the natural course of idiopathic subclinical hypothyroidism in childhood and adolescence. *Eur J Endocrinol.* 2009;160:417–421.
72. Sklar CA, Qazi R, David R. Juvenile autoimmune thyroiditis. Hormonal status at presentation and after long-term follow-up. *Am J Dis Child.* 1986;140:877–880.
73. Crocker MK, Kaplowitz P. Treatment of paediatric hyperthyroidism but not hypothyroidism has a significant effect on weight. *Clin Endocrinol.* 2010;73:752–759.
74. Guiral J, Fisas R, Martin-Herraez A, Garcia-Velazquez J. Slipped capital femoral epiphysis in primary juvenile hypothyroidism. *Acta Orthop Belg.* 1994;60:343–345.
75. Anasti JN, Flack MR, Froehlich J, Nelson LM, Nisula BC. A potential novel mechanism for precocious puberty in juvenile hypothyroidism. *J Clin Endocrinol Metab.* 1995;80:276–279.
76. Rao NS, Sriprakash ML, Dash RJ. Primary juvenile hypothyroidism with precocious puberty, galactorrhoea and multicystic ovaries. *J Assoc Physicians India.* 1987;35:161–163.
77. Piziak VK, Hahn Jr HB. Isolated menarche in juvenile hypothyroidism. *Clin Pediatr.* 1984;23:177–179.
78. Pringle PJ, Stanhope R, Hindmarsh P, Brook CG. Abnormal pubertal development in primary hypothyroidism. *Clin Endocrinol.* 1988;28:479–486.
79. Nebesio TD, Wise MD, Perkins SM, Eugster EA. Does clinical management impact height potential in children with severe acquired hypothyroidism? *J Pediatr Endocrinol Metabol.* 2011;24:893–896.
80. Watanabe T, Minamitani K, Minagawa M, et al. Severe juvenile hypothyroidism: treatment with GH and GnRH agonist in addition to thyroxine. *Endocr J.* 1998;45(Suppl):S159–162.
81. Minamitani K, Murata A, Ohnishi H, Wataki K, Yasuda T, Niimi H. Attainment of normal height in severe juvenile hypothyroidism. *Arch Dis Child.* 1994;70:429–430. discussion 30–31.
82. Quintos JB, Salas M. Use of growth hormone and gonadotropin releasing hormone agonist in addition to L-thyroxine to attain

- normal adult height in two patients with severe Hashimoto's thyroiditis. *J Pediatr Endocrinol Metabol.* 2005;18:515–521.
83. Teng L, Bui H, Bachrach L, et al. Catch-up growth in severe juvenile hypothyroidism: treatment with a GnRH analog. *J Pediatr Endocrinol Metabol.* 2004;17:345–354.
 84. Watanabe T, Minamitani K, Minagawa M, et al. Severe juvenile hypothyroidism: treatment with GH and GnRH agonist in addition to thyroxine. *Endocr J.* 1998;45(Suppl):S159–S162.
 85. Miyazaki R, Yanagawa N, Higashino H, Kobayashi Y. LHRH analogue and growth hormone did not improve the final height of a patient with juvenile hypothyroidism accompanied by precocious puberty. *Arch Dis Child.* 2000;83:87.
 86. Mitchell HS. Recommended dietary allowances up to date. *J Am Diet Assoc.* 1974;64:149–150.
 87. Leung AM, Braverman LE. Iodine-induced thyroid dysfunction. *Curr Opin Endocrinol Diabetes Obes.* 2012;19:414–419.
 88. Woebler KA. Iodine and thyroid disease. *Med Clin North Am.* 1991;75:169–178.
 89. Bona G, Chiorboli E, Rapa A, Weber G, Vigone MC, Chiumello G. Measurement of urinary iodine excretion to reveal iodine excess in neonatal transient hypothyroidism. *J Pediatr Endocrinol Metabol.* 1998;11:739–743.
 90. Koga Y, Sano H, Kikukawa Y, Ishigouka T, Kawamura M. Effect on neonatal thyroid function of povidone-iodine used on mothers during perinatal period. *J Obstet Gynaecol.* 1995;21:581–585.
 91. Gordon CM, Rowitch DH, Mitchell ML, Kohane IS. Topical iodine and neonatal hypothyroidism. *Arch Pediatr Adolesc Med.* 1995;149:1336–1339.
 92. l'Allemand D, Gruters A, Beyer P, Weber B. Iodine in contrast agents and skin disinfectants is the major cause for hypothyroidism in premature infants during intensive care. *Horm Res.* 1987;28:42–49.
 93. Brown RS, Bloomfield S, Bednarek FJ, Mitchell ML, Braverman LE. Routine skin cleansing with povidone-iodine is not a common cause of transient neonatal hypothyroidism in North America: a prospective controlled study. *Thyroid.* 1997;7:395–400.
 94. Grosso S, Berardi R, Cioni M, Morgese G. Transient neonatal hypothyroidism after gestational exposure to amiodarone: a follow-up of two cases. *J Endocrinol Invest.* 1998;21:699–702.
 95. Costigan DC, Holland FJ, Daneman D, Hesslein PS, Vogel M, Ellis G. Amiodarone therapy effects on childhood thyroid function. *Pediatrics.* 1986;77:703–708.
 96. Bogazzi F, Tomisti L, Bartalena L, Aghini-Lombardi F, Martino E. Amiodarone and the thyroid: a 2012 update. *J Endocrinol Invest.* 2012;35:340–348.
 97. Delange F. The disorders induced by iodine deficiency. *Thyroid.* 1994;4:107–128.
 98. Boyages SC, Halpern JP. Endemic cretinism: toward a unifying hypothesis. *Thyroid.* 1993;3:59–69.
 99. Matovinovic I, Trowbridge FL, Nichaman MZ, Child MA. Iodine nutrition and prevalence of endemic goiter in USA. *Acta Endocrinol Suppl (Copenh).* 1973;179:17–18.
 100. Lee K, Bradley R, Dwyer J, Lee SL. Too much versus too little: the implications of current iodine intake in the United States. *Nutr Rev.* 1999;57:177–181.
 101. Gallego G, Goodall S, Eastman CJ. Iodine deficiency in Australia: is iodine supplementation for pregnant and lactating women warranted? *Med J Aust.* 2010;192:461–463.
 102. Sherman SI, Gopal J, Haugen BR, et al. Central hypothyroidism associated with retinoid X receptor-selective ligands. *N Engl J Med.* 1999;340:1075–1079.
 103. Samuels MH, Lillehei K, Kleinschmidt-Demasters BK, Stears J, Ridgway EC. Patterns of pulsatile pituitary glycoprotein secretion in central hypothyroidism and hypogonadism. *J Clin Endocrinol Metab.* 1990;70:391–395.
 104. Trejbal D, Sulla I, Trejbalova L, Lazurova I, Schwartz P, Machanova Y. Central hypothyroidism—various types of TSH responses to TRH stimulation. *Endocr Regul.* 1994;28:35–40.
 105. Hanna CE, Krainz PL, Skeels MR, Miyahira RS, Sesser DE, LaFranchi SH. Detection of congenital hypopituitary hypothyroidism: ten-year experience in the Northwest Regional Screening Program. *J Pediatr.* 1986;109:959–964.
 106. Adams LM, Emery JR, Clark SJ, Carlton EI, Nelson JC. Reference ranges for newer thyroid function tests in premature infants. *J Pediatr.* 1995;126:122–127.
 107. Nebesio TD, McKenna MP, Nabhan ZM, Eugster EA. Newborn screening results in children with central hypothyroidism. *J Pediatr.* 2010;156:990–993.
 108. Persani L. Hypothalamic thyrotropin-releasing hormone and thyrotropin biological activity. *Thyroid.* 1998;8:941–946.
 109. Carrozza V, Csako G, Yanovski JA, et al. Levothyroxine replacement therapy in central hypothyroidism: a practice report. *Pharmacotherapy.* 1999;19:349–355.
 110. Slawik M, Klawitter B, Meiser E, et al. Thyroid hormone replacement for central hypothyroidism: a randomized controlled trial comparing two doses of thyroxine (T4) with a combination of T4 and triiodothyronine. *J Clin Endocrinol Metab.* 2007;92:4115–4122.
 111. Zwaveling-Soonawala N, Naafs JC, Verkerk PH, van Trotsenburg ASP. Mortality in children with early detected congenital central hypothyroidism. *J Clin Endocrinol Metab.* 2018;103(8):3078–3082.
 112. Huang SA, Tu HM, Harney JW, et al. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N Engl J Med.* 2000;343:185–189.
 113. Boice Jr JD. Radiation-induced thyroid cancer—what's new? *J Natl Cancer Inst.* 2005;97:703–705.
 114. Brignardello E, Corrias A, Isolato G, et al. Ultrasound screening for thyroid carcinoma in childhood cancer survivors: a case series. *J Clin Endocrinol Metab.* 2008;93:4840–4843.
 115. Madanat LM, Lahteenmaki PM, Hurme S, Dyba T, Salmi TT, Sankila R. Hypothyroidism among pediatric cancer patients: a nationwide, registry-based study. *Int J Cancer.* 2008;122:1868–1872.
 116. Rivkees SA. The evolving field of 'onco-endocrinology'. *J Pediatr Endocrinol Metabol.* 2008;21:915–916.
 117. Bonato C, Severino RF, Elneceve RH. Reduced thyroid volume and hypothyroidism in survivors of childhood cancer treated with radiotherapy. *J Pediatr Endocrinol Metabol.* 2008;21:943–949.
 118. Topliss D. Thyroid incidentaloma: the ignorant in pursuit of the palpable. *Clin Endocrinol.* 2004;60:18–20.
 119. Brent GA. The molecular basis of thyroid hormone action. *N Engl J Med.* 1994;331:847–853.
 120. Chatterjee VK. Resistance to thyroid hormone. *Horm Res.* 1997;48:43–46.
 121. Refetoff S. Resistance to thyroid hormone. *Curr Ther Endocrinol Metab.* 1997;6:132–134.
 122. Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. *Best Pract Res Clin Endocrinol Metab.* 2007;21:277–305.
 123. Refetoff S. The syndrome of resistance to thyroid stimulating hormone. *J Chin Med Assoc.* 2003;66:441–452.
 124. Beck-Peccoz P, Forloni F, Cortelazzi D, et al. Pituitary resistance to thyroid hormones. *Horm Res.* 1992;38:66–72.
 125. Stein MA, Weiss RE, Refetoff S. Neurocognitive characteristics of individuals with resistance to thyroid hormone: comparisons with individuals with attention-deficit hyperactivity disorder. *J Dev Behav Pediatr.* 1995;16:406–411.
 126. Weiss RE, Refetoff S. Treatment of resistance to thyroid hormone—*primum non nocere*. *J Clin Endocrinol Metab.* 1999;84:401–404.
 127. Canadas KT, Rivkees SA, Udelsman R, Breuer CK. Resistance to thyroid hormone associated with a novel mutation of the thyroid beta receptor gene in a four-year-old female. *Int J Pediatr Endocrinol.* 2011;2011:3.
 128. Anselmo J, Refetoff S. Regression of a large goiter in a patient with resistance to thyroid hormone by every other day treatment with triiodothyronine. *Thyroid.* 2004;14:71–74.
 129. Rivkees SA. Pediatric Graves' disease: controversies in management. *Horm Res Paediatr.* 2010;74:305–311.
 130. Bahn Chair RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Thyroid.* 2011;21:593–646.
 131. Committee on Pharmaceutical Affairs JSfPE, the Pediatric Thyroid Disease Committee JTA, Minamitani K, et al. Guidelines for the treatment of childhood-onset Graves' disease in Japan, 2016. *Clin Pediatr Endocrinol.* 2017;26:29–62.
 132. Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association Guidelines for Diagnosis and Management of

- Hyperthyroidism and Other Causes of Thyrotoxicosis. *Thyroid*. 2016;26:1343–1421.
133. Hedberg CW, Fishbein DB, Janssen RS, et al. An outbreak of thyrotoxicosis caused by the consumption of bovine thyroid gland in ground beef. *N Engl J Med*. 1987;316:993–998.
 134. Rivkees SA. Controversies in the management of Graves' disease in children. *J Endocrinol Invest*. 2016;39:1247–1257.
 135. van Veenendaal NR, Rivkees SA. Treatment of pediatric Graves' disease is associated with excessive weight gain. *J Clin Endocrinol Metab*. 2011;96:3257–3263.
 136. Simon M, Rigou A, Le Moal J, et al. Epidemiology of childhood hyperthyroidism in France: a nationwide population-based study. *J Clin Endocrinol Metab*. 2018;103(8):2980–2987.
 137. Leger J, Gelwane G, Kaguelidou F, Benmerad M, Alberti C. Positive impact of long-term antithyroid drug treatment on the outcome of children with Graves' disease: national long-term cohort study. *J Clin Endocrinol Metab*. 2012;97:110–119.
 138. Kaguelidou F, Alberti C, Castanet M, Guitteny MA, Czernichow P, Leger J. Predictors of autoimmune hyperthyroidism relapse in children after discontinuation of antithyroid drug treatment. *J Clin Endocrinol Metab*. 2018;93(10):3017–3026.
 139. Rabon S, Burton AM, White PC. Graves' disease in children: long-term outcomes of medical therapy. *Clin Endocrinol*. 2016;85:632–635.
 140. Ohye H, Minagawa A, Noh JY, et al. Antithyroid drug treatment for graves' disease in children: a long-term retrospective study at a single institution. *Thyroid*. 2014;24:200–207.
 141. Havgaard Kjaer R, Smedegard Andersen M, Hansen D. Increasing Incidence of Juvenile Thyrotoxicosis in Denmark: A Nationwide Study, 1998–2012. *Horm Res Paediatr*. 2015;84:102–107.
 142. Neil-Dwyer G, Bartlett J, McAinsh J, Cruickshank JM. Beta-adrenoceptor blockers and the blood-brain barrier. *Br J Clin Pharmacol*. 1981;11:549–553.
 143. Morales DR, Lipworth BJ, Donnan PT, Jackson C, Guthrie B. Respiratory effect of beta-blockers in people with asthma and cardiovascular disease: population-based nested case control study. *BMC Med*. 2017;15:18.
 144. Chapman EM. History of the discovery and early use of radioactive iodine. *JAMA*. 1983;250:2042–2044.
 145. Cooper DS. Antithyroid drugs. *N Engl J Med*. 2005;352:905–917.
 146. Rivkees SA, Sklar C, Freemark M. Clinical review 99: The management of Graves' disease in children, with special emphasis on radioiodine treatment. *J Clin Endocrinol Metab*. 1998;83:3767–3776.
 147. Cooper DS. Antithyroid drugs for the treatment of hyperthyroidism caused by Graves' disease. *Endocrinol Metab Clin North Am*. 1998;27:225–247.
 148. Rivkees SA, Mattison DR. Ending propylthiouracil-induced liver failure in children. *N Engl J Med*. 2009;360:1574–1575.
 149. Abalovich M, Amino N, Barbour LA, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2007;92: S1–47.
 150. Bahn RS, Burch HS, Cooper DS, et al. The Role of Propylthiouracil in the Management of Graves' Disease in Adults: report of a meeting jointly sponsored by the American Thyroid Association and the Food and Drug Administration. *Thyroid*. 2009;19:673–674.
 151. Rivkees SA. The treatment of Graves' disease in children. *J Pediatr Endocrinol Metabol*. 2006;19:1095–1111.
 152. Nakamura H, Noh JY, Itoh K, Fukata S, Miyauchi A, Hamada N. Comparison of methimazole and propylthiouracil in patients with hyperthyroidism caused by Graves' disease. *J Clin Endocrinol Metab*. 2007;92:2157–2162.
 153. Rivkees SA, Stephenson K, Dinauer C. Adverse events associated with methimazole therapy of graves' disease in children. *Int J Pediatr Endocrinol*. 2010;2010: 176970.
 154. Tajiri J, Noguchi S. Antithyroid drug-induced agranulocytosis: how has granulocyte colony-stimulating factor changed therapy? *Thyroid*. 2005;15:292–297.
 155. Cooper DS, Goldminz D, Levin AA, et al. Agranulocytosis associated with antithyroid drugs. Effects of patient age and drug dose. *Ann Intern Med*. 1983;98:26–29.
 156. Sriussadaporn S, Pumchumpol W, Lertwattanak R, Kunavisarut T. Efficacy of once daily versus divided daily administration of low daily dosage (15 mg/day) of methimazole in the induction of euthyroidism in Graves' hyperthyroidism: a randomized controlled study. *Int J Endocrinol*. 2017;2017: 2619695.
 157. Takata K, Kubota S, Fukata S, et al. Methimazole-induced agranulocytosis in patients with Graves' disease is more frequent with an initial dose of 30 mg daily than with 15 mg daily. *Thyroid*. 2009;19:559–563.
 158. Glaser NS, Styne DM. Predicting the likelihood of remission in children with Graves' disease: a prospective, multicenter study. *Pediatrics*. 2008;121:e481–e488.
 159. Shulman DI, Muhar I, Jorgensen EV, Diamond FB, Bercu BB, Root AW. Autoimmune hyperthyroidism in prepubertal children and adolescents: comparison of clinical and biochemical features at diagnosis and responses to medical therapy. *Thyroid*. 1997;7:755–760.
 160. Glaser NS, Styne DM. Predictors of early remission of hyperthyroidism in children. *J Clin Endocrinol Metab*. 1997;82:1719–1726.
 161. Lazar L, Kalter-Leibovici O, Pertzalan A, Weintrob N, Josefsberg Z, Phillip M. Thyrotoxicosis in prepubertal children compared with pubertal and postpubertal patients. *J Clin Endocrinol Metab*. 2000;85:3678–3682.
 162. Hamburger JI. Management of hyperthyroidism in children and adolescents. *J Clin Endocrinol Metab*. 1985;60:1019–1024.
 163. Puthenpura V, Desai K, Bauer A, Marshall I. Delayed methimazole-induced agranulocytosis in a 6-year old patient with Graves' disease. *Int J Pediatr Endocrinol*. 2016;16: 2016.
 164. Read Jr CH, Tansey MJ, Menda Y. A thirty-six year retrospective analysis of the efficacy and safety of radioactive iodine in treating young Graves' patients. *JCEM*. 2004;89(9):4229–4233.
 165. Levy WJ, Schumacher OP, Gupta M. Treatment of childhood Graves' disease. A review with emphasis on radioiodine treatment. *Clev Clin J Med*. 1988;55:373–382.
 166. Nebesio TD, Siddiqui AR, Pescovitz OH, Eugster EA. Time course to hypothyroidism after fixed-dose radioablation therapy of Graves' disease in children. *J Pediatr*. 2002;141:99–103.
 167. Rivkees SA, Cornelius EA. Influence of iodine-131 dose on the outcome of hyperthyroidism in children. *Pediatrics*. 2003;111:745–749.
 168. Rivkees SA, Dinauer C. An optimal treatment for pediatric Graves' disease is radioiodine. *J Clin Endocrinol Metab*. 2007;92:797–800.
 169. Dolphin GW. The risk of thyroid cancers following irradiation. *Health Phys*. 1968;15:219–228.
 170. Boice Jr JD. Thyroid disease 60 years after Hiroshima and 20 years after Chernobyl. *JAMA*. 2006;295:1060–1062.
 171. Kadmon PM, Noto RB, Boney CM, Goodwin G, Gruppuso PA. Thyroid storm in a child following radioactive iodine (RAI) therapy: a consequence of RAI versus withdrawal of antithyroid medication. *J Clin Endocrinol Metab*. 2001;86:1865–1867.
 172. Rivkees SA. Graves' disease therapy in children: truth and inevitable consequences. *J Pediatr Endocrinol Metabol*. 2007;20:953–955.
 173. Sigurdson AJ, Ronckers CM, Mertens AC, et al. Primary thyroid cancer after a first tumour in childhood (the Childhood Cancer Survivor Study): a nested case-control study. *Lancet*. 2005;365:2014–2023.
 174. Ron E, Lubin J, RE S, et al. Thyroid Cancer after exposure to external radiation: a pooled analysis of seven studies. *Radiat Res*. 1995;141:259–277.
 175. Toderian AB, Lawson ML. Use of antihistamines after serious allergic reaction to methimazole in pediatric Graves' disease. *Pediatrics*. 2014;133: e1401–e1404.
 176. Kobayashi S, Noh JY, Mukasa K, et al. Characteristics of agranulocytosis as an adverse effect of antithyroid drugs in the second or later course of treatment. *Thyroid*. 2014;24:796–801.
 177. Watanabe N, Narimatsu H, Noh JY, et al. Antithyroid drug-induced hematopoietic damage: a retrospective cohort study of agranulocytosis and pancytopenia involving 50,385 patients with Graves' disease. *J Clin Endocrinol Metab*. 2012;97: E49–E53.
 178. Shiran A, Shechner C, Dickstein G. Propylthiouracil-induced agranulocytosis in four patients previously treated with the drug. *JAMA*. 1991;266:3129–3130.
 179. Rivkees SA, Mandel SJ. Thyroid disease in pregnancy. *Horm Res Paediatr*. 2011;76(Suppl 1):91–96.

180. Van Vliet G, Polak M, Ritzen EM. Treating fetal thyroid and adrenal disorders through the mother. *Nat Clin Pract Endocrinol Metab.* 2008;4:675–682.
181. Polak M, Legac I, Vuillard E, Guibourdenche J, Castanet M, Luton D. Congenital hyperthyroidism: the fetus as a patient. *Horm Res.* 2006;65:235–242.
182. Luton D, Le Gac I, Vuillard E, et al. Management of Graves' disease during pregnancy: the key role of fetal thyroid gland monitoring. *J Clin Endocrinol Metab.* 2005;90:6093–6098.
183. Check JH, Rezvani I, Goodner D, Hopper B. Prenatal treatment of thyrotoxicosis to prevent intrauterine growth retardation. *Obstet Gynecol.* 1982;60:122–124.
184. Skuza KA, Sills IN, Stene M, Rapaport R. Prediction of neonatal hyperthyroidism in infants born to mothers with Graves disease. *J Pediatr.* 1996;128:264–268.
185. McKenzie JM, Zakarija M. Fetal and neonatal hyperthyroidism and hypothyroidism due to maternal TSH receptor antibodies. *Thyroid.* 1992;2:155–159.
186. Zimmerman D. Fetal and neonatal hyperthyroidism. *Thyroid.* 1999;9:727–733.
187. Rohrs 3rd HJ, Silverstein JH, Weinstein DA, Amdur RJ, Haller MJ. Thyroid storm following radioactive iodine (RAI) therapy for pediatric graves disease. *Am J Case Rep.* 2014;15:212–215.
188. Daneman D, Howard NJ. Neonatal thyrotoxicosis: intellectual impairment and craniosynostosis in later years. *J Pediatr.* 1980;97:257–259.
189. Bruinse HW, Vermeulen-Meiners C, Wit JM. Fetal treatment for thyrotoxicosis in non-thyrotoxic pregnant women. *Fetal Ther.* 1988;3:152–157.
190. Momotani N, Noh J, Oyanagi H, Ishikawa N, Ito K. Antithyroid drug therapy for Graves' disease during pregnancy. Optimal regimen for fetal thyroid status. *N Engl J Med.* 1986;315:24–28.
191. Tamaki H, Amino N, Iwatani Y, et al. Evaluation of TSH receptor antibody by 'natural in vivo human assay' in neonates born to mothers with Graves' disease. *Clin Endocrinol.* 1989;30:493–503.
192. Sunshine P, Kusumoto H, Kriss JP. Survival time of circulating long-acting thyroid stimulator in neonatal thyrotoxicosis: implications for diagnosis and therapy of the disorder. *Pediatrics.* 1965;36:869–876.
193. Jeng LB, Lin JD, Chen MF. Acute suppurative thyroiditis: a ten-year review in a Taiwanese hospital. *Scand J Infect Dis.* 1994;26:297–300.
194. Vitti P, Rago T, Barbesino G, Chiovato L. Thyroiditis: clinical aspects and diagnostic imaging. *Rays.* 1999;24:301–314.
195. Szabo SM, Allen DB. Thyroiditis. Differentiation of acute suppurative and subacute. Case report and review of the literature. *Clin Pediatr.* 1989;28:171–174.
196. Geva T, Theodor R. Atypical presentation of subacute thyroiditis. *Arch Dis Child.* 1988;63:845–846.
197. Lugo-Vicente H, Ortiz VN, Irizarry H, Camps JJ, Pagan V. Pediatric thyroid nodules: management in the era of fine needle aspiration. *J Pediatr Surg.* 1998;33:1302–1305.
198. Tonacchera M, Vitti P, Agretti P, et al. Activating thyrotropin receptor mutations in histologically heterogeneous hyperfunctioning nodules of multinodular goiter. *Thyroid.* 1998;8:559–564.
199. Esapa C, Foster S, Johnson S, Jameson JL, Kendall-Taylor P, Harris PE. G protein and thyrotropin receptor mutations in thyroid neoplasia. *J Clin Endocrinol Metab.* 1997;82:493–496.
200. Vattimo A, Bertelli P, Cintorino M, et al. Hurthle cell tumor dwelling in hot thyroid nodules: preoperative detection with technetium-99m-MIBI dual-phase scintigraphy. *J Nucl Med.* 1998;39:822–825.
201. David E, Rosen IB, Bain J, James J, Kirsh JC. Management of the hot thyroid nodule. *Am J Surg.* 1995;170:481–483.
202. Hamburger JI, Hamburger SW. Diagnosis and management of large toxic multinodular goiters. *J Nucl Med.* 1985;26:888–892.
203. Derwahl M, Studer H. Multinodular goitre: 'much more to it than simply iodine deficiency'. *Baillieres Best Pract Res Clin Endocrinol Metab.* 2000;14:577–600.
204. Barrio-Barrio J, Sabater AL, Bonet-Farriol E, Velazquez-Villoria A, Galofre JC. Graves' ophthalmopathy: VISA versus EUOGO Classification, assessment, and management. *J Ophthalmol.* 2015;2015:249125.
205. Bahn RS. Current Insights into the Pathogenesis of Graves' Ophthalmopathy. *Horm Metab Res.* 2015;47:773–778.
206. Wiersinga WM, Prummel MF. Graves' ophthalmopathy: a rational approach to treatment. *Trends Endocrinol Metab.* 2002;13:280–287.
207. Bahn RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Endocr Pract.* 2011;17:456–520.
208. Safa AM. Treatment of hyperthyroidism with a large initial dose of sodium iodine I 131. *Arch Intern Med.* 1975;135:673–675.
209. Barnes HV, Blizzard RM. Antithyroid drug therapy for toxic diffuse goiter (Graves disease): thirty years experience in children and adolescence. *J Pediatr.* 1977;91:313–320.
210. Sarkar SD, Beierwaltes WH, Gill SP, Cowley BJ. Subsequent fertility and birth histories of children and adolescents treated with 131I for thyroid cancer. *J Nucl Med.* 1976;17:460–464.
211. Flynn RW, Macdonald TM, Jung RT, Morris AD, Leese GP. Mortality and vascular outcomes in patients treated for thyroid dysfunction. *J Clin Endocrinol Metab.* 2006;91:2159–2164.
212. Franklyn JA, Maisonneuve P, Sheppard MC, Betteridge J, Boyle P. Mortality after the treatment of hyperthyroidism with radioactive iodine. *N Engl J Med.* 1998;338:712–718.
213. Franklyn JA, Sheppard MC, Maisonneuve P. Thyroid function and mortality in patients treated for hyperthyroidism. *JAMA.* 2005;294:71–80.
214. Goldman MB, Monson RR, Maloof F. Cancer mortality in women with thyroid disease. *Cancer Res.* 1990;50:2283–2289.
215. Holm PH, Wiklund, et al. Cancer risk after iodine-131 therapy for hyperthyroidism. *J Natl Cancer Inst.* 1991;83:1072–1077.
216. Metso S, Auvinen A, Huhtala H, Salmi J, Oksala H, Jaatinen P. Increased cancer incidence after radioiodine treatment for hyperthyroidism. *Cancer.* 2007;109:1972–1979.
217. Ron E, Doody MM, Becker DV, et al. Cancer mortality following treatment for adult hyperthyroidism. Cooperative Thyrotoxicosis Therapy Follow-up Study Group. *JAMA.* 1998;280:347–355.
218. Toohey RE, Stabin MG, Watson EE. The AAPM/RSNA physics tutorial for residents: internal radiation dosimetry: principles and applications. *Radiographics.* 2000;20:533–546.
219. Toohey RE, Stabin MG. *Comparative analysis of dosimetry parameters for nuclear medicine. ORISE Report 99-1064.* 1999. Gatlinburg, TN: Proceedings of the Sixth International Radiopharmaceutical Dosimetry Symposium; 1996:532–551.
220. Health Risks from Exposure to Low Levels of Ionizing Radiation. *BEIR VII – Phase 2.* Washington D.C: National Academies Press; 2006.
221. Miccoli P, Vitti P, Rago T, et al. Surgical treatment of Graves' disease: subtotal or total thyroidectomy? *Surgery.* 1996;120:1020–1024. discussion 4–5.
222. Ching T, Warden MJ, Fefferman RA. Thyroid surgery in children and teenagers. *Arch Otolaryngol.* 1977;103:544–546.
223. Buckingham BA, Costin G, Roe TF, Weitzman JJ, Kogut MD. Hyperthyroidism in children. A reevaluation of treatment. *Am J Dis Child.* 1981;135:112–117.
224. Rudberg C, Johansson H, Akerstrom G, Tuvemo T, Karlsson FA. Graves' disease in children and adolescents. Late results of surgical treatment. *Eur J Endocrinol.* 1996;134:710–715.
225. Peters H, Fischer C, Bogner U, Reiners C, Schleusener H. Reduction in thyroid volume after radioiodine therapy of Graves' hyperthyroidism: results of a prospective, randomized, multicentre study. *Eur J Clin Invest.* 1996;26:59–63.
226. Peters H, Fischer C, Bogner U, Reiners C, Schleusener H. Treatment of Graves' hyperthyroidism with radioiodine: results of a prospective randomized study. *Thyroid.* 1997;7:247–251.
227. Erbil Y, Ozluk Y, Giris M, et al. Effect of lugol solution on thyroid gland blood flow and microvessel density in the patients with Graves' disease. *J Clin Endocrinol Metab.* 2007;92:2182–2189.
228. Sosa JA, Tuggle CT, Wang TS, et al. Clinical and economic outcomes of thyroid and parathyroid surgery in children. *J Clin Endocrinol Metab.* 2008;93:3058–3065.
229. Breuer CK, Solomon D, Donovan P, Rivkees SA, Udelsman R. Effect of patient Age on surgical outcomes for Graves' disease: a case-control study of 100 consecutive patients at a high volume thyroid surgical center. *Int J Pediatr Endocrinol.* 2013;2013:1.
230. Lal G, Ituarte P, Kebebew E, Siperstein A, Duh QY, Clark OH. Should total thyroidectomy become the preferred procedure for

- surgical management of Graves' disease? *Thyroid*. 2005;15:569–574.
231. Boger MS, Perrier ND. Advantages and disadvantages of surgical therapy and optimal extent of thyroidectomy for the treatment of hyperthyroidism. *Surg Clin North Am*. 2004;84:849–874.
 232. Witte J, Goretzki PE, Dotzenrath C, et al. Surgery for Graves' disease: total versus subtotal thyroidectomy—results of a prospective randomized trial. *World J Surg*. 2000;24:1303–1311.
 233. Sosa JA, Bowman HM, Tielsch JM, Powe NR, Gordon TA, Udelsman R. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. *Ann Surg*. 1998;228:320–330.
 234. Breuer C, Tuggle C, Solomon D, Sosa JA. Pediatric thyroid disease: when is surgery necessary, and who should be operating on our children? *J Clin Res Pediatr Endocrinol*. 2013;5(Suppl 1):79–85.
 235. Peroni E, Angiolini MR, Vigone MC, et al. Surgical management of pediatric Graves' disease: an effective definitive treatment. *Pediatr Surg Intern*. 2012;28:609–614.
 236. Chen M, Lash M, Nebesio T, Eugster E. Change in BMI after radioactive iodine ablation for graves disease. *Int J Pediatr Endocrinol*. 2017;2017:5.
 237. Reddy PA, Harinarayan CV, Sachan A, Suresh V, Rajagopal G. Bone disease in thyrotoxicosis. *Indian J Med Res*. 2012;135:277–286.
 238. Wartofsky L. Bone disease in thyrotoxicosis. *Hosp Pract (Off Ed)*. 1994;29(80):69–72, 7–78.
 239. Thornton MD. Lower-Extremity Weakness in a Teenager Due to Thyrotoxic Periodic Paralysis. *J Emerg Med*. 2017;52:e133–e137.
 240. Salih M, van Kinschot CMJ, Peeters RP, et al. Thyrotoxic periodic paralysis: an unusual presentation of hyperthyroidism. *Neth J Med*. 2017;75:315–320.
 241. Lam L, Nair RJ, Tingle L. Thyrotoxic periodic paralysis. *Proc (Bayl Univ Med Cent)*. 2006;19:126–129.
 242. Benavides VC, Rivkees SA. Myopathy associated with acute hypothyroidism following radioiodine therapy for Graves disease in an adolescent. *Int J Pediatr Endocrinol*. 2010, pii, 717303.
 243. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med*. 1990;322:1829–1836.
 244. Aslam M, Inayat M. Fetal and neonatal Graves disease: a case report and review of the literature. *South Med J*. 2008;101:840–841.
 245. Samuels SL, Namoc SM, Bauer AJ. Neonatal thyrotoxicosis. *Clin Perinatol*. 2018;45:31–40.
 246. Chester J, Rotenstein D, Ringknanont U, et al. Congenital neonatal hyperthyroidism caused by germline mutations in the TSH receptor gene. *J Pediatr Endocrinol Metabol*. 2008;21:479–486.
 247. Watkins MG, Dejkhamron P, Huo J, Vazquez DM, Menon RK. Persistent neonatal thyrotoxicosis in a neonate secondary to a rare thyroid-stimulating hormone receptor activating mutation: case report and literature review. *Endocr Pract*. 2008;14:479–483.
 248. Ferrara AM, Onigata K, Ercan O, Woodhead H, Weiss RE, Refetoff S. Homozygous thyroid hormone receptor beta-gene mutations in resistance to thyroid hormone: three new cases and review of the literature. *J Clin Endocrinol Metab*. 2012;97:1328–1336.
 249. Hebrant A, van Staveren WC, Maenhaut C, Dumont JE, Leclere J. Genetic hyperthyroidism: hyperthyroidism due to activating TSHR mutations. *Eur J Endocrinol*. 2011;164:1–9.
 250. Bhat MH, Bhadada S, Dutta P, Bhansali A, Mittal BR. Hyperthyroidism with fibrous dysplasia: an unusual presentation of McCune-Albright syndrome. *Exp Clin Endocrinol Diabetes*. 2007;115:331–333.
 251. Gelfand IM, Eugster EA, DiMeglio LA. Presentation and clinical progression of pseudohypoparathyroidism with multi-hormone resistance and Albright hereditary osteodystrophy: a case series. *J Pediatr*. 2006;149:877–880.
 252. Mastorakos G, Mitsiades NS, Doufas AG, Koutras DA. Hyperthyroidism in McCune-Albright syndrome with a review of thyroid abnormalities sixty years after the first report. *Thyroid*. 1997;7:433–439.
 253. Lafferty AR, Chrousos GP. Pituitary tumors in children and adolescents. *J Clin Endocrinol Metab*. 1999;84:4317–4323.
 254. Nygaard B, Hegedus L, Ulriksen P, Nielsen KG, Hansen JM. Radioiodine therapy for multinodular toxic goiter. *Arch Intern Med*. 1999;159:1364–1368.
 255. Nygaard B, Hegedus L, Nielsen KG, Ulriksen P, Hansen JM. Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. *Clin Endocrinol*. 1999;50:197–202.
 256. Cohen-Lehman J, Dahl P, Danzi S, Klein I. Effects of amiodarone therapy on thyroid function. *Nat Rev Endocrinol*. 2010;6:34–41.
 257. Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. *Endocr Rev*. 2001;22:240–254.
 258. Gupta A, Ly S, Castroneves LA, et al. A standardized assessment of thyroid nodules in children confirms higher cancer prevalence than in adults. *J Clin Endocrinol Metab*. 2013;98:3238–3245.
 259. Niedziela M. Pathogenesis, diagnosis and management of thyroid nodules in children. *Endocr Relat Cancer*. 2006;13:427–453.
 260. Dermody S, Walls A, Harley Jr EH. Pediatric thyroid cancer: An update from the SEER database 2007-2012. *Int J Pediatr Otorhinolaryngol*. 2016;89:121–126.
 261. Golpanian S, Perez EA, Tashiro J, Lew JJ, Sola JE, Hogan AR. Pediatric papillary thyroid carcinoma: outcomes and survival predictors in 2504 surgical patients. *Pediatr Surg Int*. 2016;32:201–208.
 262. Francis GL, Waguespack SG, Bauer AJ, et al. Management guidelines for children with thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2015;25:716–759.
 263. Hodax JK, Reinert SE, Quintos JB. Autonomously functioning thyroid nodules in patients <21 years of age: The Rhode Island Hospital experience from 2003-2013. *Endocr Pract*. 2016;22:328–337.
 264. Boi F, Maurelli I, Pinna G, et al. Calcitonin measurement in wash-out fluid from fine needle aspiration of neck masses in patients with primary and metastatic medullary thyroid carcinoma. *J Clin Endocrinol Metab*. 2007;92:2115–2118.
 265. Wells Jr SA, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. 2015;25:567–610.
 266. Tessler FN, Middleton WD, Grant EG, et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. *J Am Coll Radiol*. 2017;14:587–595.
 267. Gannon AW, Langer JE, Bellah R, et al. Diagnostic accuracy of ultrasound with color flow doppler in children with thyroid nodules. *J Clin Endocrinol Metab*. 2018;103:1958–1965.
 268. Martinez-Rios C, Daneman A, Bajno L, van der Kaay DCM, Moineddin R, Wasserman JD. Utility of adult-based ultrasound malignancy risk stratifications in pediatric thyroid nodules. *Pediatr Radiol*. 2018;48:74–84.
 269. Leboulleux S, Girard E, Rose M, et al. Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. *J Clin Endocrinol Metab*. 2007;92:3590–3594.
 270. Gannon AW, Langer JE, Bellah R, et al. Diagnostic accuracy of ultrasound with color flow doppler in children with thyroid nodules. *J Clin Endocrinol Metab*. 2018;103(5):1958–1965.
 271. Stevens C, Lee JK, Sadatsafavi M, Blair GK. Pediatric thyroid fine-needle aspiration cytology: a meta-analysis. *J Pediatr Surg*. 2009;44:2184–2191.
 272. Lale SA, Morgenstern NN, Chiara S, Wasserman P. Fine needle aspiration of thyroid nodules in the pediatric population: a 12-year cyto-histological correlation experience at North Shore-Long Island Jewish Health System. *Diagn Cytopathol*. 2015;43:598–604.
 273. Monaco SE, Pantanowitz L, Khalbuss WE, et al. Cytomorphological and molecular genetic findings in pediatric thyroid fine-needle aspiration. *Cancer Cytopathol*. 2012;120:342–350.
 274. Bauer AJ. Thyroid nodules in children and adolescents. *Curr Opin Endocrinol Diabetes Obes*. 2019;26(5):266–274. <https://doi.org/10.1097/MED.0000000000000495>. PMID: 31361657.
 275. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2016;26:1–133.
 276. Middleton WD, Teefey SA, Reading CC, et al. Multiinstitutional analysis of thyroid nodule risk stratification using the American College of Radiology Thyroid imaging reporting and data system. *AJR Am J Roentgenol*. 2017;208:1331–1341.

277. Luster M, Lassmann M, Freudenberg LS, Reiners C. Thyroid cancer in childhood: management strategy, including dosimetry and long-term results. *Hormones (Athens)*. 2007;6:269–278.
278. Zaydfudim V, Feurer ID, Griffin MR, Phay JE. The impact of lymph node involvement on survival in patients with papillary and follicular thyroid carcinoma. *Surgery*. 2008;144:1070–1077. discussion 7–8.
279. Al-Qurayshi Z, Hauch A, Srivastav S, Aslam R, Friedlander P, Kandil EA. National perspective of the risk, presentation, and outcomes of pediatric thyroid cancer. *JAMA Otolaryngol-Head Neck Surg*. 2016;142:472–478.
280. Lee YS, Lim YS, Lee JC, et al. Clinical implications of bilateral lateral cervical lymph node metastasis in papillary thyroid cancer: a risk factor for lung metastasis. *Ann Surg Oncol*. 2011;18:3486–3492.
281. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in thyroid cancer incidence and mortality in the United States, 1974–2013. *JAMA*. 2017;317:1338–1348.
282. Schlumberger M, De Vathaire F, Travagli JP, et al. Differentiated thyroid carcinoma in childhood: long term follow-up of 72 patients. *J Clin Endocrinol Metab*. 1987;65:1088–1094.
283. Hung W, Sarlis NJ. Current controversies in the management of pediatric patients with well-differentiated nonmedullary thyroid cancer: a review. *Thyroid*. 2002;12:683–702.
284. Lazar L, Lebenthal Y, Steinmetz A, Yackobovitch-Gavan M, Phillip M. Differentiated thyroid carcinoma in pediatric patients: comparison of presentation and course between pre-pubertal children and adolescents. *J Pediatr*. 2009;154:708–714.
285. Machens A, Lorenz K, Nguyen Thanh P, Brauckhoff M, Dralle H. Papillary thyroid cancer in children and adolescents does not differ in growth pattern and metastatic behavior. *J Pediatr*. 2010;157:648–652.
286. Powers PA, Dinauer CA, Tuttle RM, Robie DK, McClellan DR, Francis GL. Tumor size and extent of disease at diagnosis predict the response to initial therapy for papillary thyroid carcinoma in children and adolescents. *J Pediatr Endocrinol Metabol*. 2003;16:693–702.
287. O’Gorman CS, Hamilton J, Rachmiel M, Gupta A, Ngan BY, Daneman D. Thyroid cancer in childhood: a retrospective review of childhood course. *Thyroid*. 2010;20:375–380.
288. Rachmiel M, Charron M, Gupta A, et al. Evidence-based review of treatment and follow up of pediatric patients with differentiated thyroid carcinoma. *J Pediatr Endocrinol Metabol*. 2006;19:1377–1393.
289. Gafanovich A, Ramu N, Krichevsky S, Pe’er J, Amir G, Ben-Yehuda D. Microsatellite instability and p53 mutations in pediatric secondary malignant neoplasms. *Cancer*. 1999;85:504–510.
290. Hay ID, Johnson TR, Kaggal S, et al. Papillary thyroid carcinoma (PTC) in children and adults: comparison of initial presentation and long-term postoperative outcome in 4432 patients consecutively treated at the Mayo clinic during eight decades (1936–2015). *World J Surg*. 2018;42:329–342.
291. Brink JS, van Heerden JA, McIver B, et al. Papillary thyroid cancer with pulmonary metastases in children: long-term prognosis. *Surgery*. 2000;128:881–886. discussion 6–7.
292. Bal CS, Garg A, Chopra S, Ballal S, Soundararajan R. Prognostic factors in pediatric differentiated thyroid cancer patients with pulmonary metastases. *J Pediatr Endocrinol Metabol*. 2014;28(7–8):745–751.
293. Pawelczak M, David R, Franklin B, Kessler M, Lam L, Shah B. Outcomes of children and adolescents with well-differentiated thyroid carcinoma and pulmonary metastases following (1)(3)(1)I treatment: a systematic review. *Thyroid*. 2010;20:1095–1101.
294. Duffy PJF. Cancer of the thyroid in children: a report of twenty-eight cases. *J Clin Endocrinol Metab*. 1950;10:1296–1308.
295. Winship T, Rosvold RV. A study of thyroid cancer in children. *Am J Surg*. 1961;102:747–752.
296. Bauer AJ, Davies L. Why the data from the Fukushima health management survey after the Daiichi nuclear power station accident are important. *JAMA Otolaryngol Head Neck Surgery*. 2019;145(1):11–13.
297. van Santen HM, Tytgat GA, van de Wetering MD, et al. Differentiated thyroid carcinoma after (131)I-MIBG treatment for neuroblastoma during childhood: description of the first two cases. *Thyroid*. 2012;22:643–646.
298. Astakhova IN, Anspaugh LR, Beebe GW, et al. Chernobyl-related thyroid cancer in children of Belarus: a case-control study. *Radiat Res*. 1998;150:349–356.
299. Tronko MD, Bogdanova TI, Komissarenko IV, et al. Thyroid carcinoma in children and adolescents in Ukraine after the Chernobyl nuclear accident: statistical data and clinicomorphologic characteristics. *Cancer*. 1999;86:149–156.
300. Clement SC, Kremer LCM, Verburg FA, et al. Balancing the benefits and harms of thyroid cancer surveillance in survivors of Childhood, adolescent and young adult cancer: Recommendations from the international Late Effects of Childhood Cancer Guideline Harmonization Group in collaboration with the PanCare-SurFup Consortium. *Cancer Treat Rev*. 2018;63:28–39.
301. Togawa K, Ahn HS, Auvinen A, et al. Long-term strategies for thyroid health monitoring after nuclear accidents: recommendations from an Expert Group convened by IARC. *Lancet Oncol*. 2018;19:1280–1283.
302. Charke ND. On the prevalence of familial nonmedullary thyroid cancer in multiply affected kindreds. *Thyroid*. 2006;16:181–186.
303. Bauer AJ. Clinical behavior and genetics of nonsyndromic. *familial nonmedullary thyroid cancer Front Horm Res*. 2013;41:141–148.
304. Klubo-Gwiedzinska J, Yang L, Merkel R, et al. Results of screening in familial non-medullary thyroid cancer. *Thyroid*. 2017;27:1017–1024.
305. Capezzone M, Marchisotta S, Cantara S, et al. Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity. *Endocr Relat Cancer*. 2008;15:1075–1081.
306. Park YJ, Ahn HY, Choi HS, Kim KW, Park do J, Cho BY. The long-term outcomes of the second generation of familial nonmedullary thyroid carcinoma are more aggressive than sporadic cases. *Thyroid*. 2012;22:356–362.
307. Rosario PW, Mineiro AF, Lacerda RX, Prates BS, Silva LC, Calsolari MR. Ultrasonographic screening for thyroid cancer in siblings of patients with apparently sporadic papillary carcinoma. *Thyroid*. 2012;22(8):805–808.
308. Ngeow J, Eng C. PTEN hamartoma tumor syndrome: clinical risk assessment and management protocol. *Methods*. 2015;77–78:11–19.
309. Smith JR, Marqusee E, Webb S, Nose V, Fishman SJ, Shamberger RC, Frates MC, Huang SA. Thyroid nodules and cancer in children with PTEN hamartoma tumor syndrome. *Clin Endocrinol Metab*. 2011;96(1):34–37.
310. Septer S, Slowik V, Morgan R, Dai H, Attard T. Thyroid cancer complicating familial adenomatous polyposis: mutation spectrum of at-risk individuals. *Hereditary Cancer Clin Pract*. 2013;11:13.
311. Schultz KAP, Williams GM, Kamihara J, et al. DICER1 and associated conditions: identification of at-risk individuals and recommended surveillance strategies. *Clin Cancer Res*. 2018;24:2251–2261.
312. Bauer AJ, Stratakis CA. The lentiginoses: cutaneous markers of systemic disease and a window to new aspects of tumorigenesis. *J Med Genet*. 2005;42:801–810.
313. Carney JA, Lyssikatos C, Seethala RR, et al. The spectrum of thyroid gland pathology in carney complex: the importance of follicular carcinoma. *Am J Surg Pathol*. 2018;42:587–594.
314. Stratakis CA. Carney complex: A familial lentiginosis predisposing to a variety of tumors. *Rev Endocr Metab Disord*. 2016;17:367–371.
315. American Institute of Ultrasound in M, American College of R, Society for Pediatric R, Society of Radiologists in U. AIUM practice guideline for the performance of a thyroid and parathyroid ultrasound examination. *J Ultrasound Med*, 32, 1319–1329.
316. Goyal N, Pakdaman M, Kamani D, Caragacianu D, Goldenberg D, Randolph GW. Mapping the distribution of nodal metastases in papillary thyroid carcinoma: Where exactly are the nodes? *Laryngoscope*. 2017;127:1959–1964.
317. Yeh MW, Bauer AJ, Bernet VA, et al. American Thyroid Association statement on preoperative imaging for thyroid cancer surgery. *Thyroid*. 2015;25:3–14.
318. Sohn SY, Choi JH, Kim NK, et al. The impact of iodinated contrast agent administered during preoperative computed tomography scan on body iodine pool in patients with differentiated thyroid

- cancer preparing for radioactive iodine treatment. *Thyroid*. 2014;24:872–877.
319. Tuggle CT, Roman SA, Wang TS, et al. Pediatric endocrine surgery: who is operating on our children? *Surgery*. 2008;144:869–877. discussion 77.
 320. Adam MA, Thomas S, Youngwirth L, et al. Is there a minimum number of thyroidectomies a surgeon should perform to optimize patient outcomes? *Ann Surg*. 2017;265:402–407.
 321. Lazar L, Lebenthal Y, Segal K, et al. Pediatric thyroid cancer: post-operative classifications and response to initial therapy as prognostic factors. *J Clin Endocrinol Metab*. 2016;101:1970–1979.
 322. Pires B, Alves-Junior PA, Bordallo MA, et al. Prognostic factors for early and long-term remission in pediatric differentiated thyroid cancer: the role of gender, age, clinical presentation and the newly proposed American Thyroid Association risk stratification system. *Thyroid*. 2016;26(10):1480–1487.
 323. Lee YA, Jung HW, Kim HY, et al. Pediatric patients with multifocal papillary thyroid cancer have higher recurrence rates than adult patients: a retrospective analysis of a large pediatric thyroid cancer cohort over 33 years. *J Clin Endocrinol Metab*. 2015;100:1619–1629.
 324. Hay ID, Gonzalez-Losada T, Reinalda MS, Honetschlager JA, Richards ML, Thompson GB. Long-term outcome in 215 children and adolescents with papillary thyroid cancer treated during 1940 through 2008. *World J Surg*. 2010;34:1192–1202.
 325. Handkiewicz-Junak D, Wloch J, Roskosz J, et al. Total thyroidectomy and adjuvant radioiodine treatment independently decrease locoregional recurrence risk in childhood and adolescent differentiated thyroid cancer. *J Nucl Med*. 2007;48:879–888.
 326. Bargren AE, Meyer-Rochow GY, Delbridge LW, Sidhu SB, Chen H. Outcomes of surgically managed pediatric thyroid cancer. *J Surg Res*. 2009;156:70–73.
 327. Hahn SY, Shin JH, Oh YL, Kim TH, Lim Y, Choi JS. Role of ultrasound in predicting tumor invasiveness in follicular variant of papillary thyroid carcinoma. *Thyroid*. 2017;27:1177–1184.
 328. Kluijfhout WP, Pasternak JD, van der Kaay D, Vriens MR, Propst EJ, Wasserman JD. Is it time to reconsider lobectomy in low-risk paediatric thyroid cancer? *Clin Endocrinol*. 2017;86:591–596.
 329. Samuels SL, Surrey LF, Hawkes CP, et al. Characteristics of follicular variant papillary thyroid carcinoma in a pediatric cohort. *J Clin Endocrinol Metab*. 2018;103(4):1639–1648.
 330. Jeon MJ, Kim YN, Sung TY, et al. Practical initial risk stratification based on lymph node metastases in pediatric and adolescent differentiated thyroid cancer. *Thyroid*. 2018;28:193–200.
 331. Kim J, Sun Z, Adam MA, et al. Predictors of nodal metastasis in pediatric differentiated thyroid cancer. *J Pediatr Surg*. 2017;52:120–123.
 332. Spinelli C, Tognetti F, Strambi S, Morganti R, Massimino M, Collini P. Cervical lymph node metastases of papillary thyroid carcinoma, in the central and lateral compartments, in children and adolescents: predictive factors. *World J Surg*. 2018;42:2444–2453.
 333. Thompson GB, Hay ID. Current strategies for surgical management and adjuvant treatment of childhood papillary thyroid carcinoma. *World J Surg*. 2004;28:1187–1198.
 334. Bilimoria KY, Bentrem DJ, Ko CY, et al. Extent of surgery affects survival for papillary thyroid cancer. *Ann Surg*. 2007;246:375–381. discussion 81–84.
 335. Youngwirth LM, Adam MA, Thomas SM, Roman SA, Sosa JA, Scheri RP. Pediatric thyroid cancer patients referred to high-volume facilities have improved short-term outcomes. *Surgery*. 2018;163:361–366.
 336. Patel NA, Bly RA, Adams S, et al. A clinical pathway for the postoperative management of hypocalcemia after pediatric thyroidectomy reduces blood draws. *Int J Pediatr Otorhinolaryngol*. 2018;105:132–137.
 337. Freire AV, Ropelato MG, Ballerini MG, et al. Predicting hypocalcemia after thyroidectomy in children. *Surgery*. 2014;156:130–136.
 338. Grodski S, Serpell J. Evidence for the role of perioperative PTH measurement after total thyroidectomy as a predictor of hypocalcemia. *World J Surg*. 2008;32:1367–1373.
 339. Sam AH, Dhillon WS, Donaldson M, et al. Serum phosphate predicts temporary hypocalcaemia following thyroidectomy. *Clin Endocrinol*. 2011;74:388–393.
 340. Demidchik YE, Demidchik EP, Reiners C, et al. Comprehensive clinical assessment of 740 cases of surgically treated thyroid cancer in children of Belarus. *Ann Surg*. 2006;243:525–532.
 341. Randolph GW, Kamani D. Intraoperative electrophysiologic monitoring of the recurrent laryngeal nerve during thyroid and parathyroid surgery: Experience with 1,381 nerves at risk. *Laryngoscope*. 2017;127:280–286.
 342. Irkocurcu O. Approach to the assessment of vocal cord movement by ultrasonography. *World J Surg*. 2018;42:1214.
 343. Ongkasuwan J, Ocampo E, Tran B. Laryngeal ultrasound and vocal fold movement in the pediatric cardiovascular intensive care unit. *Laryngoscope*. 2017;127:167–172.
 344. Schneider R, Randolph GW, Dionigi G, et al. International neural monitoring study group guideline 2018 part I: staging bilateral thyroid surgery with monitoring loss of signal. *Laryngoscope*. 2018;128(Suppl 3):S1–S17.
 345. Seidlin SM, Oshry E, Yalow AA. Spontaneous and experimentally induced uptake of radioactive iodine in metastases from thyroid carcinoma; a preliminary report. *J Clin Endocrinol Metab*. 1948;8:423–432.
 346. Coliez R. Results of examination of 85 cases of cancer of the thyroid with radioactive iodine. *J Radiol Electrol Arch Electr Med*. 1954;32:881–895.
 347. Kulkarni K, Van Nostrand D, Atkins F, Mete M, Wexler J, Wartofsky L. Does lemon juice increase radioiodine reaccumulation within the parotid glands more than if lemon juice is not administered? *Nucl Med Commun*. 2014;35:210–216.
 348. Van Nostrand D, Bandaru V, Chennupati S, et al. Radiopharmacokinetics of radioiodine in the parotid glands after the administration of lemon juice. *Thyroid*. 2010;20:1113–1119.
 349. Marti JL, Jain KS, Morris LG. Increased risk of second primary malignancy in pediatric and young adult patients treated with radioactive iodine for differentiated thyroid cancer. *Thyroid*. 2015;25:681–687.
 350. Chow SM, Law SC, Mendenhall WM, et al. Differentiated thyroid carcinoma in childhood and adolescence-clinical course and role of radioiodine. *Pediatr Blood Cancer*. 2004;42:176–183.
 351. Grigsby PW, Gal-or A, Michalski JM, Doherty GM. Childhood and adolescent thyroid carcinoma. *Cancer*. 2002;95:724–729.
 352. Jarzab B, Handkiewicz-Junak D, Wloch J, et al. Multivariate analysis of prognostic factors for differentiated thyroid carcinoma in children. *Eur J Nucl Med*. 2000;27:833–841.
 353. Reiners C, Biko J, Haenscheid H, et al. Twenty-five years after Chernobyl: outcome of radioiodine treatment in children and adolescents with very high-risk radiation-induced differentiated thyroid carcinoma. *J Clin Endocrinol Metab*. 2013;98:3039–3048.
 354. Chapter 8. Thyroid. In: Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, eds. *AJCC Cancer Staging Manual, 7th Ed*. 7th ed New York, NY: Springer; 2010:87–96.
 355. Handkiewicz-Junak D, Gawlik T, Rozkosz J, et al. Recombinant human thyrotropin preparation for adjuvant radioiodine treatment in children and adolescents with differentiated thyroid cancer. *Eur J Endocrinol*. 2015;173:873–881.
 356. Luster M, Handkiewicz-Junak D, Grossi A, et al. Recombinant thyrotropin use in children and adolescents with differentiated thyroid cancer: a multicenter retrospective study. *J Clin Endocrinol Metab*. 2009;94:3948–3953.
 357. Schoelwer MJ, Zimmerman D, Shore RM, Josefson JL. The use of 123I in diagnostic radioactive iodine scans in children with differentiated thyroid carcinoma. *Thyroid*. 2015;25:935–941.
 358. Avram AM, Fig LM, Frey KA, Gross MD, Wong KK. Preablation 131-I scans with SPECT/CT in postoperative thyroid cancer patients: what is the impact on staging? *J Clin Endocrinol Metab*. 2013;98:1163–1171.
 359. Lee JI, Chung YJ, Cho BY, Chong S, Seok JW, Park SJ. Postoperative-stimulated serum thyroglobulin measured at the time of 131I ablation is useful for the prediction of disease status in patients with differentiated thyroid carcinoma. *Surgery*. 2013;153:828–835.
 360. Verburg FA, Biko J, Diessl S, et al. I-131 Activities as High as Safely Administrable (AHASA) for the treatment of children and adolescents with advanced differentiated thyroid cancer. *J Clin Endocrinol Metab*. 2011;96. E1268–1271.

361. Verburg FA, Reiners C, Hanscheid H. Approach to the patient: role of dosimetric RAI Rx in children with DTC. *J Clin Endocrinol Metab.* 2013;98:3912–3919.
362. Urhan M, Dadparvar S, Mavi A, et al. Iodine-123 as a diagnostic imaging agent in differentiated thyroid carcinoma: a comparison with iodine-131 post-treatment scanning and serum thyroglobulin measurement. *Eur J Nucl Med Mol Imaging.* 2007;34:1012–1017.
363. Cooper DS, Specker B, Ho M, et al. Thyrotropin suppression and disease progression in patients with differentiated thyroid cancer: results from the National Thyroid Cancer Treatment Cooperative Registry. *Thyroid.* 1998;8:737–744.
364. Burmeister LA, Goumaz MO, Mariash CN, Oppenheimer JH. Levothyroxine dose requirements for thyrotropin suppression in the treatment of differentiated thyroid cancer. *J Clin Endocrinol Metab.* 1992;75:344–350.
365. Batrinos ML. The problem of exogenous subclinical hyperthyroidism. *Hormones (Athens).* 2006;5:119–125.
366. Osman F, Gammage MD, Franklyn JA. Hyperthyroidism and cardiovascular morbidity and mortality. *Thyroid.* 2002;12:483–487.
367. Rivkees SA. Pediatric Graves' disease: controversies in management. *Horm Res Paediatr.* 2010;74(5):305–311.
368. Spencer C, Fatemi S. Thyroglobulin antibody (TgAb) methods - Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. *Best Pract Res Clin Endocrinol Metab.* 2013;27:701–712.
369. Verburg FA, Luster M, Cupini C, et al. Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. *Thyroid.* 2013;23:1211–1225.
370. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies. *Curr Opin Endocrinol Diabetes Obes.* 2014;21:394–404.
371. Bannas P, Derlin T, Groth M, et al. Can (18)F-FDG-PET/CT be generally recommended in patients with differentiated thyroid carcinoma and elevated thyroglobulin levels but negative I-131 whole body scan? *Ann Nucl Med.* 2012;26:77–85.
372. Jarzab B, Handkiewicz-Junak D. Differentiated thyroid cancer in children and adults: same or distinct disease? *Hormones (Athens).* 2007;6:200–209.
373. Hay ID, Lee RA, Davidge-Pitts C, Reading CC, Charboneau JW. Long-term outcome of ultrasound-guided percutaneous ethanol ablation of selected "recurrent" neck nodal metastases in 25 patients with TNM stages III or IVA papillary thyroid carcinoma previously treated by surgery and 131I therapy. *Surgery.* 2013;154:1448–1454. discussion 54–55.
374. Biko J, Reiners C, Kreissl MC, Verburg FA, Demidchik Y, Drozd V. Favourable course of disease after incomplete remission on (131)I therapy in children with pulmonary metastases of papillary thyroid carcinoma: 10 years follow-up. *Eur J Nucl Med Mol Imaging.* 2011;38:651–655.
375. Padovani RP, Robenshtok E, Brokhin M, Tuttle RM. Even without additional therapy, serum thyroglobulin concentrations often decline for years after total thyroidectomy and radioactive remnant ablation in patients with differentiated thyroid cancer. *Thyroid.* 2012;22:778–783.
376. Covell LL, Ganti AK. Treatment of advanced thyroid cancer: role of molecularly targeted therapies. *Target Oncol.* 2015;10:311–324.
377. Rao SN, Cabanillas ME. Navigating systemic therapy in advanced thyroid carcinoma: from standard of care to personalized therapy and beyond. *J Endocr Soc.* 2018;2:1109–1130.
378. Jaber T, Waguespack SG, Cabanillas ME, et al. Targeted therapy in advanced thyroid cancer to resensitize tumors to radioactive iodine. *J Clin Endocrinol Metab.* 2018;103:3698–3705.
379. Tuttle RM, Brose MS, Grande E, Kim SW, Tahara M, Sabra MM. Novel concepts for initiating multitargeted kinase inhibitors in radioactive iodine refractory differentiated thyroid cancer. *Best Pract Res Clin Endocrinol Metab.* 2017;31:295–305.
380. Waguespack SG, Rich TA, Perrier ND, Jimenez C, Cote GJ. Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol.* 2011;7:596–607.
381. Margraf RL, Crockett DK, Krautscheid PM, et al. Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. *Hum Mutat.* 2009;30:548–556.
382. Ji JH, Oh YL, Hong M, et al. Identification of driving ALK fusion genes and genomic landscape of medullary thyroid cancer. *PLoS Genet.* 2015;11. e1005467.
383. Frank-Raue K, Raue F. Hereditary medullary thyroid cancer genotype-phenotype correlation. *Recent Result Cancer Res.* 2015;204:139–156.
384. Brauckhoff M, Machens A, Hess S, et al. Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: An exploratory analysis. *Surgery.* 2008;144:1044–1050. discussion 50–53.
385. Wray CJ, Rich TA, Waguespack SG, Lee JE, Perrier ND, Evans DB. Failure to recognize multiple endocrine neoplasia 2B: more common than we think? *Ann Surg Oncol.* 2008;15:293–301.
386. Brauckhoff M, Machens A, Lorenz K, Bjoro T, Varhaug JE, Dralle H. Surgical curability of medullary thyroid cancer in multiple endocrine neoplasia 2B: a changing perspective. *Ann Surg.* 2014;259:800–806.
387. Machens A, Schneyer U, Holzhausen HJ, Dralle H. Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. *J Clin Endocrinol Metab.* 2005;90:2029–2034.
388. Meijer JA, Bakker LE, Valk GD, et al. Radioactive iodine in the treatment of medullary thyroid carcinoma: a controlled multicenter study. *Eur J Endocrinol.* 2013;168:779–786.
389. Tran K, Khan S, Taghizadehasl M, et al. Gallium-68 Dotatate PET/CT is superior to other imaging modalities in the detection of medullary carcinoma of the thyroid in the presence of high serum calcitonin. *Hell J Nucl Med.* 2015;18:19–24.
390. Laure Giraudet A, Al Ghulzan A, Auperin A, et al. Progression of medullary thyroid carcinoma: assessment with calcitonin and carcinoembryonic antigen doubling times. *Eur J Endocrinol.* 2008;158:239–246.
391. Weitzman SP, Cabanillas ME. The treatment landscape in thyroid cancer: a focus on cabozantinib. *Cancer Manag Res.* 2015;7:265–278.
392. Brose MS, Bible KC, Chow LQM, et al. Management of treatment-related toxicities in advanced medullary thyroid cancer. *Cancer Treat Rev.* 2018;66:64–73.

14 The Adrenal Cortex and Its Disorders

Walter L. Miller, Christa E. Flück, David T. Breault, Brian J. Feldman

CHAPTER OUTLINE

HISTORY, EMBRYOLOGY, AND ANATOMY 425

History 425
Embryology 426
Anatomy 427

STEROID HORMONE SYNTHESIS 428

Early Steps: Cholesterol Uptake, Storage, and Transport 428
Steroidogenic Enzymes 428
Fetal Adrenal Steroidogenesis 433

REGULATION OF STEROIDOGENESIS 434

The Hypothalamic-Pituitary-Adrenal Axis 434
Mineralocorticoid Secretion: The Renin-Angiotensin System 436
Adrenal Androgen Secretion and the Regulation of Adrenarche 437

PLASMA STEROIDS AND THEIR DISPOSAL 437

Structure and Nomenclature 437
Circulating Steroids 438
Steroid Catabolism 439

CLINICAL AND LABORATORY EVALUATION OF

ADRENAL FUNCTION 440

Clinical Evaluation 440
Laboratory Evaluation 440

GENETIC LESIONS IN STEROIDOGENESIS 446

Disorders in Cholesterol Synthesis and Trafficking 446
Congenital Lipoid Adrenal Hyperplasia 448
Disorders Resembling Lipoid Congenital Adrenal Hyperplasia:
P450scc Deficiency and Steroidogenic Factor 1
Deficiency 449

3 β -Hydroxysteroid Dehydrogenase Deficiency 449
17 α -Hydroxylase/17,20-Lyase Deficiency 450
17,20 Lyase Deficiency: P450c17, Cytochrome b5 and Other
Factors 450

P450 Oxidoreductase Deficiency 451
21-Hydroxylase Deficiency 451
Lesions in Isozymes of P450c11 459
Lesions in Isozymes of 11 β -Hydroxysteroid
Dehydrogenase 460
PAPSS2 Deficiency 460

ADRENAL INSUFFICIENCY 460

Acute Primary Adrenal Insufficiency 461
Chronic Primary Adrenal Insufficiency 461
Secondary Adrenal Insufficiency 466

ADRENAL EXCESS 467

Cushing Syndrome 467
Primary Hyperaldosteronism 471
Virilizing and Feminizing Adrenal Tumors 472
Other Disorders 472

GLUCOCORTICOID THERAPY AND WITHDRAWAL 473

Mechanisms of Glucocorticoid Action 474
Replacement Therapy 474
Pharmacologic Therapy 475
Commonly Used Glucocorticoid Preparations 475
Withdrawal of Glucocorticoid Therapy 476
Stress Doses of Glucocorticoids 477
Mineralocorticoid Replacement 477

CONCLUDING REMARKS 477

References 477

HISTORY, EMBRYOLOGY, AND ANATOMY

The adrenal cortex produces three principal categories of steroid hormones that regulate a wide variety of physiologic processes from fetal to adult life. Mineralocorticoids, principally aldosterone, regulate renal retention of sodium and thus profoundly influence electrolyte balance, intravascular volume, and blood pressure. Glucocorticoids, principally cortisol, are named for their carbohydrate-mobilizing activity, but they are ubiquitous physiologic regulators, influencing a wide variety of bodily functions. Adrenal androgens serve no known physiologic role but do mediate some secondary sexual characteristics in women (e.g., pubic and axillary hair), and their overproduction may result in virilism. Thus the adrenal cortex is of considerable interest because of the widespread effects of its secretions and because derivatives of these secreted steroids are widely used as pharmacologic agents. Disorders of the adrenal cortex, once thought to be rare, are being recognized with increasing

frequency. The severe congenital adrenal hyperplasias (CAHs) affect nearly 1 in 10,000 persons, and the very mild forms may affect as many as 1 in 100 in some populations. Cushing disease, once regarded as a true rarity in pediatrics, may affect as many children as adults. Primary aldosteronism is a common cause of hypertension, especially in adults.

History

The history of adrenal research has been reviewed recently.¹ The adrenal glands apparently were first described in 1563 by the Italian anatomist Bartolomeo Eustaccio, better known for his description of the eustacian tube of the ear. Medical interest in the adrenals as something other than an anatomic curiosity began in the mid-19th century with Addison's classical description of adrenal insufficiency and Brown-Sequard's experimental creation of similar disorders in animals subjected to adrenalectomy. The signs and symptoms of glucocorticoid

excess caused by adrenal tumors were well known by 1932, when Cushing described the pituitary tumors that cause what is now known as *Cushing disease*. Effects of adrenalectomy on salt and water metabolism were reported in 1927, Loeb showed that saline administration extended the life of Addisonian patients in 1933, and by the late 1930s, Selye had proposed the terms *glucocorticoid* and *mineralocorticoid* to distinguish the two broad categories of actions of adrenal extracts.

Numerous adrenal steroids were painstakingly isolated and their structures determined during the 1930s in the laboratories of Reichstein and Kendall. Many of these steroids were synthesized chemically, providing pure material for experimental purposes. In 1949 Kendall and Hench reported that glucocorticoids ameliorated the symptoms of rheumatoid arthritis, greatly stimulating interest in synthesizing new pharmacologically active analogues of naturally occurring steroids. Kendall, Reichstein, and Hench shared the 1950 Nobel Prize in Medicine. The structures of the various adrenal steroids suggested precursor/product relationships, leading in 1950 to the first treatment of CAH with cortisone by both Wilkins and Bartter. This opened a vigorous era of clinical investigation of the pathways of steroidogenesis in a variety of inherited adrenal and gonadal disorders. The association of cytochrome P450 with 21-hydroxylation was made in 1965, and some of the steroidogenic enzymes were then isolated in the 1970s, but it was not until the genes for most of these enzymes were cloned in the 1980s that it became clear which proteins participated in which steroidal transformations.² The identification of these genes (Table 14.1) then led to an understanding of the genetic lesions causing heritable disorders of steroidogenesis. At the same time, studies of steroid hormone action led to the discovery of steroid hormone receptors in the 1960s, but it was not until they were cloned in the 1980s that their biology has begun to be understood.³

Embryology

The cells of the adrenal cortex are of mesodermal origin, in contrast to cells of the adrenal medulla, which are derived from the neuroectoderm. In human embryos, adrenogonadal progenitor cells first appear at around the fourth week of gestation as a thickening of the coelomic epithelium (or intermediate mesoderm) between the urogenital ridge and dorsal mesentery⁴ (Fig. 14.1). These progenitor cells give rise to the steroidogenic cells of the gonads and to the adrenal cortex. The adrenal and gonadal cells then separate, with the adrenal cells migrating retroperitoneally to the cranial pole of the mesonephros and the gonadal cells migrating caudally. Between the seventh and eighth week of development, the adrenal primordium is invaded by sympathetic cells derived from the neural crest that give rise to the adrenal medulla. By the end of the eighth week, the rudimentary adrenal has become encapsulated and is clearly associated with the upper pole of the kidney, which at this time is much smaller than the adrenal.⁵

The fetal adrenal cortex consists of an outer “definitive” zone, the principal site of glucocorticoid and mineralocorticoid synthesis, and a much larger “fetal” zone that makes androgenic precursors (dehydroepiandrosterone [DHEA], dehydroepiandrosterone sulfate [DHEAS]) that the placenta converts to estriol. A putative “transitional” zone exists between these regions toward the end of fetal development, but its role is unclear. The fetal adrenal glands are huge in proportion to other structures, and continue to grow well into the third trimester (Fig. 14.2). At birth, the adrenals weigh 8 to 9 g, about the same size of adult adrenals, and represent approximately 0.4% of total body weight. Following birth, cells of the fetal zone undergo apoptosis, and the fetal adrenal zone rapidly involutes and has virtually disappeared by 6 to 12 months of

TABLE 14.1 Physical Characteristics of Human Genes Encoding Steroidogenic Enzymes

Enzyme	Gene	Gene Size (kb)	Chromosomal Location	Exons (n)	mRNA Size (kb)
StAR	<i>STAR</i>	8	8p11.2	8	1.6
P450 _{scc}	<i>CYP11A1</i>	30	15q23–q24	9	2
P450 _{c11β}	<i>CYP11B1</i>	9.5	8q21–22	9	4.2
P450 _{c11AS}	<i>CYP11B2</i>	9.5	8q21–22	9	4.2
P450 _{c17}	<i>CYP17A1</i>	6.6	10q24.3	8	1.9
P450 _{c21}	<i>CYP21A2</i>	3.4	6p 21.1	10	2
P450 _{aro}	<i>CYP19A1</i>	130	15q21.1	10	1.5–4.5
3βHSD1	<i>HSD3B1</i>	8	1p13.1	4	1.7
3βHSD2	<i>HSD3B2</i>	8	1p13.1	4	1.7
11βHSD1	<i>HSD11B1</i>	7	1q32–q41	6	1.6
11βHSD2	<i>HSD11B2</i>	6.2	16q22	5	1.6
17βHSD1	<i>HSD17B1</i>	3.3	17q11–q21	6	1.4, 2.4
17βHSD2	<i>HSD17B2</i>	63	16q24.1–q24.2	5	1.5
17βHSD3	<i>HSD17B3</i>	67	9q22	11	1.2
17βHSD6 (RODH)	<i>HSD17B6</i>	24.5	12q13	5	1.6
AKR1C1	<i>AKR1C1</i>	14.3	10p14–p15	9	1.2
AKR1C2	<i>AKR1C2</i>	13.8	10p14–p15	9	1.3
AKR1C3	<i>AKR1C3</i>	13	10p14–p15	9	1.2
AKR1C4	<i>AKR1C4</i>	22.1	10p14–p15	9	1.2
5α-Reductase 1	<i>SRD5A1</i>	36	5p15	5	2.4
5α-Reductase 2	<i>SRD5A2</i>	56	2p23	5	2.4
SULT2A1	<i>SULT2A1</i>	17	19q13.3	6	2
PAPSS2	<i>PAPSS2</i>	85	10q24	13	3.9
P450 Oxidoreductase	<i>POR</i>	69	7q11.2	16	2.5
Ferredoxin	<i>FDX1</i>	35	11q22	5	1.0, 1.4, 1.7, 3.2
Ferredoxin Reductase	<i>FDXR</i>	11	17q24–q25	12	2
Cytochrome <i>b</i> ₅	<i>CYB5A</i>	32	18q23	5	0.9
H6PDH	<i>H6PD</i>	36.5	1p36	5	9

mRNA, Messenger ribonucleic acid.

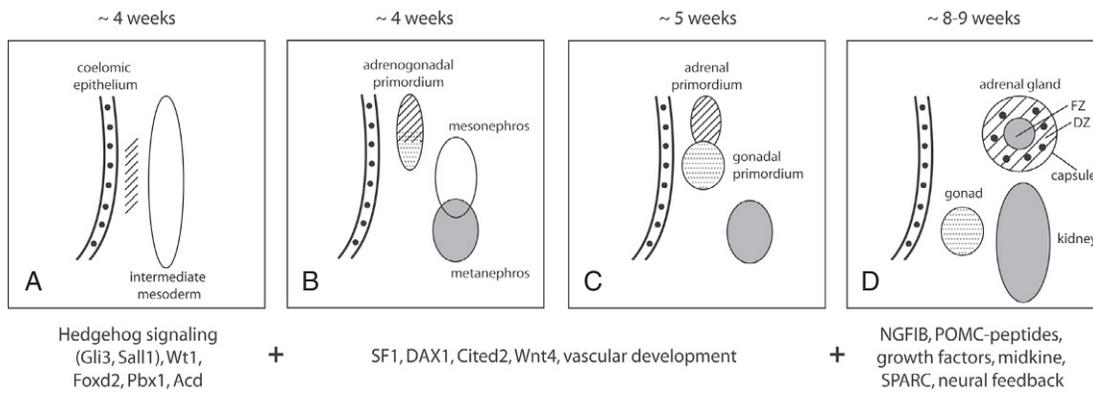


Fig. 14.1 Overview of human adrenal development. **A–C**, The adrenogonadal primordium develops at around 4 weeks, gestation, after which the adrenal primordium becomes a distinct structure that then migrates retroperitoneally to the cranial pole of the mesonephros. **D**, By 8 to 9 weeks' gestation, the adrenal gland is encapsulated, contains chromaffin cells (black) and has distinct fetal (FZ) and definitive zones (DZ). Some of the signaling molecules, transcription factors, and growth factors implicated in adrenal development are shown later, although the exact timing and interaction of many of these factors remains poorly understood at present. (Modified from Else, T., & Hammer, G. D. (2005). Genetic analysis of adrenal absence: agenesis and aplasia. *Trends Endocrinol Metab*, 16, 458–468. With permission.)

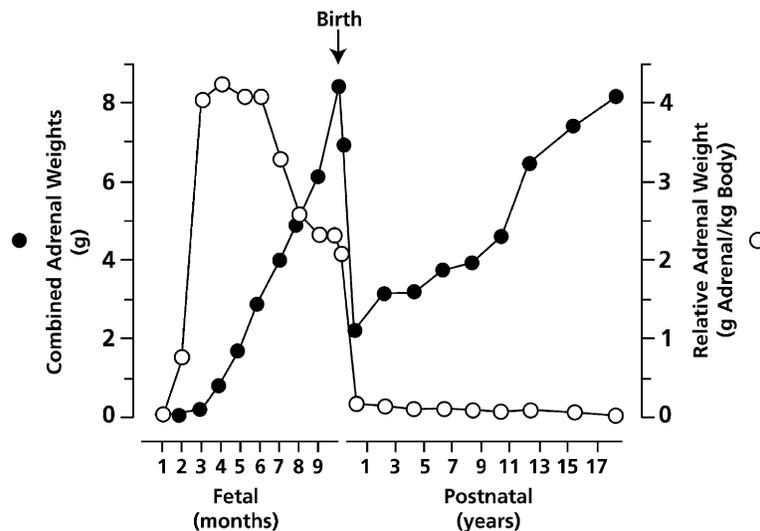


Fig. 14.2 Combined adrenal weight (filled circles) and relative adrenal weight (open circles) from the first trimester through to early adulthood. (From Mesiano, S., & Jaffe, R. B. (1997). Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev*, 18, 378–403.)

postnatal life. Thereafter adrenal growth is comparatively slow, so that the adrenal glands represent only 0.01% of body weight in the adult.

The complex mechanisms regulating adrenal development are still relatively poorly understood. However, important insight into key factors has been obtained from studies of transgenic mice and from patients with disorders of adrenal development.⁶ For example, the early stages of adrenal differentiation and development involve a number of signaling pathways (hedgehog/GLI3, WNT3/WNT4/WNT11, midkine), transcription factors (SALL1, FOXD2, PBX1, WT1, SF1 [NR5A1], DAX1 [NR0B1]), coregulators (CITED2), matrix proteins (SPARC), and regulators of telomerase activity (ACD).⁷ Subsequent fetal adrenal growth is highly dependent on the trophic effects of adrenocorticotropic hormone (ACTH), its receptor (MC2R), and its downstream signaling pathways, as well as growth factor signaling pathways, such as insulin-like growth factor-2 (IGF-2), basic fibroblast growth factor (bFGF, now termed *FGF2*), and epidermal growth factor (EGF).

Anatomy

The adrenals, once termed *suprarenal glands*, derive their name from their anatomic location, sitting on top of the upper pole of each kidney. Unlike most other organs, the arteries and veins serving the adrenal do not run parallel. Arterial blood is provided by several small arteries arising from the renal and phrenic arteries, the aorta, and sometimes the ovarian and left spermatic arteries. The veins are more conventional, with the left adrenal vein draining into the left renal vein and the right adrenal vein draining directly into the vena cava. Arterial blood enters the sinusoidal circulation of the cortex and drains toward the medulla, so that medullary chromaffin cells are bathed in very high concentrations of steroid hormones. High concentrations of cortisol are required for expression of medullary phenylethanolamine-N-methyltransferase, which converts norepinephrine to epinephrine, linking the adrenal cortical and medullary responses to stress.⁸

The adrenal cortex consists of three histologically recognizable zones: the zona glomerulosa is immediately below the

capsule, the zona fasciculata is in the middle, and the zona reticularis lies next to the medulla. The glomerulosa, fasciculata, and reticularis, respectively, constitute about 15, 75, and 10% of the adrenal cortex of the older child and adult. These zones appear to be distinct functionally, as well as histologically, but considerable overlap exists, and immunocytochemical data show that the zones physically interdigitate. After birth, the large fetal zone begins to involute and disappears by about 3 to 6 months of age. The definitive zone simultaneously enlarges, but two of the adult zones, the glomerulosa and fasciculata, are not fully differentiated until about 3 years of age, and the reticularis may not be fully differentiated until about 15 years of age. The origin of the distinct adrenocortical zones and the mechanisms that regulate their proliferation are still poorly understood. One model suggests that a population of undifferentiated stem cells exists between the zona glomerulosa and zona fasciculata, which represents a pool of common precursor cells that can contribute to either the inner or outer zones. In contrast, the “centripetal migration” theory proposes that a subcapsular population of progenitor/stem cells first differentiates into cells of the zona glomerulosa, which then convert into zona fasciculata cells (and presumably later into zona reticularis cells), as they migrate centripetally toward the adrenal medulla, where they complete their life span and undergo apoptosis.⁹

STEROID HORMONE SYNTHESIS

Early Steps: Cholesterol Uptake, Storage, and Transport

Much is now known about steroid biosynthesis,¹⁰ and about the early steps in the intracellular cholesterol trafficking.^{11,12} The human adrenal can synthesize cholesterol *de novo* from acetate, but most of its supply of cholesterol comes from plasma low-density lipoproteins (LDLs) derived from dietary cholesterol. Rodent adrenals derive most of their cholesterol from high-density lipoproteins via a receptor termed *SR-B1*, but this pathway plays a minor role in human steroidogenesis. Adequate concentrations of LDL will suppress 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. ACTH, which stimulates adrenal steroidogenesis, also stimulates the activity of HMG-CoA reductase, LDL receptors, and uptake of LDL cholesterol. LDL cholesterol esters are taken up by receptor-mediated endocytosis, then are stored directly or converted to free cholesterol and used for steroid hormone synthesis. Cholesterol can be esterified by acyl-CoA: cholesterol transferase (ACAT), stored in lipid droplets, and accessed by activation of hormone-sensitive lipase (HSL) and by the so-called NPC proteins, which derive their name from their causative role in Niemann-Pick type C disease. ACTH stimulates HSL and inhibits ACAT, thus increasing the availability of free cholesterol for steroid hormone synthesis.

Steroidogenic Enzymes

Cytochrome P450

Most steroidogenic enzymes are cytochrome P450 enzymes.¹⁰ “Cytochrome P450” designates a group of oxidative enzymes, all of which have about 500 amino acids and contain a single heme group. “P450” means “pigment 450” because all absorb light at 450 nm in their reduced states. Human beings have 57 genes for P450 enzymes; seven human P450s are targeted to mitochondria and 50 are targeted to the endoplasmic reticulum, especially in the liver, where they metabolize countless toxins, drugs, xenobiotics, and environmental pollutants.¹³ Each P450 enzyme, including the steroidogenic P450s, can metabolize multiple substrates, catalyzing a broad array of oxidations.

Five P450s are involved in adrenal steroidogenesis (Fig. 14.3). Mitochondrial P450_{sc} (CYP11A1) is the cholesterol

side-chain cleavage enzyme catalyzing the series of reactions formerly termed *20,22 desmolase*. The two mitochondrial isozymes of P450c11, P450c11 β (CYP11B1) and P450c11AS (CYP11B2), catalyze 11 β -hydroxylase, 18-hydroxylase, and 18-methyl oxidase activities. P450c17 (CYP17A1), found in the endoplasmic reticulum, catalyzes both 17 α -hydroxylase and 17,20 lyase activities, and P450c21 (CYP21A2) catalyzes the 21-hydroxylation of both glucocorticoids and mineralocorticoids. In the gonads and elsewhere P450aro (CYP19A1) in the endoplasmic reticulum catalyzes aromatization of androgens to estrogens.

Hydroxysteroid Dehydrogenases

The hydroxysteroid dehydrogenases (HSDs) have molecular masses of about 35 to 45 kDa, do not have heme groups, and require nicotinamide adenine dinucleotide (NAD⁺) or nicotinamide adenine dinucleotide phosphate (NADP⁺) as cofactors.^{14,15} Whereas most steroidogenic reactions mediated by P450 enzymes are catalyzed by a single form of P450, each of the reactions mediated by HSDs can be catalyzed by at least two isozymes. The HSDs include the 3 α - and 3 β -hydroxysteroid dehydrogenases, two 11 β -hydroxysteroid dehydrogenases, and a series of 17 β -hydroxysteroid dehydrogenases; the 5 α -reductases are unrelated to this family. Based on their structures, the HSDs fall into two groups: the short-chain dehydrogenase/reductase (SDR) family, structurally characterized by a “Rossmann fold,” and the aldo-keto reductase (AKR) family, characterized by a triosephosphate isomerase barrel motif.^{16,17} The SDR enzymes include 11 β -HSDs 1 and 2, and 17 β -HSDs 1, 2, 3, and 4; the AKR enzymes include 17 β -HSD5, which is important in extraglandular activation of androgenic precursors, and the 3 α -hydroxysteroid dehydrogenases that participate in the so-called *backdoor pathway* of fetal androgen synthesis (see later). Based on their activities, it is physiologically more useful to classify them as dehydrogenases or reductases. The dehydrogenases use NAD⁺ as their cofactor to oxidize hydroxysteroids to ketosteroids, and the reductases mainly use NADPH to reduce ketosteroids to hydroxysteroids. Although these enzymes are typically bidirectional *in vitro*, they tend to function in only one direction in intact cells, with the direction determined by the cofactor(s) available.^{16,17}

P450_{sc}

Conversion of cholesterol to pregnenolone in mitochondria is the first, rate-limiting and hormonally regulated step in the synthesis of all steroid hormones.^{10,11} This involves cholesterol 20 α -hydroxylation, 22-hydroxylation, and scission of its side chain to yield pregnenolone and isocaproic acid. Because 20-hydroxycholesterol, 22-hydroxycholesterol, and 20,22-hydroxycholesterol could be isolated from adrenals in significant quantities, it was previously thought that three separate enzymes were involved. However, a single protein, termed P450_{sc} (‘sc’ refers to the side-chain cleavage of cholesterol) encoded by the *CYP11A1* gene on chromosome 15 catalyzes all the steps between cholesterol and pregnenolone. Deletion of the mouse or rabbit *cyp11a1* gene eliminates all steroidogenesis, indicating that all steroidogenesis is initiated by this one enzyme.

Transport of Electrons to P450_{sc}: Ferredoxin Reductase and Ferredoxin

Mitochondrial P450 enzymes (P450_{sc}, P450c11 β , P450c11AS, and the vitamin D 1 α - and 24-hydroxylases) are terminal oxidases in an electron transport system.¹⁸ NADPH donates electrons to ferredoxin reductase (FDR, also termed *adrenodoxin reductase*), a flavoprotein that is loosely associated with the inner mitochondrial membrane. FDR transfers the electrons to ferredoxin (FDX, also termed *adrenodoxin*), a 14-kDa iron/sulfur protein, which then transfers the electrons to P450_{sc} (Fig. 14.4).

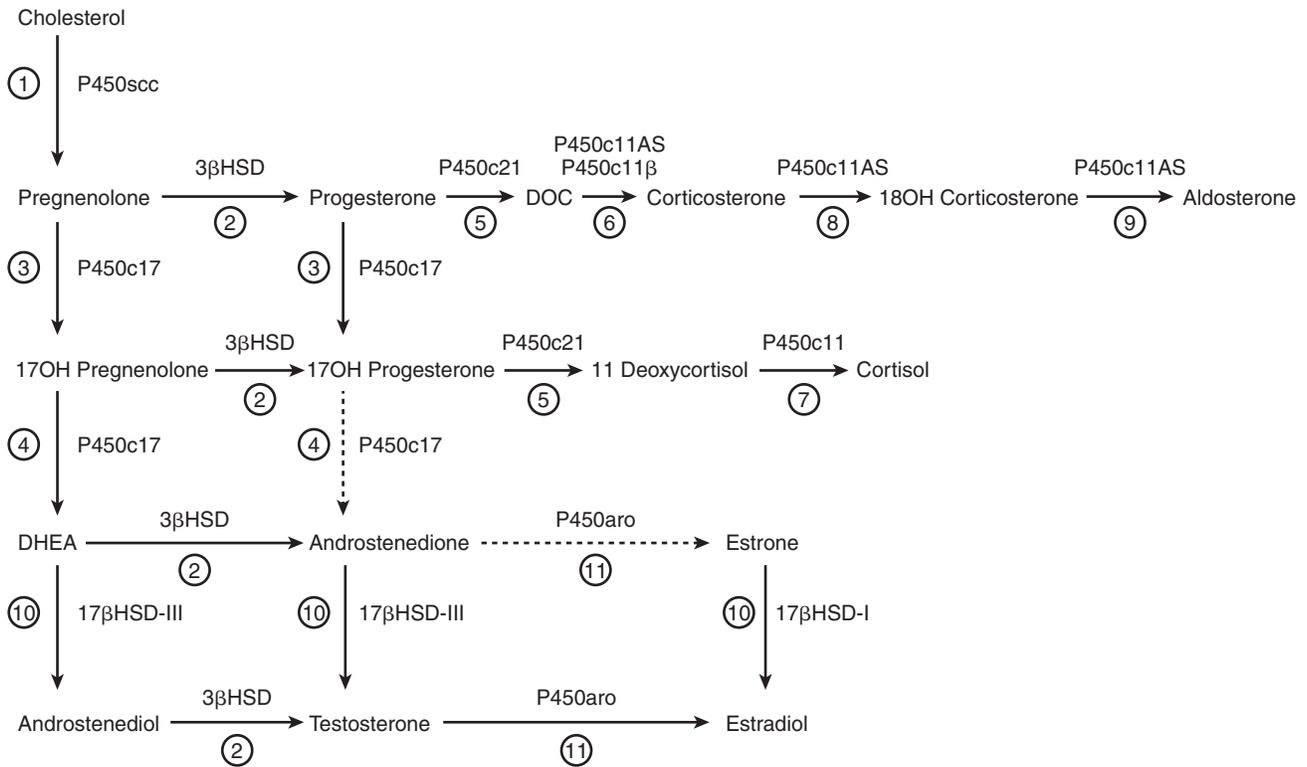


Fig. 14.3 Principal pathways of human adrenal steroid hormone synthesis. Other quantitatively and physiologically minor steroids are also produced. The names of the enzymes are shown by each reaction, and the traditional names of the enzymatic activities correspond to the circled numbers. Reaction 1: Mitochondrial cytochrome P450scc mediates 20 α -hydroxylation, 22-hydroxylation, and scission of the C20-22 carbon bond. Reaction 2: 3 β HSD mediates 3 β -hydroxysteroid dehydrogenase and isomerase activities, converting Δ^5 steroids to Δ^4 steroids. Reaction 3: P450c17 catalyzes the 17 α -hydroxylation of pregnenolone to 17OH-pregnenolone and of progesterone to 17OH-progesterone. Reaction 4: The 17,20 lyase activity of P450c17 converts 17OH-pregnenolone to dehydroepiandrosterone (DHEA); only insignificant amounts of 17OH-progesterone are converted to Δ^4 androstenedione by human P450c17, although this reaction occurs in other species. Reaction 5: P450c21 catalyzes the 21-hydroxylation of progesterone to deoxycorticosterone (DOC) and of 17OH-progesterone to 11-deoxycortisol. Reaction 6: DOC is converted to corticosterone by the 11-hydroxylase activity of P450c11AS in the zona glomerulosa and by P450c11 β in the zona fasciculata. Reaction 7: 11-Deoxycortisol undergoes 11 β -hydroxylation by P450c11 β to produce cortisol in the zona fasciculata. Reactions 8 and 9: The 18-hydroxylase and 18-methyl oxidase activities of P450c11AS convert corticosterone to 18OH-corticosterone and aldosterone, respectively, in the zona glomerulosa. Reactions 10 and 11 are found principally in the testes and ovaries. Reaction 10: 17 β HSD-III converts DHEA to androstenedione and androstenedione to testosterone, whereas 17 β HSD-I converts estrone to estradiol. Reaction 11: Testosterone may be converted to estradiol and androstenedione may be converted to estrone by P450aro.

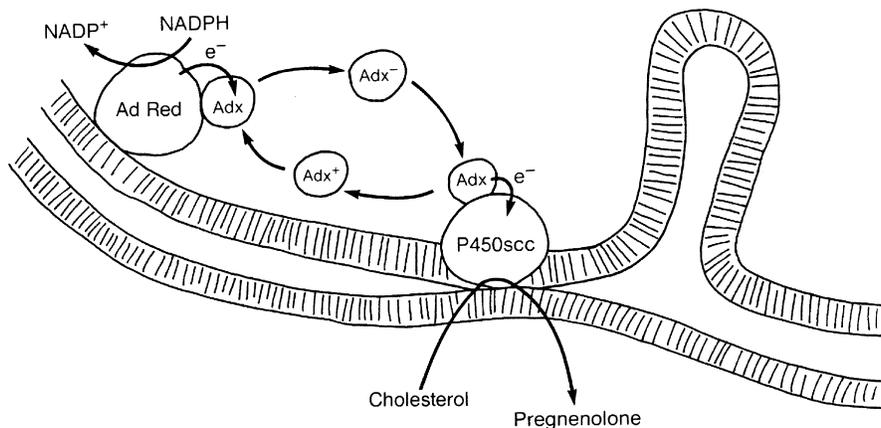


Fig. 14.4 Electron transport to mitochondrial forms of cytochrome P450. Adrenodoxin reductase (AdRed), a flavoprotein loosely bound to the inner mitochondrial membrane, accepts electrons (e^-) from nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), converting it to NADP $^+$. These electrons are passed to adrenodoxin (Adx), an iron-sulfur protein in solution in the mitochondrial matrix that functions as a freely diffusible electron shuttle mechanism. Electrons from charged adrenodoxin (Adx $^-$) are accepted by any available cytochrome P450, such as P450c11, or P450scc shown here. The uncharged adrenodoxin (Adx $^+$) may then be again bound to adrenodoxin reductase to receive another pair of electrons. For P450scc, three pairs of electrons must be transported to the P450 to convert cholesterol to pregnenolone. The flow of cholesterol into the mitochondria is facilitated by steroidogenic acute regulatory protein, which is not shown in this diagram. (©W.L. Miller.)

FDX forms a 1:1 complex with FDXR, dissociates, then reforms an analogous 1:1 complex with the P450, thus functioning as a diffusible electron shuttle mechanism. FDXR and FDX are widely expressed in human tissues, but FDXR expression is two orders of magnitude higher in steroidogenic tissues.¹⁹ The primary ribonucleic acid (RNA) transcript from the *FDXR* gene is alternatively spliced yielding two messenger RNA (mRNA) species that encode proteins differing by six amino acids,²⁰ but only the shorter protein is active in steroidogenesis.²¹ FDXR is also essential in the formation of the iron/sulfur centers used by many enzymes. There are two human FDX isozymes encoded by genes on different chromosomes: FDX1 interacts with mitochondrial P450 enzymes; FDX2 is used for synthesis of iron-sulfur clusters, but not for steroidogenesis.^{22,23} FDX1 is ubiquitously expressed but is especially abundant in adrenal cortex. Human mutations in FDX have not been described, but several FDXR mutations that partially impair formation of iron/sulfur centers are associated with neuropathic hearing loss.^{24–26}

Mitochondrial Cholesterol Uptake: the Steroidogenic Acute Regulatory Protein, StAR

ACTH regulates steroidogenic capacity (chronic regulation) by inducing the transcription of genes for steroidogenic enzymes, but acute regulation, where steroids are released within minutes of a stimulus, is at the level of cholesterol access to P450scc.^{27,28} Treating either steroidogenic cells or intact rats with inhibitors of protein synthesis (e.g., cycloheximide) eliminated the acute steroidogenic response, suggesting that a short-lived protein triggers the response. A long search led to the identification and cloning of the steroidogenic acute regulatory protein, StAR.^{27–29} The central role of StAR in steroidogenesis was proven by finding that mutations of StAR caused congenital lipid adrenal hyperplasia.^{30,31} Thus StAR is the acute trigger that is required for the rapid flux of cholesterol from the outer to the inner mitochondrial membrane that is needed for the acute response of aldosterone to angiotensin II, of cortisol to ACTH, and of sex steroids to a luteinizing hormone (LH) pulse.

Some adrenal steroidogenesis is independent of StAR; this StAR-independent steroidogenesis is about 14% of the StAR-induced rate.^{30,31} The placenta uses P450scc to initiate steroidogenesis but does not express StAR. The mechanism of StAR-independent steroidogenesis is unclear; it may occur without a triggering protein, or some other protein may exert StAR-like activity to promote cholesterol flux, but without StAR's rapid kinetics.¹² The mechanism of StAR's action is unclear, but it is established that StAR acts on the outer mitochondrial membrane (OMM), does not need to enter the mitochondria to be active, and undergoes conformational changes on the OMM that are required for StAR's activity.^{28,32} Some studies suggest that StAR functions as a component of a molecular machine that consists of StAR, TSPO (the translocator protein formerly known as the peripheral benzodiazepine receptor), and other proteins on the OMM,³³ although studies with TSPO-knockout mice question its role.²⁹ The precise fashion in which cholesterol is loaded into the OMM and moves from the OMM to P450scc with the assistance of StAR remains unclear, and remains under active investigation.³⁴

3 β -Hydroxysteroid Dehydrogenase/ $\Delta^5 \rightarrow \Delta^4$ Isomerase

Once pregnenolone is produced from cholesterol, it may undergo 17 α -hydroxylation by P450c17 to yield 17-hydroxypregnenolone (17-Preg), or it may be converted to progesterone. Two 42-kDa isozymes of 3 β -hydroxysteroid dehydrogenase (3 β HSD), encoded by the *HSD3B1* and

HSD3B2 genes,³⁵ can catalyze both conversion of the hydroxyl group to a keto group on carbon 3 and the isomerization of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids).¹⁰ These isozymes share 93.5% amino acid sequence identity and are enzymatically very similar: both can convert pregnenolone to progesterone, 17-Preg to 17 α -hydroxypregnenolone (17OHP), DHEA to androstenedione, and androstenediol to testosterone. However, 3 β HSD2, the isozyme expressed in the adrenals and gonads, has a high Michaelis-Menten constant (Km) of about 5.5 μ M,²⁰⁵ about 10 times higher than 3 β HSD1 expressed in placenta, brain, and "extraglandular" tissues.²⁰⁶ The low Km of extraglandular 3 β HSD1 permits it to act on the low concentrations of some steroids found in the circulation. Ultrastructural data show that 3 β HSD can be found in the mitochondria, endoplasmic reticulum, and cytoplasm.³⁶ It is not clear if this subcellular distribution differs in various types of steroidogenic cells, but this could be a novel point regulating the direction of steroidogenesis.³⁴

P450c17

P450c17 (CYP17A1) catalyzes both 17 α -hydroxylase and 17,20-lyase activities. The 17 α -hydroxylase activity of P450c17 can convert pregnenolone to 17-Preg and progesterone to 17OHP. The 17,20-lyase activity of P450c17 can convert 17-Preg to DHEA, but very little 17OHP is converted to androstenedione because human P450c17 catalyzes this reaction at only around 2% to 3% of the rate for conversion of 17-Preg to DHEA.³⁷ P450c17 is the key branch point in steroid hormone synthesis: in its absence, as in the adrenal zona glomerulosa, 17 α -hydroxylase and 17,20-lyase activities are absent, so that pregnenolone is converted to mineralocorticoids; in the presence of its 17 α -hydroxylase activity in the zona fasciculata, only the 17 α -hydroxylase activity is present so that pregnenolone is sequentially converted to cortisol; in the zona reticularis where both activities are present, pregnenolone is sequentially converted to sex steroids (see Fig. 14.3).¹⁰

17 α -Hydroxylase and 17,20 lyase were once thought to be separate enzymes. The adrenals of prepubertal children synthesize ample cortisol but virtually no DHEA (i.e., have 17 α -hydroxylase activity but not 17,20 lyase activity) until adrenarche initiates production of adrenal androgens (i.e., turns on 17,20 lyase activity). Furthermore, patients had been described lacking 17,20 lyase activity but retaining normal 17 α -hydroxylase activity.³⁸ However, both 17 α -hydroxylase and 17,20 lyase activities reside in the same active site, and cells transfected with a vector expressing P450c17 acquire both 17 α -hydroxylase and 17,20 lyase activities. P450c17 is encoded by the *CYP17A1* gene on chromosome 10q24.3, and is structurally related to the *CYP21A2* gene for P450c21 (21-hydroxylase). Thus the distinction between 17 α -hydroxylase and 17,20 lyase is functional and not genetic or structural. Human P450c17 catalyzes 17 α -hydroxylation of pregnenolone and progesterone equally well, but its 17,20 lyase activity uses 17-Preg almost exclusively, and not 17OHP,³⁷ consistent with the large amounts of DHEA secreted by both the fetal and adult adrenal. The principal factor regulating the 17,20 lyase reaction is electron transport from NADPH via P450 oxidoreductase (POR).³⁸

Electron Transport to P450c17: P450 Oxidoreductase and Cytochrome b₅

POR, a membrane-bound flavoprotein that is unrelated to mitochondrial ferredoxin reductase, receives electrons from NADPH and transfers them to the 50 microsomal forms of cytochrome P450, including P450c17, P450c21, and P450aro.¹⁸ For the 17,20 lyase reaction of P450c17, electron

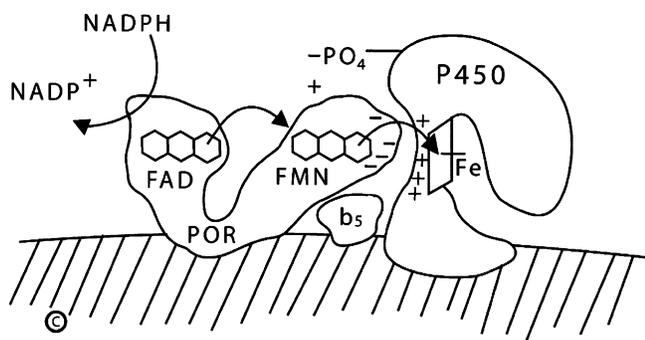


Fig. 14.5 Electron transport to microsomal forms of cytochrome P450. Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) interacts with P450 oxidoreductase (POR), bound to the endoplasmic reticulum, and gives up a pair of electrons (e^-), which are received by the flavin adenine dinucleotide (FAD) moiety. Electron receipt elicits a conformational change, permitting the isoalloxazine rings of the FAD and flavin mononucleotide (FMN) moieties to come close together, so that the electrons pass from the FAD to the FMN. Following another conformational change that returns the protein to its original orientation, the FMN domain of POR interacts with the redox-partner binding site of the P450. Electrons from the FMN domain of POR reach the heme group to mediate catalysis. The interaction of POR and the P450 is coordinated by negatively charged acidic residues on the surface of the FMN domain of POR, and positively charged basic residues in the concave redox-partner binding site of the P450. The active site containing the steroid lies on the iron side of heme ring (Fe) opposite from the redox-partner binding site. In the case of human P450c17, this interaction is facilitated by the allosteric action of cytochrome b_5 , and by the serine phosphorylation of P450c17. (©W.L. Miller)

transfer is facilitated by the action of cytochrome b_5 , acting as an allosteric factor rather than as an alternate electron donor;³⁷ 17,20 lyase activity is also increased by the serine/threonine phosphorylation of P450c17 by p38 α , a cyclic adenosine monophosphate (cAMP)-dependent protein kinase³⁹ (Fig. 14.5). Thus the availability of electrons determines whether P450c17 performs only 17 α -hydroxylation, or also performs 17,20 bond scission: increasing the ratio of POR or cytochrome b_5 to P450c17 increases the ratio of 17,20 lyase activity to 17 α -hydroxylase activity. Thus the regulation of 17,20 lyase activity, and consequently of DHEA production, depends on factors that facilitate the flow of electrons to P450c17: high concentrations of POR, the presence of cytochrome b_5 , and serine phosphorylation of P450c17.³⁸

P450c21

After the synthesis of progesterone and 17OHP, these steroids are 21-hydroxylated to yield deoxycorticosterone (DOC) and 11-deoxycortisol, respectively¹⁰ (see Fig. 14.3). The nature of the 21-hydroxylating step is of great interest because disordered 21-hydroxylation causes more than 90% of all cases of CAH. The clinical findings in CAH are complex and potentially devastating. Decreased cortisol and aldosterone synthesis often lead to sodium loss, potassium retention, acidosis, and hypotension, which will lead to cardiovascular collapse and death, usually within a month after birth if not treated appropriately. Decreased adrenal steroidogenesis in utero leads to overproduction of adrenal androgens via several pathways (see later), resulting in severe prenatal virilization of female fetuses.⁴⁰ Adrenal 21-hydroxylation is catalyzed by P450c21 found in smooth endoplasmic reticulum, using POR to receive electrons from NADPH. Duplicated *CYP21A1P* and *CYP21A2* genes lie on chromosome 6p21, but only the human *CYP21A2* gene encodes P450c21.¹⁰ As this gene lies in the middle of the major

histocompatibility locus, disorders of adrenal 21-hydroxylation are closely linked to specific human leukocyte antigen (HLA) types. Extraadrenal 21-hydroxylase activity is found in many adult and fetal tissues, especially in the liver, but is catalyzed by other enzymes, notably CYP2C19 and CYP3A4, which are principally involved in drug metabolism. CYP2C19 and CYP3A4 can 21-hydroxylate progesterone but not 17OHP, and hence may contribute to the synthesis of mineralocorticoids but not glucocorticoids.⁴¹

P450c11 β and P450c11AS

The closely related P450c11 β and P450c11AS enzymes catalyze the final steps in the synthesis of glucocorticoids and mineralocorticoids.^{10,42} These two isozymes have 93% amino acid sequence identity and are encoded by tandemly duplicated genes on chromosome 8q21-22. Like P450scc, the two forms of P450c11 are found on the inner mitochondrial membrane, and use ferredoxin and ferredoxin reductase to receive electrons from NADPH. By far the more abundant of the two isozymes is P450c11 β (encoded by *CYP11B1*), which is the classic 11 β -hydroxylase that converts 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. The less abundant isozyme, P450c11AS (encoded by *CYP11B2*), is found only in the zona glomerulosa, where it has 11 β -hydroxylase, 18-hydroxylase, and 18-methyl oxidase (aldosterone synthase) activities; thus P450c11AS is able to catalyze all the reactions needed to convert DOC to aldosterone. *CYP11B1* is induced by ACTH via cAMP and is suppressed by glucocorticoids; *CYP11B2* is induced by angiotensin II and K^+ . Patients with mutations in *CYP11B1* have classic 11 β -hydroxylase deficiency but can still produce aldosterone, whereas patients with mutations in *CYP11B2* have rare forms of aldosterone deficiency (so-called *corticosterone methyl oxidase deficiency*), while retaining the ability to produce cortisol.^{10,42}

17 β -Hydroxysteroid Dehydrogenase

Androstenedione is converted to testosterone, DHEA is converted to androstenediol, and estrone is converted to estradiol by the 17 β -hydroxysteroid dehydrogenases (17 β HSD; HSD17B), sometimes also termed 17-oxidoreductases or 17-ketosteroid reductases. The terminologies for these enzymes vary, depending on the direction of the reaction being considered. The literature about the 17 β HSDs can be confusing because (1) there are several different 17 β HSDs; (2) some are preferential oxidases, whereas others are preferential reductases; (3) they differ in their substrate preference and sites of expression; (4) the nomenclature is inconsistent; and (5) some proteins termed 17 β HSD actually have very little 17 β HSD activity, and are principally involved in other reactions.¹⁰

Type 1 17 β HSD (17 β HSD1, encoded by *HSD17B1*), is a 34-kDa reductive, estrogenic SDR enzyme expressed in ovarian granulosa cells (where it produces estradiol) and placenta (where it produces estriol).^{10,15,43-47} 17 β HSD1 uses NADPH as its cofactor, acts as a dimer, and only accepts steroid substrates with an aromatic A ring, so that its activity is confined to activating estrogens. No genetic deficiency syndrome for 17 β HSD1 has been described.

17 β HSD2 is a microsomal oxidase that uses NAD^+ to inactivate estradiol to estrone and testosterone to Δ^4 androstenedione. 17 β HSD2 is found in the placenta, liver, small intestine, prostate, secretory endometrium, and ovary. Whereas 17 β HSD1 is found in placental syncytiotrophoblast cells, 17 β HSD2 is expressed in endothelial cells of placental intravillous vessels, consistent with its apparent role in defending the fetal circulation from transplacental passage of maternal estradiol or testosterone.^{10,15,43-46} No deficiency state for 17 β HSD2 has been reported.

Microsomal 17 β HSD3 is the principal androgenic form of 17 β HSD, apparently expressed only in the testis. This enzyme is disordered in the classic syndrome of male pseudohermaphroditism that is often termed *17-ketosteroid reductase deficiency*.^{10,15,43,45,46}

An enzyme termed 17 β HSD4 was initially identified as an NAD⁺-dependent oxidase with activities similar to 17 β HSD2, but this peroxisomal protein is primarily an enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase.¹⁰ Deficiency of 17 β HSD4 causes a form of Zellweger syndrome, in which bile acid biosynthesis is disturbed but steroidogenesis is not.

17 β HSD5, originally cloned as a 3 α -hydroxysteroid dehydrogenase, is an AKR enzyme (whereas 17 β HSD types 1-4 are SDR enzymes) termed AKR1C3 that catalyzes the reduction of Δ^4 androstenedione to testosterone.^{10,48} The 17 β HSD activity of 17 β HSD5 is quite labile in vitro, and this enzyme catalyzes different activities under different conditions.⁴⁸ The adrenal zona reticularis expresses this enzyme at low levels, accounting for the small amount of testosterone produced by the adrenal.⁴⁹

17 β HSD6, encoded by the *HSD17B6* gene on chromosome 12q13.3, is also known as RoDH for its homology to retinol dehydrogenases.⁵⁰ 17 β HSD6 is expressed at low but detectable levels in the fetal testes, where it appears to catalyze oxidative 3 α HSD activities in the so-called "backdoor pathway" to dihydrotestosterone (DHT) synthesis.⁵¹

Steroid Sulfotransferase and Sulfatase

Steroid sulfates may be synthesized directly from cholesterol sulfate or may be formed by sulfation of steroids by cytosolic sulfotransferase (SULT) enzymes.^{52,53} SULTs use 3'-phosphoadenine-5'-phosphosulfate (PAPS) as sulfate donor, synthesized from adenosine triphosphate (ATP) and sulfate by two isozymes of PAPS synthase: PAPSS1, which is ubiquitously expressed, and PAPSS2, principally expressed in cartilage, adrenal, and liver.⁵⁴ At least 44 distinct isoforms of these enzymes have been identified belonging to five families of *SULT* genes; many of these genes yield alternately spliced products accounting for the large number of enzymes. The SULT enzymes that sulfonate steroids include SULT1E (estrogens), SULT2A1 (non-aromatic steroids), and SULT2B1 (sterols). SULT2A1 is the principal sulfotransferase expressed in the adrenal, where it sulfates the 3 β hydroxyl group of Δ^5 steroids (pregnenolone, 17OH-pregnenolone, DHEA, androsterone) but not cholesterol.⁵⁵ SULT2B1a will also sulfonate pregnenolone but not cholesterol, whereas cholesterol is the principal substrate for SULT2B1b in the skin, liver, and elsewhere. It is not clear whether most steroid sulfates are simply inactivated forms of steroid or if they serve specific hormonal roles. Knockout of the mouse *sult1e1* gene is associated with elevated estrogen levels, increased placental expression of tissue factor, and increased platelet activation, leading to placental thrombi and fetal loss that could be ameliorated by anticoagulant therapy. Mutations ablating the function of human SULT enzymes have not been described, but PAPSS2 mutations deplete adrenal PAPS, increasing production of unconjugated DHEA.⁵⁶ African Americans have a high rate of polymorphisms in SULT2A1, apparently influencing plasma ratios of DHEA:DHEAS, which may correlate with risk of prostatic and other cancers.

Steroid sulfates may also be hydrolyzed to the native steroid by steroid sulfatase. Deletions in the steroid sulfatase gene on chromosome Xp22.3 cause X-linked ichthyosis. The fact that males have a single copy of this gene probably accounts for males having higher DHEAS levels than females of the same age. In the fetal adrenal and placenta, diminished or absent sulfatase deficiency reduces the pool of free DHEA available for

placental conversion to estrogen, resulting in low concentrations of estriol in the maternal blood and urine. The accumulation of steroid sulfates in the stratum corneum of the skin causes the ichthyosis.

Aromatase: P450aro

Estrogens are produced by the aromatization of androgens, including adrenal androgens, by a complex series of reactions catalyzed by aromatase, P450aro.^{57,58} This microsomal cytochrome P450 is encoded by a single gene (*CYP19A1*) on chromosome 15q21.1 that uses several different promoter sequences, transcriptional start sites, and alternatively chosen first exons to encode identical P450aro in different tissues under different hormonal regulation. Aromatase expression in the extraglandular tissues, especially adipose tissue, can convert adrenal androgens to estrogens. Aromatase in the epiphyses of growing bone converts testosterone to estradiol; the tall stature, delayed epiphyseal maturation and osteopenia of males with aromatase deficiency, and their rapid reversal with estrogen replacement indicate that estrogen, not androgen, is responsible for epiphyseal maturation in males.

Rare patients with aromatase deficiency illustrate that placental aromatase activity and consequent fetoplacental estrogen synthesis are not needed for normal fetal development.⁵⁸ However, in the absence of placental aromatase activity, fetal adrenal androgens and androgen precursors pass into the maternal circulation, causing maternal virilization.⁵⁹ Furthermore, placental aromatase converts maternal androgens to estrogens, protecting the fetus from potential virilization (as in pregnant women with undertreated 21-hydroxylase deficiency [21OHD]).⁶⁰ Children with aromatase deficiency grow normally and continue to grow after puberty, because only estrogens, not androgens, mediate epiphyseal fusion. Treating aromatase-deficient patients with estrogens will fuse their epiphyses and arrest growth.⁶¹ These observations underlie the use of aromatase inhibitors for inhibiting accelerated bone maturation.

5 α -Reductases

Testosterone is converted to the more potent androgen, DHT, by 5 α -reductase, an enzyme found in testosterone's target tissues. There are two hydrophobic, 30-kDa isozymes of 5 α -reductase that share 50% identity. The type I enzyme, found in the scalp and other peripheral tissues, is encoded by the *SRD5A1* gene on chromosome 5; the type II enzyme, the predominant form found in male reproductive tissues, is encoded by the structurally related *SRD5A2* gene on chromosome 2p23.^{62,63} The syndrome of 5 α -reductase deficiency, a disorder of male sexual differentiation, is caused by a wide variety of mutations in the *SRD5A2* gene.⁶⁴ In the fetus, the *SRD5A1* gene is expressed in the testis,⁵¹ then is expressed briefly in the skin of the newborn, and then remains unexpressed until its activity and protein are again found after puberty. The *SRD5A2* gene is expressed in fetal genital skin, in the normal prostate, and in prostatic hyperplasia and adenocarcinoma. Thus the type I enzyme may be responsible for the pubertal virilization seen in patients with classic 5 α -reductase deficiency, and the type II enzyme may be involved in male pattern baldness.^{62,64}

11 β -Hydroxysteroid Dehydrogenases

Although certain steroids are typically categorized as glucocorticoids or mineralocorticoids, the "mineralocorticoid" (glucocorticoid type II) receptor has equal affinity for both aldosterone and cortisol.⁶⁵ Nevertheless, cortisol does not act as a mineralocorticoid in vivo, even though cortisol concentrations can exceed aldosterone concentrations by 100- to

1000-fold, because mineralocorticoid responsive tissues (such as the kidney) convert cortisol to cortisone, a metabolically inactive steroid.^{66,67} The interconversion of cortisol and cortisone is mediated by two membrane-bound isozymes of 11 β -hydroxysteroid dehydrogenase (11 β HSD; HSD11B), each of which can catalyze both oxidase and reductase activity, depending on the cofactor available (NADP⁺ or NADPH).^{68–74} The ratio of NADP⁺ to NADPH is regulated by hexose-6-phosphate dehydrogenase (H6PDH).⁷⁵

The type 1 enzyme (HSD11B1; 11 β HSD1) is expressed mainly in glucocorticoid-responsive tissues, such as the liver, fat, testis, and lung. HSD11B1 can catalyze both the oxidation of cortisol to cortisone using NADP⁺ as its cofactor (Km 1–2 μ M), or the reduction of cortisone to cortisol using NADPH as its cofactor (Km 0.1–0.3 μ M); the reaction catalyzed depends on which cofactor is available, but the enzyme can only function with high (micromolar) concentrations of steroid. HSD11B1 is located on the luminal side of the endoplasmic reticulum, and hence is not in contact with the cytoplasm, permitting HSD11B1 to receive NADPH provided by H6PDH.^{70,76} This links HSD11B1 to the pentose monophosphate shunt, providing a direct paracrine link between local glucocorticoid production and energy storage as fat.^{70,71} Prednisone and cortisone are inactive 11-ketosteroids that must be reduced to their active 11 β -hydroxy derivatives by HSD11B1, mainly in the liver. By activating cortisone, HSD11B1 amplifies glucocorticoid action in the tissues in which it is expressed, especially in liver and fat, potentially contributing to obesity, insulin resistance, metabolic syndrome, and possibly nonalcoholic fatty liver disease; consequently, studies of HSD11B1 inhibitors are of substantial pharmaceutical interest.^{73,74}

HSD11B2 (11 β HSD2) catalyzes the oxidation of cortisol to cortisone using NADH, and can function with low (nanomolar) steroid concentrations (Km 10–100 nM). HSD11B2 is expressed in mineralocorticoid-responsive tissues, such as the distal nephron and thus serves to “defend” the mineralocorticoid receptor (MR) by inactivating cortisol to cortisone, so that only “true” mineralocorticoids, such as aldosterone or deoxycorticosterone, can exert a mineralocorticoid effect; thus HSD11B2 prevents cortisol from overwhelming renal MRs. Placental HSD11B2 also inactivates maternal cortisol to cortisone (and prednisolone to prednisone), and the placenta has abundant NADP⁺ favoring the oxidative action of HSD11B1, so that in the placenta both enzymes protect the

fetus from high maternal concentrations of cortisol, but not from maternally administered beta-methasone or dexamethasone, which are not substrates for HSD11B enzymes. Thus the HSD11B isozymes determine whether a steroid “crosses the placenta.”

3 α -Hydroxysteroid Dehydrogenases

The 3 α -hydroxysteroid dehydrogenases (3 α HSDs) are not familiar to most endocrinologists, but are clinically important because of the recent discovery of the “backdoor pathway” of steroidogenesis.⁷⁷ This remarkable pathway (Fig. 14.6), first discovered as the mechanism by which the male marsupial fetal testis makes androgens,^{77,78} plays a central role in human male sexual differentiation.⁵¹ In this pathway, 17OHP is converted to DHT without going through DHEA, androstenedione, or testosterone, and hence provides a mechanism for 17OHP to contribute to the virilization of female fetuses with 21OHD.^{40,79,80} The reductive 3 α HSDs are AKR enzymes and the oxidative 3 α HSDs are SDR enzymes. There are four reductive 3 α HSDs, termed AKR1C4-1.¹⁵ These enzymes are structurally very similar, are encoded by a cluster of very similar genes on chromosome 10p14-15, and catalyze a wide array of steroidal conversions (notably 17-ketosteroid reduction and 20 α -reduction) and other reactions.¹⁰ AKR1C3, also known as 17 β HSD5, converts androstenedione to testosterone in the adrenal.

The backdoor pathway is characterized by both reductive and oxidative 3 α HSD activities; the reductive activity can apparently be catalyzed by either AKR1C2 or AKR1C4.⁵¹ The oxidative 3 α HSDs are SDRs that are similar to the retinol dehydrogenase or cis-retinol/androgen dehydrogenase (RoDH/CRAD) subfamily.⁸¹ The most active member of these is RoDH, also called 17 β HSD6, which probably catalyzes the final step in the backdoor pathway. In the brain, 3 α HSDs reduce 5 α -dihydroprogesterone to allopregnanolone, which is an allosteric activator of the gamma-aminobutyric acid (GABA)_A receptors.⁸² Further studies of the role of the backdoor pathway will be central to pediatric endocrinology.

Fetal Adrenal Steroidogenesis

Embryonic adrenocortical steroidogenesis begins around 7 weeks after fertilization. Steroidogenic enzymes are immunocytochemically identifiable, principally in the fetal zone, at 50 to 52 days

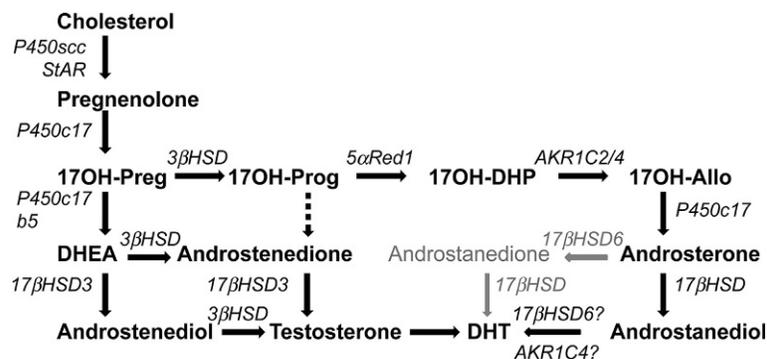


Fig. 14.6 Pathways to androgens in congenital adrenal hyperplasia (CAH). In the absence of P450_{c21} activity, the adrenal can produce androgens by three pathways. First, the pathway from cholesterol to dehydroepiandrosterone (DHEA) remains intact in 21-hydroxylase deficiency, and increased production of DHEA will lead to some DHEA being converted to androstenedione and thence to testosterone. Second, although minimal amounts of 17 α -hydroxyprogesterone (17OHP) are converted to androstenedione in the normal adrenal, the huge amounts of 17OHP produced in CAH permit some 17OHP to be converted to androstenedione and then to testosterone. Third, the so-called “backdoor pathway” depends on the 5 α and 3 α reduction of 17OHP to 17OH-allopregnenolone. This steroid is readily converted to androstenediol, which can then be oxidized to dihydrotestosterone (DHT) by a 3 α -hydroxysteroid dehydrogenase (HSD) enzyme, AKR1C2. Mass spectrometric examinations of human urinary steroids indicate this pathway is a major contributor in CAH.

postconception, and by 8 weeks postconception, the adrenal contains cortisol and responds to ACTH in primary culture systems.⁸³ This cortisol synthesis is regulated by pituitary ACTH and involves transient expression of adrenal 3 β HSD2: following the ninth week postconception, expression of 3 β HSD2 and synthesis of cortisol wane, and 3 β HSD2 is barely detectable at 10 to 11 weeks and absent at 14 weeks. At the same time, the fetal adrenal also produces 17 β HSD5,⁸³ which can convert androstenedione to testosterone. Thus the fetal adrenal makes cortisol at the same time during gestation that fetal testicular testosterone is virilizing the genitalia of the normal male fetus. This fetal adrenal cortisol apparently suppresses ACTH, which otherwise would drive adrenal androgen synthesis and virilization of 46,XX fetuses.

Fetuses affected with genetic lesions in adrenal steroidogenesis can produce sufficient adrenal androgen to virilize a female fetus to a nearly male appearance, and this masculinization of the genitalia is complete by about the 12th week of gestation. The definitive zone of the fetal adrenal produces steroid hormones according to the pathways in Fig. 14.3. In contrast, the large fetal zone of the adrenal is relatively deficient in 3 β HSD2 activity after 12 weeks.^{83,84} Robust 17,20 lyase activity and low 3 β HSD activity in the fetal adrenal account for the abundant production of DHEA and its sulfate, DHEAS, which are then converted to estrogens by the placenta (Fig. 14.7).⁸⁵ The fetal adrenal also has considerable sulfotransferase activity but little steroid sulfatase activity, also favoring conversion of DHEA to DHEAS. The resulting DHEAS is not a substrate for adrenal 3 β HSD2, and is secreted, 16 α -hydroxylated in

the fetal liver, and then acted on by placental 3 β HSD1, 17 β HSD1, and P450aro to produce estriol; steroids that escape 16 α -hydroxylation in the fetal liver yield estrone and estradiol. Placental estrogens inhibit adrenal 3 β HSD activity, providing a feedback system to promote production of DHEAS. Fetal adrenal steroids account for 50% of the estrone and estradiol and 90% of the estriol in the maternal circulation.

Although the fetoplacental unit produces huge amounts of DHEA, DHEAS, and estriol (and other steroids), they do not appear to serve an essential role.⁸⁵ Successful pregnancy requires placental synthesis of progesterone, which suppresses uterine contractility and prevents spontaneous abortion; however, fetuses with genetic disorders of adrenal and gonadal steroidogenesis develop normally, reach term gestation, and undergo normal parturition and delivery. Mineralocorticoid production is only required postnatally, estrogens are not required, and androgens are only needed for male sexual differentiation. It appears that human fetal glucocorticoids are needed at about 8 to 12 weeks,⁸³ but it is not clear that they are needed thereafter; if they are, the small amount of maternal cortisol that escapes placental inactivation suffices. A single newborn has been described with profound glucocorticoid resistance who was homozygous for a frameshift mutation at codon 772 in the glucocorticoid-binding domain of the glucocorticoid receptor (GR).⁸⁶ Although the infant had severe hypoglycemia and hypertension postnatally, pulmonary and other aspects of fetal development were normal, suggesting that glucocorticoid action is not required for normal human fetal development.

The regulation of steroidogenesis and growth of the fetal adrenal are not fully understood, but both are related to ACTH. ACTH effectively stimulates steroidogenesis by fetal adrenal cells in vitro, and excess ACTH is clearly involved in the adrenal growth and overproduction of androgens in fetuses affected with CAH. Experimental prenatal treatment of such fetuses by administering pharmacologic doses of dexamethasone to the mother at 6 to 10 weeks' gestation can significantly reduce fetal adrenal androgen production and thus reduce the virilization of female fetuses, indicating that the hypothalamic-pituitary-adrenal (HPA) axis functions very early in fetal life.⁴⁰ In contrast, anencephalic fetuses lacking pituitary ACTH have adrenals that contain a fairly normal complement of steroidogenic enzymes and retain their capacity for steroidogenesis. Thus fetal adrenal steroidogenesis may be regulated by both ACTH-dependent and ACTH-independent mechanisms.

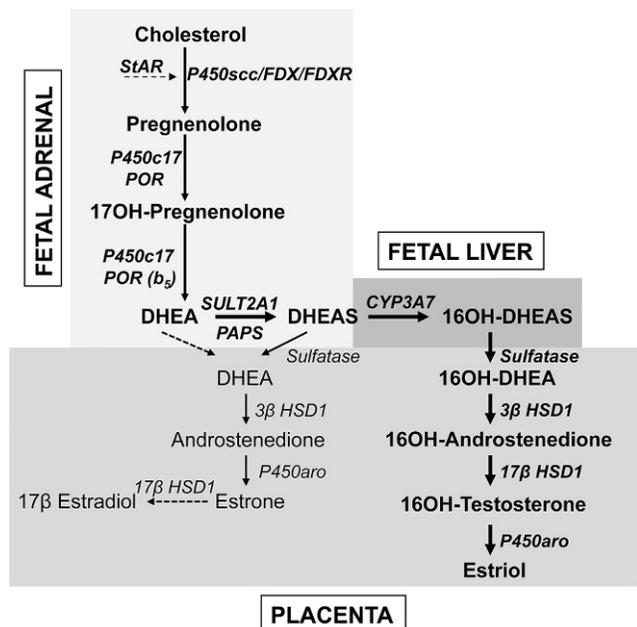


Fig. 14.7 Steroid synthesis by the fetoplacental system. The fetal adrenal has minimal 3 β -hydroxysteroid dehydrogenase (3 β HSD) activity, hence the pathway from cholesterol to dehydroepiandrosterone (DHEA) predominates. Most DHEA is converted to DHEAS by the sulfotransferase SULT2A1 and is then 16 α -hydroxylated by CYP3A7 in the fetal liver. The 16 α -hydroxy DHEAS reaches the placenta, where the sequential action of steroid sulfatase, 3 β HSD1, 17 β HSD1 and aromatase (P450aro) yield estriol, the principal steroidal product of the placenta. Small amounts of DHEA reach the placenta without being 16 α -hydroxylated, where it can be converted to estrone by 3 β HSD1 and P450aro; small amounts of estrone may also be converted to estradiol. About 80% of placental estrogen is estriol, 15% is estrone, and only 5% is estradiol.

REGULATION OF STEROIDOGENESIS

The Hypothalamic-Pituitary-Adrenal Axis

Hypothalamus: Corticotropin-Releasing Factor and Arginine Vasopressin

The principal steroidal product of the human adrenal is cortisol, which is mainly secreted in response to ACTH (corticotropin) produced in the pituitary; secretion of ACTH is stimulated primarily by corticotropin-releasing factor (CRF) from the hypothalamus. The history of the discovery of the components of the HPA axis and their interrelationships has been reviewed recently.⁸⁷ Hypothalamic CRF is a 41-amino acid peptide synthesized mainly by neurons in the paraventricular nucleus. These same hypothalamic neurons also produce the decapeptide arginine vasopressin (AVP, also known as antidiuretic hormone or ADH).⁸⁸ Both CRF and AVP are proteolytically derived from larger precursors, with the AVP precursor containing the sequence for neurophysin, which is the AVP-binding protein.

CRF and AVP travel through axons to the median eminence, which releases them into the pituitary portal circulation, although most AVP axons terminate in the posterior pituitary. AVP is cosecreted with CRF in response to stress, and both CRF and AVP stimulate the synthesis and release of ACTH, but they appear to do so by different mechanisms. CRF binds to a G protein-coupled receptor on the membranes of pituitary corticotropes and activates adenylyl cyclase, increasing cAMP, which activates the protein kinase A (PKA) signaling pathway. PKA triggers ACTH secretion by concerted regulation of cellular potassium and calcium fluxes, and enhances proopiomelanocortin (POMC) gene transcription. AVP binds to its G protein-coupled receptor and activates phospholipase C, which leads to the release of intracellular Ca^{++} and to the activation of protein kinase C (PKC). AVP seems to amplify the effects of CRF on ACTH secretion without affecting synthesis. However, CRF is the more important physiologic stimulator of ACTH release, although maximal doses of AVP can elicit a maximal ACTH response. When given together, CRF and AVP act synergistically, as would be expected from their independent mechanisms of action.

Pituitary: Adrenocorticotrophic Hormone and Proopiomelanocortin

Pituitary ACTH is a 39-amino acid peptide derived from POMC, a 241-amino acid protein.⁸⁹ POMC undergoes a series of proteolytic cleavages, yielding several biologically active peptides (Fig. 14.8). The N-terminal glycopeptide (POMC 1-75) can stimulate steroidogenesis and may function as an adrenal mitogen. POMC 112-150 is ACTH 1-39, POMC 112-126 and POMC 191-207 constitute α - and β -MSH (melanocyte stimulating hormone), respectively, and POMC 210-241 is β -endorphin. POMC is also produced in small amounts by the brain, testis, liver, kidney, and placenta, but this extrapituitary POMC does not contribute significantly to circulating ACTH. Malignant tumors will commonly produce "ectopic ACTH" in adults and rarely in children; this ACTH derives from ectopic biosynthesis of the same POMC precursor. Only the first 20 to 24 amino acids of ACTH are needed for its full biologic activity, and synthetic ACTH 1-24 is widely used in diagnostic tests of adrenal function. However, these shorter forms of ACTH have a shorter half-life than does native ACTH 1-39. POMC gene transcription is stimulated by CRF and inhibited by glucocorticoids.⁸⁹

Actions of Adrenocorticotrophic Hormone

ACTH stimulates the G protein-coupled melanocortin 2 receptor (MCR2), which is located almost exclusively in the adrenal cortex. Activation of MCR2 triggers the production of cAMP, activating PKA that catalyzes the phosphorylation of many proteins involved in steroidogenesis, thereby modifying their activity. ACTH elicits both acute and long-term effects. ACTH stimulates the biosynthesis of LDL receptors and the uptake of LDL, which provides most of the cholesterol used for steroidogenesis, and stimulates transcription of the gene for HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis, but adrenal biosynthesis of cholesterol is quantitatively much less important than the uptake of LDL cholesterol.¹¹ Cholesterol is stored in steroidogenic tissues as cholesterol esters in lipid droplets. ACTH stimulates the activity of cholesterol esterase, while inhibiting cholesterol ester synthetase, thus increasing the intracellular pool of free cholesterol, the substrate for P450_{scc}. Finally, ACTH facilitates transport of cholesterol into mitochondria, by stimulating the synthesis and phosphorylation of StAR, thus increasing the flow of free cholesterol into the mitochondria.¹¹ All of these actions are mediated by cAMP and occur within minutes, constituting the "acute" effect of ACTH on steroidogenesis.²⁸ The adrenal contains relatively modest amounts of steroid hormones; thus release of preformed cortisol does not contribute significantly to the acute response to ACTH; acute responses occur by the rapid provision of large supplies of cholesterol to mitochondrial P450_{scc}.^{11,28}

The long-term "chronic" effects of ACTH are mediated directly at the level of the steroidogenic enzymes. ACTH via cAMP stimulates the accumulation of the steroidogenic enzymes and their mRNAs by stimulating the transcription of their genes.^{2,10} ACTH also increases adrenal blood flow, increasing the influx of oxygen and metabolic fuel and the delivery of newly secreted hormones to the circulation.⁹⁰ Thus ACTH increases both the uptake of the cholesterol substrate and its conversion to steroidal products. The stimulation of this steroidogenesis occurs at each step in the pathway, and not only at the rate-limiting step, P450_{scc}.

The role of ACTH and other peptides derived from POMC, in stimulating growth of the adult adrenal, is supported by the observations that lack of pituitary POMC causes severe adrenal hypoplasia, and chronic ACTH excess causes adrenal hyperplasia. In the fetal adrenal, ACTH stimulates the local production

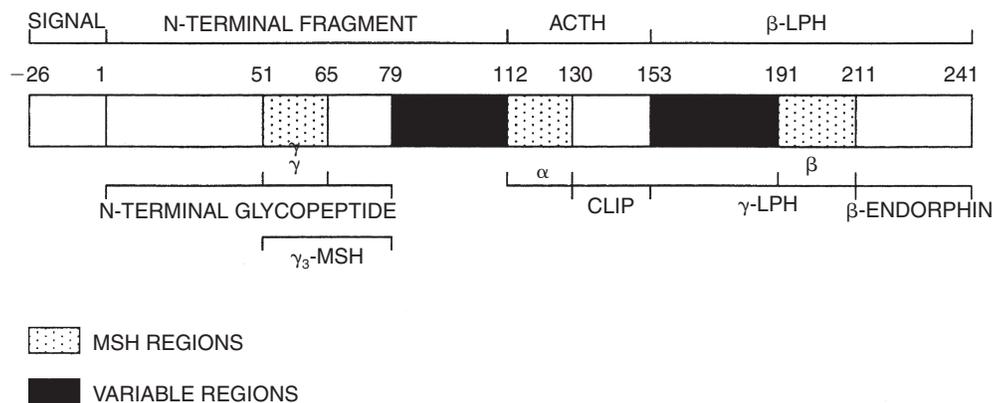


Fig. 14.8 Structure of human preproopiomelanocortin (POMC). The numbers refer to amino acid positions, with No. 1 assigned to the first amino acid of POMC after the 26-amino acid signal peptide. The α -, β -, and γ -MSH regions, which characterize the three "constant" regions, are indicated by diagonal lines; the "variable" regions are solid. The amino acid numbers shown refer to the N-terminal amino acid of each cleavage site; because these amino acids are removed, the numbers do not correspond exactly with the amino acid numbers of the peptides as used in the text. ACTH, Adrenocorticotrophic hormone; CLIP, corticotropin-like intermediate lobe peptide; LPH, lipotropic hormone; MSH, melanocyte-stimulating hormone.

of IGF-2, FGF2, and EGF. These, and possibly other factors work together to mediate ACTH-induced growth of the fetal adrenal.⁵

Diurnal Rhythms of Adrenocorticotrophic Hormone and Cortisol

Plasma concentrations of ACTH and cortisol tend to be high in the morning and low in the evening. Peak ACTH levels are usually seen at 4 to 6 AM and peak cortisol levels follow at about 8 AM. Both ACTH and cortisol are released episodically in pulses every 30 to 120 minutes throughout the day, but the frequency and amplitude of these is much greater in the morning. The basis of this diurnal rhythm is complex and poorly understood. The hypothalamic content of CRF itself shows a diurnal rhythm with peak content at about 4 AM. At least four factors appear to play a role in the rhythm of ACTH and cortisol: intrinsic rhythmicity of synthesis and secretion of CRF by the hypothalamus; light/dark cycles; feeding cycles; and inherent rhythmicity in the adrenal, possibly mediated by adrenal innervation. These factors are clearly interdependent and related. Dietary rhythms may play as large a role as light/dark cycles. Animal experiments show that altering the time of feeding can overcome the ACTH/cortisol periodicity established by a light/dark cycle. In normal human subjects, cortisol is released before lunch and supper, but not at these times in persons eating continuously during the day. Thus glucocorticoids, which increase blood sugar, appear to be released at times of fasting and are inhibited by feeding.

As all parents know, infants do not have a diurnal rhythm of sleep or feeding. Infants acquire such behavioral rhythms in response to their environment long before they acquire a rhythm of ACTH and cortisol. The diurnal rhythms of ACTH and cortisol begin to be established at 6 to 12 months and often are not well established until after 3 years of age. Once the rhythm is well established in the older child or adult, it is changed only with difficulty. When people move to different parts of the world, their ACTH/cortisol rhythms generally take 15 to 20 days to adjust appropriately. Reentrainment of the circadian clock following jet lag requires approximately 1 to 2 hours/day, more for eastward and less for westward travel.⁹¹

Physical stress (such as major surgery, severe trauma, blood loss, high fever, or serious illness) can increase the secretion of both ACTH and cortisol, but minor surgery and minor illnesses (such as upper respiratory infections) have little effect on ACTH and cortisol secretion.⁹²⁻⁹⁶ Infection, fever, and pyrogens can stimulate the release of cytokines, such as interleukin (IL)-1 and IL-6, which stimulate secretion of corticotropin-releasing hormone (CRH), and also stimulate IL-2 and tumor necrosis factor (TNF), which stimulate release of ACTH, providing further stimulus to cortisol secretion during inflammation⁹⁷; furthermore, IL-6 can directly stimulate adrenal synthesis and release of cortisol.⁹⁸ Conversely, glucocorticoids inhibit cytokine production in the immune system, providing a negative feedback loop. Most psychoactive drugs, such as anticonvulsants, neurotransmitters, and antidepressants, do not affect the diurnal rhythm of ACTH and cortisol, although cyproheptadine (a serotonin antagonist) can suppress ACTH release.

Adrenal: Glucocorticoid Feedback

The HPA axis is a classic example of an endocrine feedback system. ACTH increases production of cortisol, and cortisol decreases production of ACTH. Cortisol and other glucocorticoids exert feedback inhibition of both CRF and ACTH (and AVP), principally through the GR. Like the acute and chronic phases of the action of ACTH on the adrenal, there are acute and chronic phases of the feedback inhibition of ACTH (and

presumably CRF). The acute phase, which occurs within minutes, inhibits release of ACTH (and CRF) from secretory granules. With prolonged exposure, glucocorticoids inhibit ACTH synthesis by directly inhibiting the transcription of the gene for POMC (and AVP), which can result in secondary adrenal insufficiency. Some evidence also suggests that glucocorticoids can directly inhibit steroidogenesis at the level of the adrenal fasciculata cell itself, but this appears to be a physiologically minor component of the regulation of cortisol secretion.

Mineralocorticoid Secretion: The Renin-Angiotensin System

Renin is a serine protease enzyme synthesized primarily by the juxtaglomerular cells of the kidney, but it is also produced in a variety of other tissues, including the glomerulosa cells of the adrenal cortex. The role of adrenally produced renin is not well established; it appears to maintain basal levels of P450c11AS, but it is not known if angiotensin II is involved in this action. Renin is synthesized as a precursor of 406 amino acids that is cleaved to prorenin (386 amino acids) and finally to the 340-amino acid protein found in plasma.⁹⁹ Decreased blood pressure, upright posture, sodium depletion, vasodilatory drugs, kallikrein, opiates, and β -adrenergic stimulation all promote release of renin. Renin enzymatically attacks angiotensinogen, the renin substrate, in the circulation. Angiotensinogen is a highly glycosylated protein, and therefore has a highly variable molecular weight from 50,000 to 100,000 daltons. Renin proteolytically releases the amino-terminal 10 amino acids of angiotensinogen, referred to as *angiotensin I*. This decapeptide is biologically inactive until converting enzyme, an enzyme found primarily in the lungs and blood vessels, cleaves off its two carboxy-terminal amino acids, to produce an octapeptide, termed *angiotensin II*. Angiotensin II binds to specific membrane receptors located in the zona glomerulosa of the adrenal cortex to stimulate aldosterone production. Angiotensin-converting enzyme can be inhibited by captopril and related agents; alternatively angiotensin II receptors may be blocked by pharmacologic agents, such as candesartan for the diagnosis and treatment of (hyperreninemic) hypertension.

Angiotensin II has two principal actions, both of which increase blood pressure. It directly stimulates arteriolar vasoconstriction within a few seconds and it stimulates synthesis and secretion of aldosterone within minutes. Increased plasma potassium is a highly potent, direct stimulator of aldosterone synthesis and release. Aldosterone, secreted by the glomerulosa cells of the adrenal cortex, has the greatest mineralocorticoid activity of all naturally occurring steroids. Aldosterone causes renal potassium excretion and sodium retention, with a consequent increase in intravascular volume and blood pressure. Expansion of the blood volume provides the negative feedback signal for regulation of renin and aldosterone secretion. Angiotensin II binds to AT₁, its G protein-coupled receptor (G_q) to stimulate production of phosphatidylinositol, mobilize intracellular and extracellular Ca⁺⁺, and activate signaling via PKC and the calcium-calmodulin system.¹⁰⁰ These intracellular second messengers stimulate transcription of the *CYP11A1* gene for P450c11 independently of the actions of ACTH and cAMP. Increases in plasma potassium directly regulate aldosterone production by depolarizing zona glomerulosa cells, which leads to an influx of Ca⁺⁺ through voltage-dependent calcium channels. Thus angiotensin II and increased concentrations of potassium converge on the same intracellular second messenger pathway to regulate aldosterone synthesis and secretion. Although the renin-angiotensin system is clearly the major regulator of mineralocorticoid secretion, ACTH (and possibly

other POMC-derived peptides) can also promote secretion of aldosterone. Ammonium ion, hyponatremia, dopamine antagonists, and some other agents can also stimulate secretion of aldosterone, and atrial natriuretic factor is a potent physiologic inhibitor of aldosterone secretion.

The trophic roles of angiotensin II and hyperkalemia are supported by animal studies showing a dynamic expansion of the zona glomerulosa in response to chronic volume depletion, MR blockade, a low-sodium diet, and/or a high-potassium diet. In contrast, chronic volume expansion, as occurs with a high-sodium diet and/or hypokalemia, inhibits growth of the zona glomerulosa.¹⁰¹

Adrenal Androgen Secretion and the Regulation of Adrenarche

DHEA, DHEAS, and androstenedione, which are almost exclusively secreted by the adrenal zona reticularis, are generally referred to as adrenal androgens because they can be peripherally converted to testosterone. However, these steroids have little if any capacity to bind to and activate androgen receptors, hence they are only androgen precursors, and not true androgens. The fetal adrenal secretes large amounts of DHEA and DHEAS, and these steroids are abundant in the newborn, but their concentrations fall rapidly as the fetal zone of the adrenal involutes following birth. After the first year of life, the adrenals of young children secrete very small amounts of DHEA, DHEAS, and androstenedione until the onset of adrenarche, usually around age 7 to 8 years, preceding the onset of puberty by about 2 years. Adrenarche is independent of puberty, the gonads, or gonadotropins, and the mechanism by which the onset of adrenarche is triggered remains unknown. The secretion of DHEA and DHEAS continues to increase during and after puberty and reach maximal values in young adulthood, following which there is a slow, gradual decrease in the secretion of these steroids in the elderly ("adrenopause") (Fig. 14.9).¹⁰² Men have higher serum concentrations of DHEAS than women,¹⁰² probably because men have a single copy of the X-linked steroid sulfatase gene.¹⁰³ Throughout much of adult life, adrenal secretion of DHEAS exceeds that of cortisol; in adult women, adrenal secretion of androgen precursors and androgens is equal to their secretion from the ovary. Despite the huge increases in the adrenal secretion of DHEA and DHEAS during adrenarche, circulating concentrations of ACTH and cortisol do not change with age. Thus ACTH plays a permissive role in adrenarche but does not trigger it. Searches for hypothetical polypeptide hormones that might specifically stimulate the zona reticularis have been unsuccessful. Adrenarche is a unique phenomenon confined to few

higher primates, such as chimpanzees or orangutans, but the significance of adrenarche remains unknown.

Recent studies of adrenarche have focused on the roles of 3 β HSD and P450c17. The abundance of 3 β HSD in the zona reticularis appears to decrease with the onset of adrenarche, and the adrenal expression of cytochrome b₅, which fosters the 17,20 lyase activity of P450c17,^{37,38} is almost exclusively confined to the zona reticularis; these factors strongly favor the production of DHEA.¹⁰⁴ The phosphorylation of P450c17, apparently by p38 α ,³⁹ also increases 17,20 lyase activity, but its potential role in adrenarche remains unexplored. Premature and exaggerated adrenarche may be associated with insulin resistance and/or being overweight, and girls with premature exaggerated adrenarche appear to be at much higher risk of developing the polycystic ovary syndrome as adults (characterized by hyperandrogenism, fewer ovulatory cycles, insulin resistance, and hypertriglyceridemia). Recent evidence suggests that infants born small for gestational age may be at increased risk for this syndrome. Thus studies of physiology, biochemistry, and clinical correlates of adrenarche suggest premature adrenarche may be an early sign of a metabolic disorder. The suggestion that replacing the DHEA may improve memory and a sense of well-being in the elderly and in adrenal insufficiency remains controversial.¹⁰⁵

Whereas it has long been thought that DHEA, DHEAS, and androstenedione are "adrenal androgens," recent work has shown that the principal adrenal androgens are 11-oxygenated (Oxo) steroids, mainly 11-keto-testosterone (11-KT).^{106,107} In addition to being expressed in the zona fasciculata, P450c11 β , the classic 11-hydroxylase that converts 11-deoxycortisol to cortisol, is expressed in the zona reticularis, where it converts androstenedione and testosterone to 11OH-androstenedione and 11OH-testosterone, respectively. These 11-Oxo steroids can be converted into the nonaromatizable 11-keto androstenedione and 11-KT by HSD11B2 (Fig. 14.10). 11-KT may also be 5 α -reduced to 11-keto dihydrotestosterone (11-KDHT); 11-KT and 11-KDHT are androgens that bind and activate the androgen receptor with affinities similar to those of testosterone and DHT;^{108–111} these androgens are found at similar levels in men and woman,¹⁰⁹ are higher in the adrenal vein than in peripheral veins,¹¹² and are elevated in CAH and polycystic ovary syndrome. Thus 11-KT appears to be the dominant circulating bioactive androgen during both normal and premature adrenarche,¹¹³ as well as in classic CAH.^{112,114} These 11-Oxo steroids correlate with adrenal size and may be useful biomarkers for adrenal rest tumors in CAH patients.¹¹⁵

PLASMA STEROIDS AND THEIR DISPOSAL Structure and Nomenclature

All steroid hormones are derivatives of pregnenolone (Fig. 14.11). Pregnenolone and its derivatives that contain 21 carbon atoms are often termed C21 steroids. Each carbon atom is numbered, indicating the location at which the various steroidogenic reactions occur (e.g., 21-hydroxylation, 11-hydroxylation). The 17,20 lyase activity of P450c17 cleaves the bond between carbon atoms 17 and 20, yielding C19 steroids, which include all the androgens; P450aro converts C19 androgens to C18 estrogens. With the exception of estrogens, all steroid hormones have a single unsaturated carbon-carbon double bond. Steroids having this double bond between carbon atoms 4 and 5, including all the principal biologically active steroids, are termed Δ^4 steroids; their precursors having a double bond between carbon atoms 5 and 6 are termed Δ^5 steroids. The two isozymes of 3 β HSD convert Δ^5 to Δ^4 steroids.

There is a logically systematic, unambiguous chemical terminology to describe accurately the structure of all the steroid

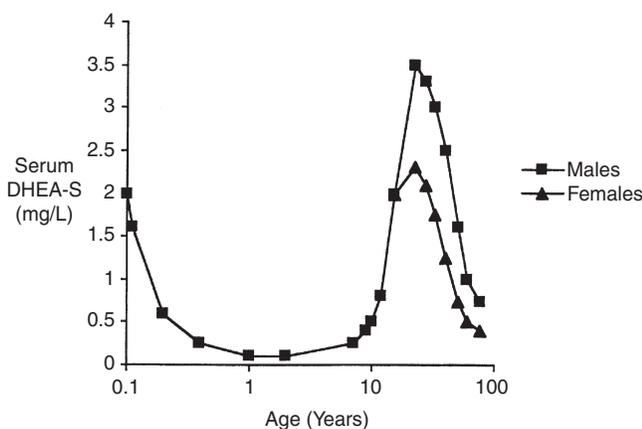


Fig. 14.9 Concentrations of dehydroepiandrosterone sulfate as a function of age. Note that the x-axis is on a log scale.

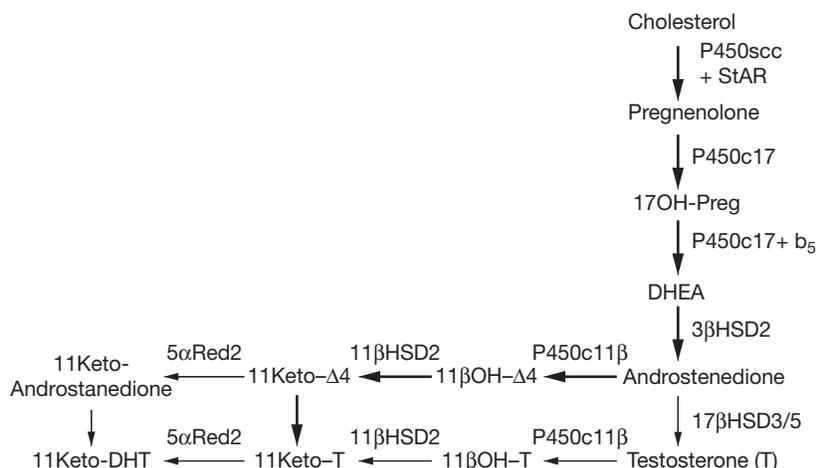


Fig. 14.10 Adrenal androgen synthesis. The traditional view of adrenal androgen synthesis is cholesterol→pregnenolone→17OH-pregnenolone (17OH-Preg)→DHEA→androstenedione, as shown on the right. However, rather than being androgens, dehydroepiandrosterone (DHEA), DHEA sulfate (S), and androstenedione have virtually no capacity to bind to and transactivate the androgen receptor, and hence are simply C19 (19-carbon) precursors of androgens. Small amounts of androstenedione may be converted to testosterone by adrenal 17 β -hydroxysteroid dehydrogenase 5 (17 β HSD5) (AKR1C3), but the principal androgenic steroid secreted by the adrenal is 11-keto-testosterone (11Keto-T). Both androstenedione and testosterone may be 11-hydroxylated by P450c11 β to yield 11OH-androstenedione (11OH- Δ 4 and 11OH-testosterone (11OH-T), respectively. These steroids can be oxidized by 11 β HSD (the same enzyme that oxidizes of cortisol to cortisone) to 11-keto-androstenedione (11Keto- Δ 4) and 11-keto-testosterone (11Keto-T), respectively. Although 11Keto-T is the principal androgen in the adrenal vein, it may be 5 α -reduced by 5 α -reductase type 2 in peripheral tissues and possibly also by 5 α -reductase type 1 in the adrenal itself, to 5 α -dihydrotestosterone, respectively. The quantitatively predominant pathway is androstenedione→11OH- Δ 4→11Keto- Δ 4→11-Keto-T, as indicated by the bold arrows. (© W.L. Miller)

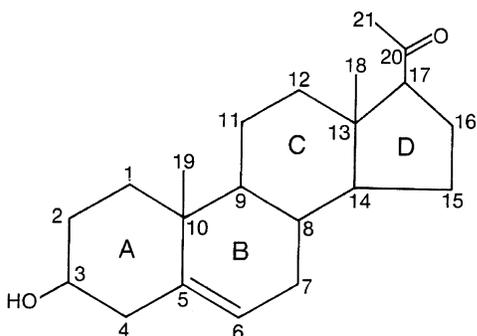


Fig. 14.11 Structure of pregnenolone. The carbon atoms are indicated by numbers and the rings are designated by letters according to standard convention. Pregnenolone is derived from cholesterol, which has a 6-carbon side chain attached to carbon #21. Pregnenolone is a “ Δ^5 compound,” having a double bond between carbons #5 and 6; the action of 3 β -hydroxysteroid dehydrogenase/isomerase moves this double bond from the B ring to carbons #4 and 5 in the A ring, forming Δ^4 compounds. All of the major biologically active steroid hormones are Δ^4 compounds.

hormones and all their conceivable derivatives. However, this terminology is unbelievably cumbersome (e.g., cortisol is 11 β ,17 α ,21-trihydroxy-pregn-4-ene-3,20-dione, and dexamethasone is 9 α -fluoro-11 β ,17 α ,21-trihydroxypregna-1,4-diene-3,20-dione). Therefore we use only the standard “trivial names.” Before the structures of the steroid hormones were determined in the 1930s, Reichstein, Kendall, and others identified them as spots on paper chromatograms and designated them A, B, C, and so on. Unfortunately, some persist in using this outmoded terminology more than 80 years later, so that corticosterone is sometimes termed compound B, cortisol compound F, and 11-deoxycortisol compound S. This archaic terminology obfuscates the precursor-product relationships of the steroids, confuses students, and should not be used.

Circulating Steroids

Although over 50 different steroids have been isolated from adrenocortical tissue, the main pathways of adrenal steroidogenesis include only a dozen or so steroids, of which only a few are secreted in sizable quantities. The adult secretory rates for DHEA and cortisol are each about 20 mg/24 hours and the secretion of corticosterone, a weak glucocorticoid, is about 2 mg/24 hours. Although glucocorticoids, such as cortisol, and mineralocorticoids, such as aldosterone, are both needed for life and hence are of physiologic importance, diagrams, such as Fig. 14.3, fail to indicate that these steroids are not secreted in molar equivalents. The adult secretion rate of aldosterone is only about 0.1 mg/24 hours. This 100- to 1000-fold molar difference in the secretory rates of cortisol and aldosterone must be borne in mind when considering the effects of steroid-binding proteins in plasma and when conceptualizing the physiologic manifestations of incomplete defects in steroidogenesis.

Most circulating steroids are bound to plasma proteins, including corticosteroid-binding globulin (CBG), albumin, and α_1 acid glycoprotein.^{116,117} CBG has a very high affinity for cortisol but a relatively low binding capacity albumin has a low affinity and high capacity and α_1 acid glycoprotein is intermediate for both variables. The result is that about 90% of circulating cortisol is bound to CBG and a little more is bound to other proteins. Interestingly, absence of CBG does not cause a detectable physiologic disorder. Therefore these plasma proteins are thought to act as a nonessential reservoir for steroids that facilitates broad and even distribution of cortisol throughout the body. Most synthetic glucocorticoids used in therapy do not bind significantly to CBG and bind poorly to albumin, partially accounting for their increased potencies, which are also associated with increased receptor-binding affinities. Aldosterone is not bound well by any plasma protein; hence changes in plasma protein concentration do not affect plasma aldosterone concentrations, but greatly influence plasma cortisol concentrations. Estradiol and testosterone bind

strongly to a different plasma protein termed sex steroid-binding globulin and also bind weakly to albumin.

Because steroids are hormones, it is often thought that the concentration of “free” (i.e., unbound) circulating steroids determines biologic activity. However, the target tissues for many steroid hormones contain enzymes that modify those steroids. Thus many actions of testosterone are actually caused by DHT produced by local 5α -reductase. Similarly, cortisol will have differential actions on various tissues because of the presence or absence of the two isozymes of 11β HSD, which can inactivate cortisol to cortisone or reactivate cortisone back to cortisol. Analogous peripheral metabolism occurs via “extraglandular” 21 -hydroxylase, $P450_{aro}$, 3β HSD, and 17β HSD. Thus circulating steroids are both classic hormones and precursors to locally acting autocrine or paracrine factors.

Steroid Catabolism

Only about 1% of circulating plasma cortisol and aldosterone are excreted unchanged in the urine; the remainder is metabolized by the liver. Technologies, such as labeled tracers and mass spectroscopy, have enhanced our understanding of adrenal physiology and have advanced clinical care. Understanding the processes and pathways of steroid metabolism can facilitate more rapid and precise diagnoses and avoid unwanted side effects from drugs. Liquid chromatography-mass spectrometry (LC-MS/MS) can now specifically and simultaneously identify and quantify major and minor metabolites. The major enzymes that metabolize steroids are summarized later.

Major Enzymes That Metabolize Steroids

The liver is the primary site of glucocorticoid metabolism. Cortisol is reduced, oxidized, or hydroxylated and then conjugated with sulfate or glucuronic acid, rendering it water soluble, and is excreted in the urine. 3α -Hydroxysteroid dehydrogenase (e.g., AKR1C2 and AKR1C4) is the major enzyme group that reduces cortisol at the 3-keto group, and 5α - or 5β -reductases (SRD5A1 and AKR1D1, respectively) reduce the 4-5 double bond in the A ring. The tetrahydrocortisols and tetrahydrocortisones that result from these reactions can be reduced by 20 -hydroxysteroid dehydrogenases, producing cortols and cortolones. Depending on cofactor availability, 11β -hydroxysteroid dehydrogenase type 1 (HSD11B1) can have either dehydrogenase (inactivation) or reductase (activation) activities, but in the liver, this enzyme usually functions as a reductase to activate cortisone to cortisol.^{68,118} Cortisol, and the metabolites generated in the reactions described earlier, are oxidized. This process removes the C20-C21 side chain, resulting in C19 steroids with a 17-ketone group, but the enzyme that drives this reaction has not yet been identified. CYP3A4 can 6β -hydroxylate cortisol; when cortisol levels are normal, the rate of this reaction is very low.¹¹⁹ However, when cortisol levels are elevated, the rate of the reaction increases, which can make the measurement of urinary 6β -hydroxycortisol a useful adjunct test for excess glucocorticoids. Finally, uridine diphosphoglucuronosyl transferases can conjugate glucuronic acid or sulfate to C19 and C21 metabolites, increasing their water solubility and facilitating their urinary excretion.¹²⁰

Similarly to cortisol, aldosterone is reduced and conjugated in the liver. $4,3$ -Ketosteroid-reductase (which also has 5β -hydroxysteroid reductase activity) and a 3α -hydroxysteroid dehydrogenase are the major enzymes that result in the conversion of aldosterone to 3α , 5β -tetrahydroaldosterone.¹²¹ Tetrahydroaldosterones are conjugated to glucuronic acid at the 3-keto position (which increases water solubility) and is the major metabolite of aldosterone that is excreted in the urine.

DHEA is the adrenal steroid produced in greatest quantity. DHEA is converted to androstenedione and 5α -reduced to androsterone, which is then converted to etiocholanolone by 5β -reductase. Finally, 17β -hydroxysteroid dehydrogenases reduce etiocholanolones into -diol derivatives, which can be conjugated and excreted in the urine. However, fecal excretion of DHEA and its metabolites is higher than other steroids. The sulfate ester of DHEA (DHEAS), on the other hand, can be directly excreted in the urine. Although these pathways can seem somewhat esoteric, as illustrated later, these pathways can be perturbed by common therapies and conditions, hence they need to be considered in clinical diagnostic and treatment plans.

Examples of Drugs That Alter Steroid Metabolism

Thyroid Hormone accelerates cortisol metabolism by inducing hepatic 5α - and 5β -reductase activity and inhibiting CYP3A enzymes.¹²² The converse process occurs in patients with hypothyroidism. In both situations, serum cortisol levels are usually normal because the HPA axis remains intact. However, in cases of hypopituitarism, it is prudent to replace cortisol before initiating thyroid hormone replacement, to avoid an acute adrenal crisis because of accelerated cortisol clearance.

Spirolactone increases serum aldosterone levels, in part by inhibition of 18 -glucuronidation of aldosterone.

Troglitazone induces CYP3A4 activity, disrupting cortisol metabolism.¹²³ Although neither rosiglitazone nor pioglitazone appear to affect CYP3A4,¹²⁴ thiazolidinedione drugs generally inhibit steroidogenic $P450_{c17}$ and HSD3B2.^{125,126}

Drugs That Alter P450 Enzymes. The results of a randomized control trial supported reports that consuming *Hypericum perforatum* (St. John’s Wort, a common herbal remedy) decreased efficacy of estradiol-based oral contraception. St. John’s Wort induces CYP3A4, CYP2C19, and CYP2C9, accelerating the metabolism of the oral contraceptives, decreasing their effectiveness.^{127,128} In contrast, the antifungal drug ketoconazole acts principally by inhibiting CYP51 (lanosterol demethylase), but it also inhibits several steroidogenic P450 enzymes and has been used as adjunctive therapy in prostate cancer¹²⁹ and Cushing syndrome.¹³⁰

Examples of Disorders That Alter Steroid Metabolism

Stress: Surprisingly, elevations in ACTH levels are usually a transient processes, even when stress is prolonged. The persistently elevated cortisol levels during chronic stress are caused by stimulating cytokines and diminished clearance of cortisol through enzymatic inactivation by 5α - and 5β -reductases and by HSD11B2.¹³¹

Cushing Syndrome: It is thought that when glucocorticoid levels remain persistently elevated, the oxidation and reduction pathways that metabolize them become saturated and CYP3A4 is induced. This results in increased levels of 6β -hydroxylation (and concomitantly lower levels of cortols, cortolones, tetrahydrocortisone, and 5α -tetrahydrocortisol) excreted, which can be confirmed by analysis of the urine.¹¹⁹ Elevations in the levels of glucocorticoids also stimulate hepatic HSD11B2 activity.

Obesity: Obese individuals have elevated levels of cortisol metabolites in their urine, even when normalized for body surface area.¹³² It is important to distinguish this process from Cushing syndrome. In obese patients, serum cortisol levels are normal as the higher excretion rate is matched by higher production rates.

Insulin Resistance: Individuals with insulin resistance, including those with hyperinsulinemia, have increased levels of hepatic SRD5A1 activity, increasing urinary excretion of 5α -reduced metabolites of cortisol.¹³³

Renal Disease: Although metabolism of cortisol is usually normal in patients with renal disease, clearance of glucuronides can be reduced, causing accumulation of the inactive conjugated compounds in the circulation.¹³⁴ This process could potentially be used to monitor disease but is not thought to contribute to pathology.

Heart Failure: Patients with severe congestive heart failure and impaired perfusion of the liver also have impaired aldosterone metabolism, resulting in lower levels of urinary tetrahydroaldosterone glucuronide.¹³⁵

CLINICAL AND LABORATORY EVALUATION OF ADRENAL FUNCTION

Clinical Evaluation

Astute clinical evaluation can generally reveal the presence of primary adrenal deficiency or hypersecretion before performing laboratory tests. Thomas Addison described adrenal insufficiency in 1849, long before immunoassays became available. Virtually all patients with chronic adrenal insufficiency will have weakness, fatigue, anorexia, weight loss, hypotension, and hyperpigmentation. Patients with acute adrenal insufficiency may have hypotension, shock, weakness, apathy, confusion, anorexia, nausea, vomiting, dehydration, abdominal or flank pain, hyperthermia, and/or hypoglycemia. Deficient adrenal androgen secretion will compromise the acquisition of virilizing secondary sexual characteristics (pubic and axillary hair, acne, axillary odor) in female adolescents. Early signs of glucocorticoid excess include increased appetite, weight gain, mood changes, and growth arrest without a concomitant delay in bone age. Chronic glucocorticoid excess in children results in typical Cushingoid facies and muscle wasting, but the “buffalo hump” and centripetal distribution of body fat that are characteristic of adult Cushing disease are seen only in long-standing undiagnosed disease in children. Mineralocorticoid excess is mainly characterized by hypertension, but patients receiving very low sodium diets (e.g., the newborn) will not be hypertensive, as mineralocorticoids increase blood pressure primarily by retaining sodium and thus increasing intravascular volume. Moderate hypersecretion of adrenal androgens is characterized by mild signs of virilization, whereas substantial hypersecretion of adrenal androgens is characterized by accelerated growth, with a disproportionate increase in bone age, increased muscle mass, acne, hirsutism, and deepening of the voice. A key feature of any physical examination of a virilized male is careful examination and measurement of the testes. Bilaterally enlarged testes suggest true (central) precocious puberty; unilateral testicular enlargement suggests testicular tumor; prepubertal testes in a virilized male indicate an extratesticular source of androgen, such as the adrenal.

Imaging studies are of limited utility in adrenocortical disease. Computed tomography (CT) will only rarely detect pituitary tumors hypersecreting ACTH, and magnetic resonance imaging (MRI) will detect less than half of these, even with gadolinium enhancement. The small size, odd shape, and location near other structures also compromise the use of imaging techniques for the adrenals. CT is currently the first choice of imaging the adrenal gland; while entailing radiation exposure, it has the advantage of higher resolution compared with MRI. Patients with Cushing disease or CAH will have modestly enlarged adrenals, but such enlargement is not detectable by imaging techniques with any useful degree of certainty. Imaging studies may be of assistance in diagnosing gross enlargement of the adrenals in congenital lipoid adrenal hyperplasia, their hypoplasia in adrenal hypoplasia congenita, or in the hereditary ACTH unresponsiveness syndromes, and with many malignant tumors; however, many adrenal

adenomas are too small (<2 mm) to be detected. Thus imaging studies may establish the presence of pituitary or adrenal tumors, but they can never establish their absence. Complementary functional tests, with radiopharmaceuticals targeted to the adrenal cortex and medulla, may enhance the diagnostic utility of imaging studies and are used in combination with scintigraphy, positron emission tomography (PET), and single-photon emission computed tomography (SPECT).

Catheter-directed hormonal sampling is rarely performed in children. Petrosal sinus sampling may be of use in suspected Cushing disease, when imaging studies fail to reveal the pituitary ACTH-secreting tumor. Adrenal vein sampling may distinguish unilateral from bilateral hypersecretory adrenal adenomas (e.g., hyperaldosteronism, Conn syndrome), but these are usually seen in adults.

Laboratory Evaluation

The diagnostic evaluation of adrenal function is essentially chemical. The nonspecificity of many of the clinical signs described earlier and the disappointing results with imaging studies remind us that any proper evaluation of HPA function must rely on a series of carefully performed physiologic maneuvers associated with hormonal assays. The development of highly specific, exquisitely sensitive assays that can be done on small volumes of blood now permit the direct examination of virtually every hormone involved in adrenal metabolism. In addition, steroid profiling and analysis of serum and urine samples, using chromatographic, mass-spectrometric methods, with which conventional and novel steroid metabolites may be detected simultaneously from a single sample, has developed into a powerful tool to identify characteristic steroidal signatures of complex disorders.

Measurement of Steroids

Measurement Techniques. Steroids are typically measured by immunoassays (IAs) or mass spectrometry (MS) methods. IAs are the traditional technique used in most routine laboratories: they are easy, fast, cheap, and widely available. IAs are limited by their specificity, which is limited by (1) a size mismatch between small steroid molecules and the binding fragment of the immunoglobulin, (2) cross-reactivity of antisera to related steroids, and (3) the quality of the antisera themselves. Furthermore, matrix effects caused by cross-reacting nonsteroidal molecules, other lipophilic compounds, or binding proteins in unprocessed samples may also affect specificity of IAs. This effect may be ameliorated by sample purification by liquid or solid phase extraction, chromatography, or precipitation. However, direct IAs on automated high-throughput instruments have pushed IAs to their limits.¹³⁶ Although the reliability of IAs has been questioned recently, especially for measuring sex steroids,^{136,137} validated IAs performed properly remain a valuable tool for clinical steroid measurements.^{136,138,139}

MS may be the most versatile and precise method for the qualitative and quantitative measurement of steroids.¹³⁹ Chromatographic methods (gas or liquid) serve as separation techniques, and the mass spectrometer serves as the detector; together, these methods achieve the highest analytical specificity and selectivity. In contrast to IAs, where one test measures a single steroid, a single run in an MS-based assay provides a steroid profile of multiple steroids. Depending on the specific methods applied, instruments may be run in a nondiscriminatory, untargeted scanning mode, which is typically used for research studies. In contrast, in clinical applications, the instruments record preselected ions corresponding to specific steroid analytes. Quantitative analysis of steroids requires

internal standards labeled with stable isotopes; these are now commercially available for most steroids of interest, including the newly identified steroid metabolites derived from the “backdoor” and 11-Oxo pathways. In general, gas chromatography followed by mass spectrometry (GC-MS) is the ideal method for separating steroids and is widely used in research laboratories for characterizing steroid metabolites in new or complex disorders of steroidogenesis. However, GC-MS/MS requires sample derivatization, it is complex and time-consuming, and is not suitable for high-throughput analysis of clinical samples. In contrast, LC-MS/MS is easier, faster, and can analyze sulfated and glucuronidated steroids. Thus LC-MS/MS is used as a high-throughput method for steroid analysis and has now become the method of choice. However, all MS-based methods require knowledge of complex analytics and biochemistry and hence are only established in specialized laboratories. Interpreting a steroid profile of a serum or urine sample is not trivial. Concentrations or excretion rates of multiple metabolites are compared with reference values and/or precursor:product ratios calculated as surrogates of enzyme activities.¹⁴⁰ Diagnosis of complex steroid disorders requires pattern recognition from quantitative datasets of multiple steroids. This may be facilitated by unbiased mathematical approaches (“machine learning”) and has been used for some steroid disorders.¹⁴¹

Plasma/Serum Concentrations of Cortisol and Other Steroids. Plasma cortisol may be measured by IAs, such as radioimmunoassay (RIA), immunoradiometric assay (IRMA), and electrochemiluminescence assay (ECLIA), the latter being the preferred method in automated high-throughput laboratories. Alternatively, cortisol can also be determined by LC-MS/MS in a standardized setting. It is essential to know what method one’s laboratory is using and precisely what this method is measuring. All immunoassays have some degree of cross-reactivity with other steroids. Most cortisol IAs will detect both cortisol and cortisone; in contrast, these are readily distinguished by mass spectrometric techniques. The newborn’s plasma contains mainly cortisone rather than cortisol, hence comparison of newborn data obtained by high-performance liquid chromatography (HPLC) or LC-MS/MS to published standards obtained by IAs may incorrectly suggest adrenal insufficiency. Tables 14.2 and 14.3 summarize the normal plasma concentrations for a variety of steroids measured by LC-MS/MS (<https://www.endocrinesciences.com/>). With the notable exception of DHEAS, most adrenal steroids exhibit a diurnal variation, based on the diurnal rhythm of ACTH. Because the stress of illness or hospitalization can increase adrenal steroid secretion and because diurnal rhythms may not be well established in children under 3 years of age, it is best to obtain two or more samples for the measurement of any steroid.

Data exist for the concentrations of a large number of steroid hormones throughout normal infancy, childhood, and adolescence (see Tables 14.2 and 14.3). Not all endocrine laboratories perform all of these assays, and, depending on the assay procedures used, various laboratories may have different “normal” values. Most central hospital and commercial laboratories are designed primarily to serve adult, rather than pediatric, patients. Thus it is important to know whether the available assays will be sufficiently sensitive with small volumes of blood to be useful in measuring pediatric values. This is especially true for the measurement of sex steroids (and gonadotropins), which can exhibit pathological elevations in children and still remain below the limit of detection of most “adult” assays. Efforts to achieve standardization of analytical methods and normative values are active topics in the field.^{138,139}

Urinary Steroid Excretion. Measurement of the 24-hour urinary excretion of steroid metabolites, one of the oldest procedures for assessing adrenal function, has recently been revived by novel analytical mass spectrometric methods. Examination of total 24-hour urinary steroid excretion eliminates the diurnal fluctuations in serum steroid concentrations, and the variations attributable to episodic bursts of ACTH and transient stress (such as a visit to the clinic or difficult venipuncture). Collection of a complete 24-hour urine sample can be difficult in the infant or small child, hence urine should also be assayed for creatinine to monitor the completeness of the collection. Because of the diurnal and episodic nature of steroid secretion, one should never infer the 24-hour excretory rate from partial collections. This is important when assessing the absolute excretion rate of a single steroid, such as free cortisol. In contrast, a spot urine (preferably the first morning void, which is most concentrated and has been produced under peak ACTH stimulation), in which multiple excreted steroids are quantified in relation to creatinine, may suffice for qualitative pattern diagnostic of a specific steroid disorder.

Contemporary procedures for urinary steroid analysis use chromatography for separating steroids, followed by an MS detection method, usually GC-MS/MS, permitting very sensitive and specific assays of urinary steroids. However, each steroid is metabolized to multiple forms before being excreted in urine, and this metabolism can vary with age and sex in pediatric populations, so that the analyses are complex and require specialized expertise that is not yet widely available.^{139,140} Urinary free cortisol (UFC) measurements are highly reliable for diagnosing Cushing syndrome in adults. Excretion of UFC and of total cortisol metabolites is closely correlated with age, body surface area, and adiposity, but are typically $11 \pm 5 \mu\text{g}/\text{m}^2/\text{day}$.^{142,143} Values vary substantially among different reference laboratories, reflecting variations in assay technologies, thus it is essential to use a laboratory with good data for normal children. It remains important to measure urinary creatinine to monitor the completeness of the collection.

Plasma Renin

Renin may be assayed by its enzymatic activity or by direct measurements of its concentration. Plasma renin activity (PRA) is simply an assay of the amount of angiotensin I generated per milliliter of serum, per hour at 37° C. In normal serum, the concentration of both renin and angiotensinogen (the renin substrate) are limiting. Another test, which is technically more challenging, directly measures plasma renin content (PRC) by immunoassay.

PRA is sensitive to dietary sodium intake, posture, diuretic therapy, activity, and sex steroids. Because PRA values can vary widely, it is best to measure PRA twice, once in the morning, after overnight supine posture, and then again after maintenance of upright posture for 4 hours. A simultaneous 24-hour urine for total sodium excretion is generally needed to interpret PRA results. Decreased dietary and urinary sodium, decreased intravascular volume, diuretics, and estrogens will increase PRA. Sodium loading, hyperaldosteronemia, and increased intravascular volume decrease PRA.

Renin measurements are used in the evaluation of hypertension and in the management of CAH. Assessment of the renin-angiotensin system is essential in children with simple virilizing (SV) CAH who lack clinical evidence of urinary salt loss (hyponatremia, hyperkalemia, acidosis, hypotension, shock), but who may nevertheless have increased PRA, especially when dietary sodium is restricted. This is a clinical sign that this form of 21OHD is simply a milder form of the more common salt-wasting form. Treatment of SV CAH with sufficient

TABLE 14.2 Sex Steroid Concentrations by Age

	Age	Prog	17OHP	DHEA	DHEA-S	Δ4A	E1	E2	T		DHT	
									F	M	F	M
	Unit	ng/dL	ng/dL	ng/dL	μg/dL	ng/dL	pg/mL	pg/mL	ng/dL	ng/dL	ng/dL	ng/dL
CHILDHOOD												
Preterms												
26–28 weeks	Postnatal D4		124–841	82–1484	123–882	63–935			5–16	59–125	2–13	10–53
31–35 weeks	Postnatal D4		26–568	56–1853	122–710	50–449			5–22	37–198	2–13	10–53
Terms	Postnatal D3		<78	41–1292	88–356	<10–279			20–64	75–400	<2–15	5–60
Infants												
	1 wk to 6 mo		13–106	<948	<112	<10–37	<15	<15	<10	20–50	<3	
	6–12 mo		13–106	<136	<49	<10–17	<15	<15	<10	<2.5–10	<3	<3
Minipuberty	2–3 mo		40–200 (M)					5–50		60–400		12–85
CHILDREN												
			<91			<10–17	<15	<15	<2.5–10	<2.5–10	<3	<3
	1–5 y			<68	<57							
	6–7 y			<111	<72							
	8–10 y			<186	<193							
MALES												
Pubertal	1–16 y	<10–15		<491								
	Tanner I		<91	<186	13–83	<10–17	<5–17	5–11		<2.5–10		<3
	Tanner II		<116	<202	42–109	<10–33	10–25	5–16		18–150		3–17
	Tanner III		10–138	<319	48–200	17–72	15–25	5–25		100–320		8–33
	Tanner IV		29–180	39–481	102–385	15–115	15–45	10–36		200–620		22–52
	Tanner V		24–175	40–491	120–370	33–192	20–45	10–36		350–970		24–65
Adult		<10–11	27–199				10–50	8–35		264–916		30–85
	20–49 y			31–701	16–523	44–186						
	>50 y			21–402	<298							
FEMALES												
Pubertal	Tanner I	<10–26	<83	<186	19–144	<10–17	4–29	5–20	<2.5–10		<3	
	Tanner II	<10–255	11–98	<202	34–129	<10–72	10–33	10–24	7–28		5–12	
	Tanner III	<10–852	11–115	<319	32–226	50–170	15–43	7–60	15–35		7–19	
	Tanner IV	<10–1076	18–230	39–481	58–260	47–208	16–77	21–85	13–32		4–13	
	Tanner V	<10–1294	20–265	40–491	44–248	50–224	29–105	34–170	20–38		3–18	
Adult				31–701	17–372	28–230			10–55		4–22	
	Follicular	<10–1563	15–70				30–100	30–100				
	Luteal	<10–2555	35–290				9–160	70–300				
	Peak (D17–23)	350–3750										
Postmenopausal		<10		21–402	<215	<10–93	<40	<15	7–40			

New steroid values in ng/dL available for adults only: 11-Hydroxy–testosterone, M 5.2–43.4, F <39.8; 11-Keto–testosterone, M 9.5–70.8, F 5–60.6; 11OH Δ 4A, M 36.4–313 F 19.2–333; androsterone, M <28, F <23.

All the steroids are measured by high-performance liquid chromatography mass spectrometry (HPLC-MS/MS).

All values are ranges in ng/dL, μg/dL, or pg/mL as indicated; to convert these values to nmol/L, multiply given values times the following factor: androstenedione, 0.035; DHEA, 0.035; DHT, 0.034; E₁, 0.0037; E₂, 0.0037; Prog, 0.032; 17OHP, 0.03; DHEA-S, 27.17; total T, 0.035; DHT, 0.034; androsterone, 0.034.

Δ4A, Androstenedione; 17OHP, 17 Hydroxyprogesterone; D4, day 4; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; E₁, estrone; E₂, estradiol; F, female; M, male; mo, month(s); PROG, progesterone; T, testosterone; y, year(s).

(From LabCorp (Laboratory Corporation of America)/Endocrine Sciences, Calabasas Hills, California, 2019.)

TABLE 14.3 Glucocorticoid and Mineralocorticoid Concentrations

	Age	Cortisol	Corticosterone	DOC	11-Deoxycortisol	21-Deoxycortisol	18OH Corticosterone	Aldosterone	Plasma Renin Activity
	Unit	μg/dL	ng/dL	ng/dL	ng/dL	ng/dL	ng/dL	ng/dL	ng/mL/h
CHILDHOOD									
Preterms									
									11000–167000
	26–28 weeks	1–11	235–1108	20–105	110–1376		10–670	5–635	
	31–35 weeks	2.5–9.1	150–1700	28–78	48–579		57–410	19–141	
	Term Newborns		<78						2000–35000
	Postnatal D3	1.7–14	70–850		13–147		31–546	7–184	
	Postnatal D7	2–11	70–850					5–175	
	Infants 1–12 mo	2.8–23	80–1500	7–49	<10–156		5–220	5–90	2000–37000
Children									
	8:00 AM 1–15 y	3–21	135–1860	2–34	12–158	<10			
	4:00 PM 1–15 y	ND	70–620						
	1–2 y						18–155	7–54 ^a	1700–11200
	3–9 y						6–85	5–80 ^b	500–6500
	10–14 y						10–72	4–48 ^b	500–3300
ADULTS									
	8:00 am	8–19	130–820	2–19	12–158	<10	9–58		167–5380
	4:00 pm	4–11	60–220						
	Supine/upright						4–21/5–46	ND/<31	

All values are given in ranges and were measured by high-performance liquid chromatography mass spectrometry (HPLC-MS/MS).

To convert given values to SI units nmol/L multiply by the following factors: cortisol, 27.59; corticosterone, 0.029; 18OH corticosterone, 0.028; aldosterone, 0.028; DOC, 0.03; 11DOC, 0.029; 21Deoxycortisol, 0.029.

Values in: ^asupine and ^bupright posture.

D3, Day 3; DOC, deoxycorticosterone; mo, month(s); ND, not determined; y, year(s).

(From LabCorp (Laboratory Corporation of America)/Endocrine Sciences, Calabasas Hills, California 2019.)

mineralocorticoid, to suppress PRA into the normal range, will reduce the child's requirement for glucocorticoids, thus maximizing final adult height and reducing exposure to excess glucocorticoids. Children with CAH need to have their mineralocorticoid replacement therapy monitored routinely by measuring PRA.⁴⁰ Measurement of angiotensin II is also possible in some research laboratories, but most antibodies to angiotensin II strongly cross-react with angiotensin I. Thus PRA remains the usual way of evaluating the renin-angiotensin-aldosterone system.

Plasma Adrenocorticotrophic Hormone and other Proopiomelanocortin Peptides

Accurate immunoassay of plasma ACTH is available in most centers, but its measurement remains more difficult and variable than the assays for most other pituitary hormones. Handling of the samples must be done with care; samples must be drawn into a plastic syringe containing heparin or ethylenediamine tetraacetic acid (EDTA) and quickly transported in plastic tubes on ice, as ACTH adheres to glass and is quickly inactivated. Thus elevated plasma ACTH concentrations can be highly informative, but most assays cannot detect low or low-normal values, and such values can be spurious if the samples are handled badly. In adults and older children who have well-established diurnal rhythms of ACTH, normal 8 AM values rarely exceed 50 pg/mL, whereas 8 PM values are usually undetectable. Patients with Cushing disease often have normal morning values, but the diagnosis can be suggested by consistently elevated afternoon and evening values; patients with the ectopic ACTH syndrome can have values up to 1000 pg/mL.

Secretory Rates

The secretory rates of cortisol and aldosterone (or other steroids) can be measured by administering a small dose of tritiated cortisol or aldosterone and measuring the specific activity of one or more known metabolites in a 24-hour urine collection. This procedure permitted measurement of certain steroids, such as aldosterone, before specific immunoassays were available. These procedures have also provided much information about the normal rate of production of various steroids. Based on this procedure, most authorities have agreed that children and adults secrete about 6 to 9 mg of cortisol per square meter of body surface area per day.^{144,145}

Dexamethasone Suppression Test

Administration of dexamethasone, a potent synthetic glucocorticoid, will suppress secretion of pituitary ACTH and adrenal cortisol; the dexamethasone suppression test is useful for distinguishing whether glucocorticoid excess is caused primarily by pituitary disease or adrenal disease. As dexamethasone also suppresses adrenal androgen secretion, this test is useful for distinguishing between adrenal and gonadal sources of sex steroids. A complete formal dexamethasone suppression test requires the measurement of basal values and those obtained in response to both low- and high-dose dexamethasone. This is described in the section on the evaluation of Cushing syndrome. Variations of this test are commonly used, notably the single dose 1.0-mg in adults or 0.3 mg/m² in children. This is a useful outpatient screening procedure for distinguishing Cushing syndrome from exogenous obesity. It can be useful for the same purpose in adolescents and older children, but is otherwise of limited utility in pediatrics. An overnight high-dose dexamethasone suppression test is probably more reliable than the standard 2-day, high-dose test in

differentiating adults with Cushing disease from those with the ectopic ACTH syndrome. However, the utility of this test in pediatric patients has not been established.

Stimulation Tests

Direct stimulation of the adrenal with ACTH is a rapid, safe, easy way to evaluate adrenocortical function. The original ACTH test consisted of a 4- to 6-hour infusion of 0.5 units/kg of ACTH(1-39). This will maximally stimulate adrenal cortisol secretion, and thus effectively distinguishes primary adrenal insufficiency (Addison disease), in which the adrenal is incapable of responding, from secondary adrenal insufficiency caused by hypopituitarism. In secondary adrenal insufficiency, some steroidogenic capacity is present, therefore some cortisol is produced in response to the ACTH; thus cortisol secretion is less than normal but greater than the negligible values seen in primary adrenal insufficiency. The 4- to 6-hour intravenous ACTH test has been replaced by the 60-minute test, wherein a single bolus of ACTH(1-24) is administered intravenously and cortisol and possibly other steroids are measured at 0 and 60 minutes. Normal responses to a 60-minute test are shown in Table 14.4.¹⁴⁶ Synthetic ACTH(1-24) (cosyntropin) is preferred, as it has a more rapid action and shorter half-life than ACTH(1-39). The usual dose is 15 µg/kg in children up to 2 years of age, and 0.25 mg for children older than 2 years and adults. All of these doses are pharmacologic. A very-low-dose (1 µg) test may be useful in assessing adrenal recovery from glucocorticoid suppression. Newer data show that maximal steroidal responses can be achieved after only 30 minutes, but the best available standards are for a 60-minute test. One of the widest uses of intravenous ACTH tests in pediatrics is in diagnosing CAH. Stimulating the adrenal with ACTH increases steroidogenesis, resulting in accumulation of steroids proximal to the disordered enzyme. For example, inspection of Fig. 14.3 shows that impaired activity of P450c21 (21-hydroxylase) should lead to the accumulation of progesterone and 17OHP. However, progesterone does not accumulate in appreciable quantities, because it is converted to 17OHP. In routine practice, measuring the response of 17OHP to a 60-minute challenge, with intravenous ACTH, is the single most powerful and reliable means of diagnosing 21OHD; genetic testing can provide a useful confirmation.⁴⁰ Comparing the patient's basal and ACTH-stimulated values of 17OHP against those from large numbers of well-studied patients usually permits the discrimination of normal persons, heterozygotes, patients with nonclassic CAH, and patients with classic CAH, although there inevitably is some overlap between groups (Fig. 14.12).⁴⁰ Measurement of testosterone or Δ⁴ androstenedione in response to ACTH can distinguish normal persons from patients with classic CAH, but heterozygotes and patients with cryptic CAH have values overlapping both normal and classic CAH.

Longer ACTH tests of up to 3 days have also been used to evaluate adrenal function. It is important to remember that ACTH has both acute and chronic effects. Thus short tests measure only the acute effects of ACTH, that is, the maximal stimulation of preexisting steroidogenic machinery. In contrast, a 3-day test will examine the more chronic effects of ACTH to stimulate increased capacity for steroidogenesis by increasing the synthesis of steroidogenic machinery. Few situations exist where a 3-day intramuscular ACTH test is indicated, although it is occasionally useful in diagnosing the rare syndromes of hereditary unresponsiveness to ACTH.¹⁴⁶

Insulin-induced hypoglycemia is an effective but potentially risky test, and hence is rarely used. Insulin (0.1 U/kg/IV) is administered and blood is obtained at 0, 30, 45, and 60 minutes. The insulin-induced hypoglycemia will stimulate the release of "counterregulatory" hormones that have actions

TABLE 14.4 Responses of Adrenal Plasma Steroids to an Intravenous 60-Minute Adrenocorticotropic Hormone Test

	Unit	1–6 Months		2–6 Years		Prepubertal		Adult Males		Adult Females	
		Basal	Stimulated	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
Cortisol	μg/dL	3–22	27–50	6–19	20–33	5–16	20–31	7–15	19–31	7–21	17–39
17OHP	ng/dL	13–173	85–250	7–114	50–269	7–100	85–280	35–150	45–258	22–140	65–250
17OH Pregnenolone	ng/dL	52–828	633–3286	10–103	45–347	10–186	70–656	20–187	240–1000	48–320	290–1382
11–Desoxycortisol	ng/dL	10–200	101–392	7–210	95–323	14–136	95–254	20–65	73–214	15–158	65–237
DOC	ng/dL	7–48	40–158	4–49	26–139	4–17	22–120	3–13	14–38	3–19	12–90
Pregnenolone	ng/dL	10–150	100–359	17–50	34–99	15–63	39–130	10–85	20–200	46–150	70–220
Δ4A	ng/dL	<10–48	<10–87	<10	<10–35	<10	<10–69	50–210	78–285	61–222	98–295

Basal values are assessed between 8–9 am.

All values are measured by high-performance liquid chromatography mass spectrometry (HPLC-MS/MS) and are given as ranges in ng/dL, except cortisol in μg/dL. Conversion factors to SI Units are given in [Tables 14.2 and 14.3](#).

Δ4A, Androstenedione; 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic hormone (corticotropin); DOC, deoxycorticosterone.

(From LabCorp (Laboratory Corporation of America)/Endocrine Sciences, Calabasas Hills, California.)

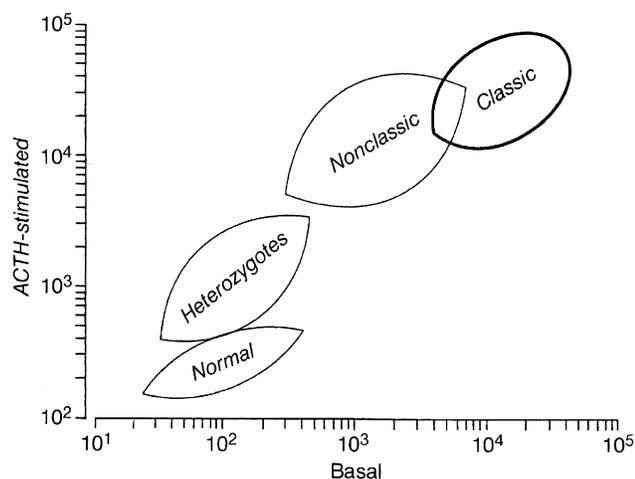


Fig. 14.12 17α -Hydroxyprogesterone values (in ng/100 mL) before and after stimulation with adrenocorticotropic hormone in normals, patients with congenital adrenal hyperplasia, and heterozygotes.

to increase plasma glucose concentrations: ACTH and cortisol, growth hormone, epinephrine, and glucagon. Because of the inherent risk of convulsions as a result of hypoglycemia, an experienced physician must be in attendance (not merely “available”) throughout the entire course of the test. Blood glucose must fall to half of the initial value or to 45 mg/dL to achieve an adequate test, and it is wise to terminate the test after this level is reached. Most patients will experience hunger, irritability, diaphoresis, and tachycardia; when these are followed by drowsiness or sleep, blood sugar levels are likely below acceptable limits. If this occurs, a blood sample should be obtained and 2 mL/kg of 20% to 25% glucose given intravenously, to a maximum of 100 mL.

Metyrapone Test

Metyrapone blocks the action of P450c11 β and, to a much lesser extent, P450sc. It is thus a chemical means of inducing a transient deficiency of 11-hydroxylase activity, which results in decreased cortisol secretion and subsequently increased ACTH secretion. Metyrapone testing is done to assess the capacity of the pituitary to produce ACTH in response to a physiologic stimulus. This test is useful in evaluating the hypothalamic-pituitary axis in the presence of central nervous system lesions, after neurosurgery, or long-term suppression by glucocorticoid therapy.¹⁴⁷ Patients with a previous history of hypothalamic, pituitary, or adrenal disease, or those who have been withdrawn from glucocorticoid therapy should be reevaluated with a metyrapone test. A normal response indicates recovery of the HPA axis and predicts that the patient will respond normally to the stress of surgery.

Metyrapone is generally given orally as 300 mg/m² every 4 hours for a total of six doses (24 hours). Unlike many other drugs, it is appropriate to continue to increase the dose in older or overweight patients, but the total dose should not exceed 3.0 g. Blood should be obtained for cortisol, 11-deoxycortisol, and ACTH, before and after the test. In a normal response to metyrapone, cortisol decreases, ACTH increases, and 11-deoxycortisol (the substrate for P450c11 β) increases greatly, to about 5 μ g/dL. Adults and older children can be tested with the administration of a single oral dose of 30 mg/kg at midnight, given with food to reduce the gastrointestinal irritation. Blood samples are drawn at 8 AM the mornings before and after administering the drug.

GENETIC LESIONS IN STEROIDOGENESIS

Autosomal recessive genetic disorders disrupt each of the steps shown in Fig. 14.3; most of these diminish cortisol synthesis. In response to hypocortisolemia, the pituitary produces increased amounts of POMC and ACTH, which promotes increased steroidogenesis; ACTH and POMC amino-terminal peptides also stimulate adrenal hypertrophy and hyperplasia. Thus the term “congenital adrenal hyperplasia” refers to a group of diseases traditionally grouped together on the basis of the most prominent finding at autopsy.

In theory, the CAHs are easy to understand: a genetic lesion in a steroidogenic enzyme interferes with normal steroidogenesis. The signs and symptoms of the disease derive from deficiency of the steroidal end product and the effects of accumulated steroidal precursors, proximal to the blocked step. Thus reference to the pathways in Fig. 14.3 and a knowledge of the biologic effects of each steroid should permit one to deduce the manifestations of the disease.

In practice, the CAHs can be clinically and scientifically confusing. Each steroidogenic enzyme has multiple activities and many extraadrenal tissues, especially the liver, express other enzymes with steroidogenic activity, hence the complete elimination of an adrenal enzyme may not result in the complete elimination of its steroidal products from the circulation. Furthermore, there are “partial deficiencies” in each enzyme, where some enzymatic activity remains, typically causing milder “nonclassic” disease with later onset. The key clinical, laboratory, and therapeutic features of each form of CAH are summarized in Table 14.5.

Disorders in Cholesterol Synthesis and Trafficking

In addition to the enzymatic steps shown in Fig. 14.3, upstream disorders in cholesterol synthesis and intracellular trafficking can impair steroidogenesis. The early fetus needs to synthesize its own cholesterol, with less being supplied transplacentally, hence disorders of cholesterol biosynthesis cause well-defined congenital malformation syndromes: Smith-Lemli-Opitz syndrome (SLOS, 7-dehydrocholesterol reductase deficiency), lathosterolosis (lathosterol Δ 5-desaturase deficiency), desmosterolosis (sterol Δ 24-reductase deficiency), CHILD syndrome (C-4 demethylation complex disorders), and Conradi-Hunermann syndrome (X-linked sterol isomerase deficiency).¹⁴⁸ Consequent impaired fetal steroidogenesis may result in 46,XY disorder of sex development (DSD), but adrenal insufficiency is not reported.¹²

Wolman Disease and Cholesteryl Ester Storage Disease

Most cellular cholesterol derives from the uptake of circulating lipoproteins that contain cholesterol esters; these esters are cleaved to free cholesterol by lysosomal acid lipase (cholesterol esterase), encoded by the *LIPA* gene on chromosome 10q23.31.¹¹ Mutations of *LIPA* cause Wolman disease (WD, primary xanthomatosis) and its milder variant, cholesteryl ester storage disease (CESD).^{149–154} In WD, cholesteryl esters and triglycerides accumulate in the liver, spleen, lymph nodes, and other tissues. In the adrenal, there is insufficient free cholesterol available for steroidogenesis, causing adrenal insufficiency. The disease is less severe than lipoid CAH with respect to steroidogenesis, and patients may survive for several months after birth. However, the disease affects all cells, not just steroidogenic cells, as all cells must store and use cholesterol; hence, the disorder is relentless and fatal. Vomiting, steatorrhea, failure to thrive, hepatosplenomegaly, jaundice, and anemia, are the usual presenting findings sometimes beginning in the first week of life, leading to

TABLE 14.5 Clinical and Laboratory Findings in the Congenital Adrenal Hyperplasias

Enzyme Deficiency	Presentation	Laboratory Findings	Therapeutic Measures
Lipoid CAH (StAR or P450scc)	Salt-wasting crisis 46,XY DSD	Low/absent levels of all steroid hormones Decreased/absent response to ACTH Decreased/absent response to hCG in 46,XY DSD ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement, salt supplementation Estrogen replacement at age ≥ 12 years Gonadectomy in 46,XY DSD and salt supplementation
3 β -HSD	Salt-wasting crisis 46,XX and 46,XY DSD	↑ Δ^5 steroids before and after ACTH ↑ Δ^5/Δ^4 serum steroids Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical correction of genitalia Sex hormone replacement as necessary
P450c21	Classic forms: Salt-wasting crisis 46,XX DSD Pre- and postnatal virilization Nonclassic form: Premature adrenarche, menstrual irregularity, hirsutism, acne, infertility	↑ 17OHP before and after ACTH ↑ Serum androgens and urine 17KS Suppression of elevated adrenal steroids after glucocorticoid R _x ↑ ACTH and PRA	Glucocorticoid and mineralocorticoid replacement Salt supplementation Surgical repair in 46,XX DSD
P450c11 β	46,XX DSD Postnatal virilization in males and females	↑ 11-deoxycortisol and DOC before and after ACTH ↑ Serum androgens and urine 17KS Suppression of elevated steroids after glucocorticoid administration ↑ ACTH and ↓ PRA Hypokalemia	Glucocorticoid administration Surgical repair in 46,XX DSD
P450c11AS	Failure to thrive Weakness Salt loss	Hyponatremia, hyperkalemia ↑ Corticosterone ↓ Aldosterone ↑ PRA	Mineralocorticoid replacement Salt supplementation
P450c17	46,XY DSD Sexual infantilism Hypertension	↑ DOC, 18-OHDOC, corticosterone, 18-hydroxycorticosterone Low 17 α -hydroxylated steroids and poor response to ACTH Poor response to hCG in 46,XY DSD Suppression of elevated adrenal steroids after glucocorticoid administration ↑ ACTH and ↓ PRA Hypokalemia	Glucocorticoid administration Surgical correction of genitalia and sex steroid replacement in 46,XY DSD consonant with sex of rearing Estrogen replacement in female at ≥ 12 years Testosterone replacement if reared as male (rare)
POR	46,XX and 46,XY DSD Antley-Bixler syndrome Infertility in adults	↑ ACTH, Prog, 17OHP ↓ DHEA, Andro, T Normal electrolytes	Glucocorticoid and sex steroid replacement Surgical correction of skeletal anomalies

17OHP, 17-Hydroxyprogesterone; 17KS, 17-ketosteroids; 18-OHDOC, 18-hydroxy deoxycorticosterone; ACTH, adrenocorticotropic hormone (corticotropin); DOC, deoxycorticosterone; DSD, disordered sexual development; hCG, human chorionic gonadotropin; PRA, plasma renin activity.

developmental delay, and malabsorptive malnutrition. Characteristic bilateral subcapsular adrenal calcification that outlines the adrenals may be seen on pre- or postnatal ultrasound scan.^{155,156} The diagnosis is established by bone marrow aspiration, yielding foam cells containing large lysosomal vacuoles, engorged with cholesterol esters, and is confirmed by finding absent cholesterol esterase activity in fibroblasts, leukocytes, bone marrow cells, or cultured amniocytes (for prenatal diagnosis). Treatment by bone marrow transplantation appears to ameliorate the course of the disease in about half of cases, but the mechanism of this effect is unclear.^{157,158}; enzyme replacement therapy may ameliorate disease progression.¹⁵⁹ Supportive treatments include corticosteroid replacement, vitamin, and mineral supplementation. One survey found WD in around 3% of pediatric adrenal insufficiency.¹⁶⁰

CESD is a milder, later-onset form of WD caused by *LIPA* mutations that retain partial activity.^{152,161} CESD typically presents in later childhood and adolescence with hypercholesterolemia, atherosclerosis, and hepatosplenomegaly (hepatic infiltration with macrophages containing cholesteryl

esters);¹⁵⁴ hepatic fibrosis may lead to esophageal varices.¹⁶¹ There may be early onset with severe cirrhosis or later onset with more slowly progressive hepatic disease. Some patients reach adulthood.

Niemann-Pick Type C Disease

The endosomal processing of cholesterol esters requires the action of NPC1 and NPC2, two proteins named for NPC disease.¹⁶² NPC is a rare neurological disorder of cholesterol trafficking characterized by accumulated cholesterol and glycosphingolipids in endosomes, leading to progressive neurodegeneration, robust glial infiltration, and death.¹⁶³ NPC typically presents at 2 to 4 years, with mild intellectual impairment, speech loss, supranuclear vertical gaze palsy, ataxia, dystonia, dementia, seizures, and extrapyramidal deficits.¹⁶⁴ An infantile, rapidly progressive form presents with severe hepatic dysfunction, psychomotor delay, supranuclear vertical gaze palsy, ataxia, spasticity, and dementia; the hepatosplenomegaly and childhood cirrhosis can be lethal. However, despite

the seemingly central role of the NPC proteins in intracellular cholesterol trafficking, adrenal insufficiency is not reported in NPC. Thus although there is good evidence for disordered steroidogenesis in the NPC brain,¹⁶⁵ the adrenal appears to escape, suggesting it may use other pathways for intracellular cholesterol trafficking.¹²

Congenital Lipoid Adrenal Hyperplasia

Classic lipoid CAH, the most severe genetic disorder of steroidogenesis, typically presents in infancy with low or absent concentrations of all steroids, high basal ACTH and plasma renin, absent steroidal responses to long-term treatment with high doses of ACTH or human chorionic gonadotropin (hCG), and grossly enlarged adrenals laden with cholesterol and cholesterol esters; these findings indicate a lesion in the conversion of cholesterol to pregnenolone.¹⁶⁶ It was initially thought that the lesion was in an enzyme, and before the role of P450_{scc} was understood, lipoid CAH was misnamed “20,22-desmolase deficiency.” However, the gene for P450_{scc} is normal in these patients, as are the mRNAs for adrenodoxin reductase and adrenodoxin;¹⁶⁷ rare disorders in P450_{scc} are clinically indistinguishable from lipoid CAH (see later). Furthermore, placental steroidogenesis persists in lipoid CAH, permitting normal term gestation. These findings suggested that the lesion may be caused by a factor involved in cholesterol transport into mitochondria. A long search led to the discovery of StAR,²⁹ and its causal role in lipoid CAH established StAR’s essential function.^{30,31} StAR facilitates the influx of cholesterol from the OMM to the inner mitochondrial membrane, where it may be converted to pregnenolone by P450_{scc}. P450_{scc} is the slowest steroidogenic enzyme, but the true rate-limiting step in steroidogenesis is import of cholesterol into mitochondria, facilitated by StAR.

Lipoid CAH is the StAR gene knockout experiment of nature, revealing the complex physiology of the StAR protein.¹⁶⁶ StAR

promotes steroidogenesis by increasing the movement of cholesterol into mitochondria, but in the absence of StAR, steroidogenic cells make steroids at about 14% of the StAR-induced level.^{10,11,31,32} The discovery of StAR-independent steroidogenesis led to the two-hit model of lipoid CAH^{31,166} (Fig. 14.13). The first hit is the loss of StAR itself, leading to a loss of most, but not all steroidogenesis, and compensatory increases in ACTH and LH. These hormones increase cellular cAMP, which increases biosynthesis of LDL receptors, their consequent uptake of LDL cholesterol, and de novo synthesis of cholesterol. In the absence of StAR, this increased intracellular cholesterol accumulates as in a storage disease, causing the second hit, which is the mitochondrial and cellular damage caused by the accumulated cholesterol, cholesterol esters, and their auto-oxidation products.^{10,11,31}

The two-hit model explains the unusual clinical findings in lipoid CAH. In the fetal testis, which normally makes large amounts of testosterone in fetal life, the Leydig cells are destroyed early in gestation, eliminating testosterone biosynthesis, hence an affected 46,XY fetus does not undergo normal virilization, and is born with female external genitalia and a blind vaginal pouch. However, Wolffian duct derivatives are well developed,¹⁶⁸ indicating the presence of some testosterone synthesis early in fetal life, as predicted by the two-hit model. The undamaged Sertoli cells produce Müllerian inhibitory hormone, so that the phenotypically female 46,XY fetus has no cervix, uterus, or fallopian tubes. The steroidogenically active fetal zone of the adrenal is similarly affected, eliminating most DHEA biosynthesis and hence eliminating the fetoplacental production of estriol, so that mid-gestation maternal and fetal estriol levels are very low.¹⁶⁹ The definitive zone of the fetal adrenal, which differentiates into the zona glomerulosa and fasciculata, normally produces very little aldosterone, and as fetal salt and water metabolism are maintained by the placenta, stimulation of the glomerulosa by angiotensin II generally does not begin until birth. Consistent with this, many

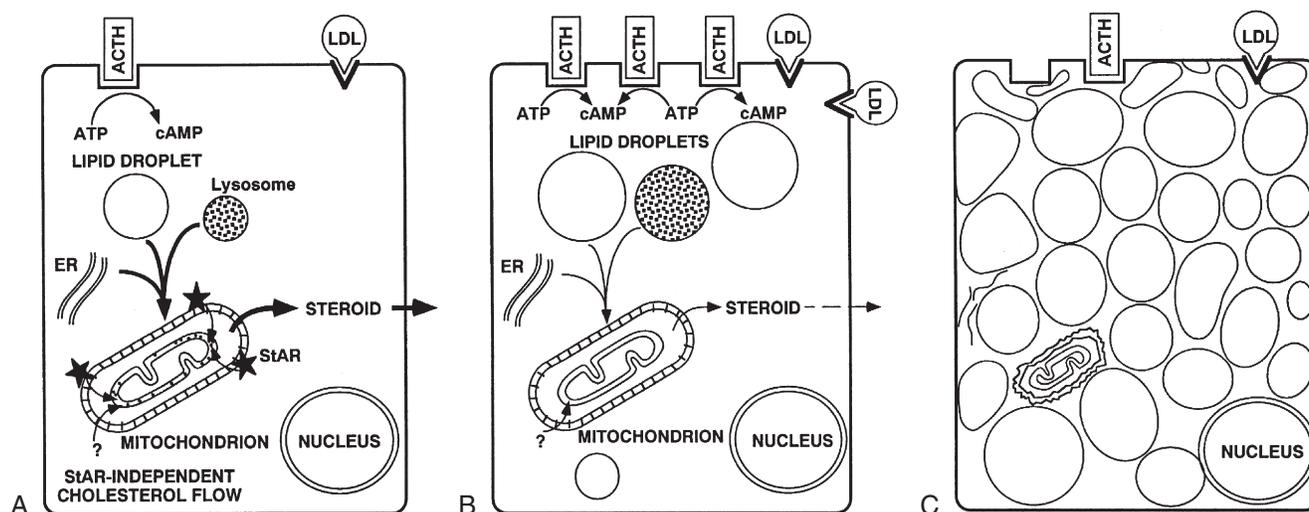


Fig. 14.13 Two-hit model of lipoid congenital adrenal hyperplasia (CAH). **A**, In a normal adrenal cell, cholesterol is primarily derived from low-density lipoprotein (LDL) by receptor-mediated endocytosis and is processed in lysosomes before entering the cellular pool, but cholesterol can also be synthesized de novo from acetyl CoA. Cholesterol from both sources is stored as cholesterol esters in lipid droplets. Cholesterol reaches the mitochondria by poorly defined processes, then travels from the outer to inner mitochondrial membrane by both steroidogenic acute regulatory protein (StAR)-dependent and StAR-independent mechanisms. **B**, In early lipoid CAH, the absence of StAR reduces cholesterol flow and steroidogenesis, but some steroidogenesis persists via the StAR-independent pathway. The decreased secretion of cortisol leads to increased adrenocorticotropic hormone, which stimulates further cholesterol uptake and synthesis; this cholesterol accumulates in lipid droplets. **C**, Accumulating lipid droplets damage the cell, both through physical disruption of cytoarchitecture and by the chemical action of autooxidation products, eventually destroying all steroidogenic capacity. In the ovary, follicular cells remain unstimulated and undamaged until they are recruited at the beginning of each cycle. They can then produce small amounts of estradiol, as in panel B, leading to feminization and anovulatory cycles in affected females.

newborns with lipid CAH do not have a salt-wasting crisis until after several weeks of life, when chronic stimulation then leads to cellular damage.^{31,170}

The two-hit model also explains the spontaneous feminization of affected 46,XX females who are treated in infancy and reach adolescence.^{10,11,31,171,172} The fetal ovary makes little or no steroids and contains no steroidogenic enzymes after the first trimester; consequently, the ovary remains largely undamaged until it is stimulated by gonadotropins at the time of puberty, when it then produces some estrogen by StAR-independent steroidogenesis. Continued stimulation results in cholesterol accumulation and cellular damage, so that biosynthesis of progesterone in the latter part of the cycle is impaired. Because gonadotropin stimulation only recruits individual follicles and does not promote steroidogenesis in the whole ovary, most follicles remain undamaged and available for future cycles. Cyclicity is determined by the hypothalamic-pituitary axis, and remains normal. With each new cycle, a new follicle is recruited and more estradiol is produced by StAR-independent steroidogenesis. Although net ovarian steroidogenesis is impaired, enough estrogen is produced to induce breast development, general feminization, monthly estrogen withdrawal, and cyclic vaginal bleeding.^{31,171} However, progesterone synthesis in the latter half of the cycle is disturbed by the accumulating cholesterol esters so that the cycles are anovulatory and ovarian cysts may develop. Measurements of estradiol, progesterone, and gonadotropins throughout the cycle in affected adult females with lipid CAH confirm this model,¹⁷² as do experiments with StAR-knockout mice.^{173,174} Thus examination of patients with lipid CAH has elucidated the physiology of the StAR protein in each steroidogenic tissue.

Studies in over 100 patients with lipid CAH have identified over 40 StAR mutations.^{10–12} Lipoid CAH is the second-most common form of CAH in Japan and Korea, where the mutation Q258X predominates,^{31,175,176} with a carrier frequency of about 1 in 300, so that one in every 250,000 to 300,000 newborns in these countries is affected.^{177,178} Lipoid CAH is also common in Arab populations, with R182L found among Palestinians,³¹ R188C in eastern Saudi Arabia,¹⁷⁰ and R182H and c.201-202delCT among other Arab populations;^{179,180} in Switzerland, L260P is a recurrent mutation.¹⁸¹

The typical clinical presentation in lipid CAH is an infant with normal-appearing female genitalia who experiences failure to thrive and salt loss in the first weeks of life.^{31,175,176,182}

Other clinical presentations include apparent sudden infant death syndrome (SIDS)¹⁸³ and late initial presentation of salt loss at about 1 year of age.¹⁷⁰ Nonclassic lipid CAH caused by mutations that retain about 20% to 25% of normal StAR activity was found in 46,XY children with normal-appearing male external genitalia children, who experience symptomatic adrenal insufficiency at several years of age.¹⁸⁴ Adults with nonclassic lipid CAH are recognized with increasing frequency and have often been mistaken for having “familial glucocorticoid deficiency,” a blanket term referring to disorders of ACTH action; many of these patients have mildly compromised mineralocorticoid secretion, with normal serum electrolytes and elevated plasma renin, and some may have mild hypergonadotropic hypogonadism.^{185–187} Most of these patients carry the same R188C mutation first described in children.¹⁸⁴ Thus the spectrum of clinical presentation of congenital lipid adrenal hyperplasia is substantially broader than initially appreciated.

Treatment of lipid CAH is straightforward if the diagnosis is made. Physiologic replacement with glucocorticoids, mineralocorticoids, and salt, in the newborn period, will permit survival to adulthood. Glucocorticoid doses are as in Addison disease and are less than in the virilizing adrenal hyperplasias because it is not necessary to oversuppress excess adrenal androgen production, so that growth in these patients should

be normal. Mineralocorticoid doses are not increased with age or size, as children become progressively more sensitive to mineralocorticoids with age; salt supplementation is weaned after about 1 year. Severely affected 46,XY newborns have normal female external genitalia and may be advised to undergo orchiectomy later in life and be raised as females, with sex hormone replacement therapy, started at age of puberty. Affected 46,XX females typically have spontaneous pubertal feminization, but anovulatory cycles and early secondary amenorrhea, then also requiring sex hormone replacement therapy. Pregnancy has been induced in two reports.^{188,189}

Disorders Resembling Lipoid Congenital Adrenal Hyperplasia: P450scc Deficiency and Steroidogenic Factor 1 Deficiency

Mutations in other genes can produce a clinical phenotype that is essentially indistinguishable from that caused by StAR mutations, but these disorders should not be called lipid CAH. Beginning in 2001, several patients were described with mutations in P450scc.¹⁹⁰ Their clinical and hormonal findings are indistinguishable from those with StAR mutations, so that gene sequencing is the only definitive way to distinguish these disorders¹⁹¹; however, to date no patient with a P450scc mutation has had the adrenal hyperplasia typically seen in lipid CAH.^{182,190–193} It would seem logical that elimination of all P450scc activity would be incompatible with term gestation, as the placenta, a fetal tissue, must produce progesterone in the second half of pregnancy to suppress maternal uterine contractions, thus preventing miscarriage. However, about 40 such cases were reported through 2018, many from southeastern Turkey.^{12,194,195} Fetuses with P450scc mutations probably reach term gestation when there is protracted maintenance of the maternal corpus luteum of pregnancy, which normally involutes in the second trimester, but this has not been investigated directly. Nonclassic P450scc deficiency that is clinically and hormonally indistinguishable from nonclassic lipid CAH has been reported in patients with P450scc mutations that retain 10% to 20% of wild-type activity.^{196,197}

More than 50 patients have also been described carrying mutations in the gene for steroidogenic factor 1 (SF1), a transcription factor required for adrenal and gonadal, but not for placental, expression of genes for the steroidogenic enzymes.^{198,199} There is broad phenotypic variability in SF1-deficient patients; some are 46,XY with a female phenotype and adrenal failure, thus resembling lipid CAH, but in most cases the gonadal phenotype predominates and there is little if any impairment of adrenal steroidogenesis. SF1 mutations may be found in about 10% of 46,XY patients who have disordered sexual development. The Leydig cells may have lipid accumulation and progressive degeneration, similar to the findings in lipid CAH.¹⁹⁹

3 β -Hydroxysteroid Dehydrogenase Deficiency

3 β HSD deficiency is a rare, potentially fatal cause of glucocorticoid, mineralocorticoid, and sex steroid deficiency.¹⁸² There are two functional human genes for 3 β HSD: the type 1 gene (*HSD3B1*) is expressed in the placenta and peripheral tissues, and the type 2 gene (*HSD3B2*) is expressed in the adrenals and gonads.¹⁰ All mutations causing 3 β HSD deficiency are found in the type 2 gene^{200,201}; mutations have never been found in 3 β HSD1, presumably because this would prevent placental biosynthesis of progesterone, resulting in a spontaneous first-trimester abortion. 3 β HSD deficiency causes DSD in both sexes: genetic females have clitoromegaly and mild virilization because the fetal adrenal overproduces large amounts of

DHEA, a small portion of which is converted to testosterone by 3β HSD1; genetic males synthesize some androgens by peripheral conversion of adrenal and testicular DHEA, but the concentrations are insufficient for complete male genital development.²⁰²

One would expect that infants with 3β HSD2 deficiency would have low concentrations of 17OHP, yet some newborns with 3β HSD deficiency have very high concentrations of serum 17OHP, approaching those seen in patients with classic 21OHD,²⁰³ and registering a positive newborn screen for 21OHD.²⁰⁴ Whereas 3β HSD2 has a high Km of around 5.5 μ M,²⁰⁵ the Km of 3β HSD1 is only around 0.2 μ M,²⁰⁶ permitting hepatic 3β HSD1 to act on the high circulating concentrations of 17-Preg in patients with 3β HSD2 deficiency, producing 17OHP. This 17OHP is not effectively picked up by the adrenal for subsequent conversion to cortisol because the circulating concentrations are below the Km of P450c17 (\sim 0.8 μ M 17OHP, or \sim 40,000 ng/dL).³⁷ The ratio of the Δ^5 to the Δ^4 compounds remains high, consistent with the adrenal and gonadal deficiency of 3β HSD.²⁰³ Thus the principal diagnostic test in 3β HSD deficiency is intravenous administration of ACTH, with measurement of the three Δ^5 compounds (Preg, 17-Preg, DHEA), and the corresponding Δ^4 compounds (progesterone, 17OHP, androstenedione). Unlike the case of 21OHD, where heterozygotes can be diagnosed by the response of 17OHP to ACTH, steroidal responses to ACTH cannot be used to identify carriers of 3β HSD deficiency.²⁰⁷

Mild or "partial" defects of adrenal 3β HSD activity have been reported on the basis of ratios of Δ^5 steroids to Δ^4 steroids, following an ACTH test that exceed 2 or 3 standard deviations (SD) above the mean; these patients are typically young girls with premature adrenarche, or young women with a history of premature adrenarche and complaints of hirsutism, virilism, and oligomenorrhea. However, these patients do not have 3β HSD deficiency as their *HSD3B2* genes are normal.²⁰⁸ Patients with mild 3β HSD2 mutations have ratios of Δ^5 to Δ^4 steroids that exceed 8 SD above the mean.^{209,210} Thus ratios of Δ^5 to Δ^4 steroids are not reliable and cannot be used to diagnose 3β HSD deficiency; the diagnosis requires an ACTH test with a rise in Δ^5 steroids (typically a rise in 17-Preg to $>$ 3000 ng/dL).²⁰⁹ The basis of the mildly elevated ratios of Δ^5 to Δ^4 steroids, in these hirsute individuals with normal *HSD3B* genes, remains unknown.³⁴ In adult women, the hirsutism can be ameliorated and regular menses can be restored by suppressing ACTH with 0.25 mg of dexamethasone given orally each day, but such treatment is contraindicated in girls who have not yet reached their final adult height.

17 α -Hydroxylase/17,20-Lyase Deficiency

P450c17 catalyzes both 17 α -hydroxylase and 17,20-lyase activities.¹⁰ 17-hydroxylase deficiency is well understood clinically and genetically,^{211,212} and is common in Brazil and China.¹⁸² Deficient 17 α -hydroxylase activity results in decreased cortisol synthesis, overproduction of ACTH, and stimulation of the steps proximal to P450c17. These patients may have mild symptoms of glucocorticoid deficiency, but this is not life-threatening, as the cortisol deficiency is compensated by overproduction of corticosterone, which has glucocorticoid activity. This is similar to the situation in rodents, whose adrenals lack P450c17 and consequently produce corticosterone as their glucocorticoid. Affected patients also typically overproduce DOC in the zona fasciculata, which causes sodium retention, hypertension, and hypokalemia, and also suppresses plasma renin activity and aldosterone secretion from the zona glomerulosa, although the suppression of aldosterone is rather variable.^{211,212} When P450c17 deficiency is treated with

glucocorticoids, DOC secretion is suppressed and plasma renin activity and aldosterone concentrations rise to normal.

The absence of 17 α -hydroxylase and 17,20-lyase activities in complete P450c17 deficiency prevents the synthesis of adrenal and gonadal sex steroids. As a result, affected females are phenotypically normal, but fail to undergo adrenarche and puberty, and genetic males have absent or incomplete development of the external genitalia (male pseudohermaphroditism; 46,XY DSD). The classic presentation is that of a teenage female with sexual infantilism and hypertension. The diagnosis is made by finding low or absent 17-hydroxylated C21 and C19 plasma steroids, which respond poorly to stimulation with ACTH, and elevated DOC, corticosterone, and 18OH-corticosterone, which are hyperresponsive to ACTH and suppressible with glucocorticoids. Physiologic glucocorticoid replacement therapy will suppress the mineralocorticoid hypertension; sex steroid replacement concordant with the sex of rearing is instituted in adolescence.

More than 100 distinct mutations have been found in the *CYP17A1* gene on chromosome 10q24.3, causing 17 α -hydroxylase deficiency.^{211,212} Four mutations appear recurrently: a duplication of four nucleotides causing a frameshift is found among descendants of Dutch Frieslanders,²¹³ 7-489 is common in Asia^{214,215}; a frameshift mutation common in Northern China and Korea,²¹⁵⁻²¹⁷; and the common W406R and R362C mutations, found among Brazilians of Spanish and Portuguese ancestry, respectively.²¹⁸ Most reported P450c17 mutations ablate all activity, whereas some others partially affect both activities, usually about equally.

17,20 Lyase Deficiency: P450c17, Cytochrome b5 and Other Factors

Early reports of selective 17,20-lyase deficiency initially led to the incorrect conclusion that 17 α -hydroxylase and 17,20-lyase are separate enzymes. Isolated 17,20 lyase deficiency can result from mutations in several genes, but all are involved with electron transfer to P450c17 from POR.³⁸ The first genetically proven cases of isolated 17,20 lyase deficiency were two patients with genital ambiguity, normal excretion of 17OHCS (17-hydroxycorticosteroids), and markedly reduced production of C19 steroids.²¹⁹ These patients were homozygous for the P450c17 mutations R347H or R358Q; both mutations changed the distribution of surface charges in the redox-partner binding site of P450c17, and enzymatic and cell biologic experiments showed that the loss in lyase activity was caused by impaired electron transfer.^{219,220} Just as altering the charge distribution in the redox-partner binding site of P450c17 from positive to negative with the R347H or R358Q mutants caused isolated 17,20 lyase activity, so changing the electrostatic change from negative to positive with the G539R mutant in the electron-donating domain of POR was also found in patients with isolated 17,20 lyase deficiency.²²¹ Thus mutations in either the redox-partner binding site of P450c17 or in the electron-donating domain of POR can cause isolated 17,20 lyase deficiency, showing that isolated 17,20 lyase deficiency is a disorder of electron transport to P450c17.³⁸

In addition to rare, specific mutations in P450c17 and POR, mutations in cytochrome b5, a small hemoprotein that stimulates 17,20 lyase activity about 10-fold,³⁷ will also cause 17,20 lyase deficiency. Adrenal expression of b5 is specific to the zona reticularis and coincides with the onset of adrenarche.^{103,104,222} The first report of b5 deficiency was in a male with DSD and methemoglobinemia who was not evaluated hormonally.²²³ Methemoglobinemia is an expected consequence of b5 deficiency because the reduction of methemoglobin is the

principal physiologic role of b5, and the usual cause of methemoglobinemia is deficiency of cytochrome b5 reductase. Elevated concentrations of methemoglobin, without clinical methemoglobinemia, may be more common.^{224,225} 17,20-Lyase activity can also be augmented by the serine/threonine phosphorylation of P450c17,^{226,227} catalyzed by p38 α (MAPK14),³⁹ but disruption of this system has not (yet) been reported to cause clinical 17,20 lyase deficiency. Interestingly, the first patients reported to have 17,20 lyase deficiency were subsequently found to have mutations in AKR1C2 and AKR1C4, two enzymes that catalyze 3 α -reduction in the backdoor pathway of androgen synthesis.⁵¹ This pathway participates in the virilization seen in 21OHD,⁷⁹ but has no role in 17,20 lyase activity. These multiple genetic causes illustrate that 17,20 lyase deficiency is a syndrome rather than a specific disease.³⁸

P450 Oxidoreductase Deficiency

POR deficiency is a recently recognized form of CAH.^{228,229} POR is the 2-flavin protein that transfers electrons from NADPH to all 50 microsomal forms of cytochrome P450, including P450c17, P450c21, and P450aro, as well as the drug-metabolizing P450 enzymes of the liver (see Fig. 14.5).¹³ Because POR participates in many functions, its mutation might be expected to yield a very severe phenotype, and POR-deficient mice die during fetal development, but many patients with POR deficiency have been reported since 2004. A wide array of POR mutations has been described, affecting various P450 enzymes to differing degrees, apparently explaining the great variability in the clinical and hormonal findings in POR deficiency.^{228–233} The serum and urinary steroids indicate defects in P450c17, with defects in P450c21 and P450aro being more variable; the clinical findings vary from severely affected infants with ambiguous genitalia, cortisol deficiency, and the Antley-Bixler skeletal malformation syndrome (ABS) to mildly affected women who appear to have a form of polycystic ovary syndrome, or mildly affected men, with gonadal insufficiency.^{228–233} ABS is characterized by craniosynostosis, brachycephaly, radioulnar or radiohumeral synostosis, bowed femora, arachnodactyly, midface hypoplasia, proptosis, and choanal stenosis. When ABS is seen in association with abnormal steroids and ambiguous genitalia in either sex, the cause is an autosomal recessive mutation in POR^{228,229}; in contrast, when ABS is seen without a lesion in steroidogenesis or genital development, the cause is an autosomal dominant, gain-of-function mutation in FGF receptor 2.²²⁹ Thus the term “Antley-Bixler syndrome” should be reserved for the phenotypic description of the skeletal malformations, and should not be equated with POR deficiency, which may or may not be associated with ABS.^{228,229} The ABS skeletal phenotype probably results from diminished activity of CYP26B1, the POR-dependent microsomal enzyme that degrades retinoic acid.²³⁴ Studies of two families with CYP26B1 mutations, and recreating such mutations in transgenic mice and zebrafish provides powerful evidence that retinoic acid must be degraded locally at embryonic sites that normally form skeletal joints and sutures; interference with this activity in POR deficiency appears to be the principal mechanism accounting for the skeletal phenotype.²³⁴ Other mechanisms, including defective signaling by *hedgehog* proteins secondary to a POR-associated defect in cholesterol synthesis, may also play a role.²³¹

In some POR-deficient patients, the impairment of P450c21 activity may generate levels of 17OHP detected by newborn screening for 21OHD.^{228,235} Patients with POR deficiency typically have normal electrolytes and mineralocorticoid function, nearly normal levels of cortisol that respond poorly to stimulation with ACTH, high concentrations of 17OHP that respond variably to ACTH, and low levels of C19 precursors to sex

steroids.^{228–233} A notable feature of POR deficiency is that there is genital ambiguity in both sexes; females may be virilized and males may be underdeveloped, although there is considerable variation among individuals. Because the 17,20 lyase activity of P450c17 is especially sensitive to perturbations in electron transport,^{18,38,219} defects in fetal testicular steroidogenesis leading to incompletely developed external genitalia in 46,XY males is the predicted outcome. In contrast, the partial virilization seen in 46,XX genetic females appears to be the result of two causes. First, placental aromatase (P450aro) requires POR. Pregnant women carrying a fetus with the POR mutation R457H (but not POR A287P) may experience virilization during pregnancy,^{228–230} similar to that experienced by women carrying a fetus with P450aro deficiency.⁵⁸ The fetus normally disposes of large amounts of adrenal C19 steroids by excreting them through the placenta, which aromatizes them to the maternal estrogens of pregnancy (see Fig. 14.7). A defect in this placental aromatase activity, either from mutation of POR or P450aro itself, will permit large amounts of fetal C19 steroids to enter and virilize the mother. This is evidenced by the low estriol values seen in women carrying a fetus with certain POR mutations. Second, analysis of urinary steroids from patients with POR deficiency indicates that the alternative backdoor pathway of androgen production (see Fig. 14.6) also contributes to the prenatal virilization of affected females.^{230,232,233}

Because POR is required by the hepatic drug-metabolizing cytochrome P450 enzymes, it is logical to expect impaired drug metabolism in POR-deficient patients. Although transgenic mice with liver-specific POR defects metabolize drugs poorly and accumulate hepatic lipids, similar problems have not yet been described in patients with POR deficiency. Numerous studies of drug-metabolizing enzymes in vitro show major impairment by POR mutations^{236,237}; however, only one study has found such an effect in a POR-deficient patient.²³⁸ Much remains to be learned about POR deficiency.

The incidence of POR deficiency varies among ethnic groups. Two mutations are especially common: A287P, the predominant mutation in patients of European ancestry,^{228,232} and R457H, the predominant mutation in patients of Japanese ancestry.^{229, 230} The clinical phenotype in females with POR deficiency depends on their POR mutation. POR A287P disrupts activity of P450c17 but not P450c21,²³⁹ and R457H disrupts P450aro activity but A287P does not.²⁴⁰ Defective placental P450aro activity may permit fetal androgen precursors to virilize the mother, hence a woman carrying a fetus with POR R457H (but not A287P) may become virilized during pregnancy; such pregnancies are characterized by low maternal estriol levels.^{228–230,240} The polymorphic variant A503V, which mildly affects many P450 activities,²³⁶ is found in around 28% of POR alleles, varying from around 19% in African Americans to around 37% in Chinese Americans.²⁴¹

Treatment of POR deficiency requires multidisciplinary management of craniosynostosis, orthopedic problems, and DSD, including sex hormone replacement therapy, starting at pubertal age in both sexes. Some patients may benefit from low-dose glucocorticoid replacement therapy, especially during severe illness; this should be determined individually by assessing the response of cortisol to ACTH.

21-Hydroxylase Deficiency

21OHD, caused by mutations in the CYP21A2 gene encoding adrenal P450c21, is one of the most common inborn errors of metabolism, and accounts for over 90% of all patients with CAH. Because of improved diagnosis and treatment in infancy, patients with severe 21OHD now routinely reach adulthood, so that the management of 21OHD concerns physicians dealing with all age groups.^{40,80,242,243}

Pathophysiology

In severe 21OHD, there is an inability to convert progesterone to DOC, resulting in aldosterone deficiency that causes severe hyponatremia, hyperkalemia, and acidosis. The associated hypotension, shock, and cardiovascular collapse may result in death in an untreated newborn. As the control of fluids and electrolytes in the fetus are maintained by the placenta and the mother's kidneys, this salt-losing crisis develops only after birth, usually during the second week of life. The inability to convert 17OHP to 11-deoxycortisol results in cortisol deficiency, which impairs postnatal carbohydrate metabolism and exacerbates cardiovascular collapse, because a permissive action of cortisol is required for full pressor action of catecholamines. High concentrations of cortisol in the adrenocortical capillary effluent that bathes the medulla are needed for the transcription of medullary phenylethanolamine N-methyltransferase, which converts norepinephrine to epinephrine, hence children with 21OHD have low epinephrine concentrations, which may exacerbate the hypoglycemia associated with cortisol deficiency.²⁴⁴

Although a role for cortisol in fetal physiology is not well established,^{83,85,86} cortisol deficiency is manifested prenatally. Low fetal cortisol stimulates ACTH secretion, which stimulates adrenal hyperplasia and transcription of the genes for all the steroidogenic enzymes, especially for P450_{sc}, the rate-limiting enzyme in steroidogenesis. This increased transcription increases enzyme production and activity, with consequent accumulation of non-21-hydroxylated steroids. There are four pathways by which these steroids are diverted to androgens (see Fig. 14.6). First, 17-Preg may be converted to DHEA by the 17,20 lyase activity of P450_{c17}; if not inactivated by sulfation to DHEAS, DHEA may be converted to androstenedione by 3 β HSD2. Androstenedione may then be converted to testosterone in the fetal adrenal or in target tissues by 17 β HSD5 (see Fig. 14.3). Second, although 17OHP is not normally an effective substrate for the 17,20 lyase activity of P450_{c17}, the very high levels of 17OHP characteristic of 21OHD will "force" some conversion to androstenedione by mass action. Third, as described in Section III (Adrenal Androgen Secretion and the Regulation of Adrenarche), androstenedione may be converted to 11-keto-testosterone, the principal adrenal androgen, in the zona reticularis. Fourth, in the alternative or backdoor pathway to DHT, 17OHP is 5 α - and 3 α -reduced to 17OH-allopregnenolone, which is readily converted by P450_{c17} to androsterone and thence by 17 β HSD to androstenediol and an oxidative 3 α HSD to DHT, so that this most

potent androgen is produced without DHEA, androstenedione, or testosterone as intermediates.⁷⁷ Recent work has established that this pathway exists in the human fetus⁵¹ and that it contributes substantially to the production of androgens in children with 21OHD.^{79,245} Also as described earlier (see Fetal Adrenal Steroidogenesis), the fetal adrenal transiently expresses 3 β HSD2 at about 7 to 12 weeks, permitting cortisol synthesis, which in turn suppresses ACTH and hence suppressing fetal adrenal production of DHEA and other C-19 steroids.⁸³ Thus fetal adrenal androgen synthesis is normally suppressed during the time when the external genitalia may become virilized. In 21OHD, this transient production of cortisol is not possible, further driving the androgenization of female fetuses.

Early in gestation, the fetal testes produce testosterone by the conventional androgenic pathway and DHT by the backdoor pathway, both of which are needed to differentiate the pluripotent embryonic precursor structures into male external genitalia.⁵¹ In the male fetus with 21OHD, the additional androgens produced in the adrenals have no phenotypic effect. In contrast, the fetal ovaries normally produce minimal sex steroids or other factors needed for differentiation of the female external genitalia. The androgens inappropriately produced by the adrenals of the 21OHD female fetus cause varying degrees of external genital virilization, which can range from mild clitoromegaly, with or without posterior fusion of the labioscrotal folds, to complete labioscrotal fusion that includes a urethra traversing the enlarged clitoris (Fig. 14.14). These infants have normal ovaries, fallopian tubes, and a uterus, but have "ambiguous" external genitalia or may be sufficiently virilized so that they appear to be male, resulting in errors of sex assignment at birth. 21OHD is the most common form of 46, XX DSD.

The diagnosis of 21OHD is suggested by DSD in females, a salt-losing episode in either sex, or rapid growth and virilization in males or females in infancy. Plasma 17OHP is markedly elevated (>2000 ng/dL or 60 nmol/L, and often exceeding 40,000 ng/dL or 1200 nmol/L after 24 h of age, in an otherwise healthy full-term infant), and hyperresponsive to stimulation with ACTH (see Fig. 14.12). The clinician must also measure 11-deoxycortisol, DHEA, and androstenedione, both to distinguish among the forms of CAH and because adrenal and testicular tumors can also produce 17OHP. ACTH will also induce a substantial rise in serum 21-deoxycortisol in all forms of 21OHD, but not in normals, providing a useful test when this steroid can be measured. High newborn 17OHP values that rise further after ACTH can also be seen in 3 β HSD2 deficiency (because of the activity of hepatic 3 β HSD1) and in

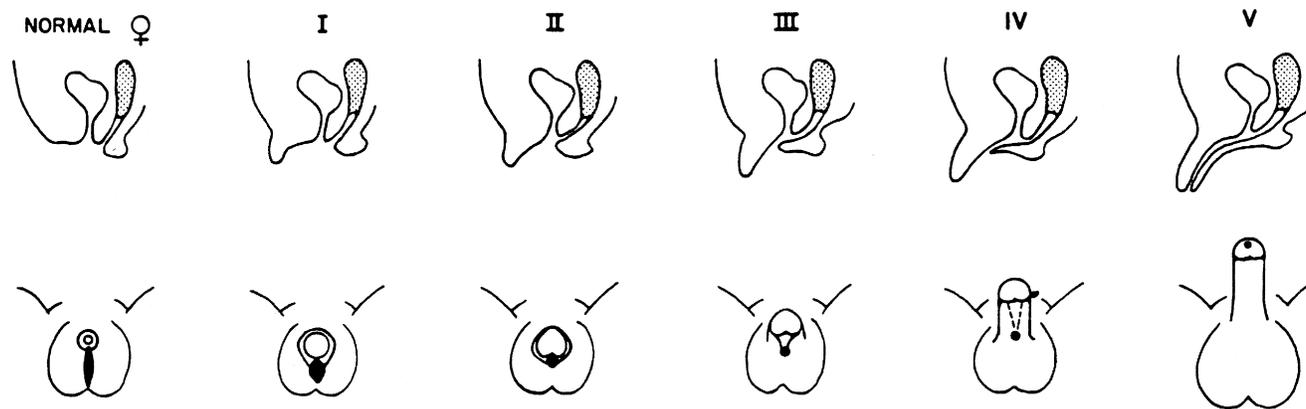


Fig. 14.14 Virilization of the external genitalia. A continuous spectrum is shown from normal female to normal male in both sagittal section (above) and perineal views (below), using the staging system of Prader. Disorders of external genitalia can occur either by the virilization of a normal female, as in congenital adrenal hyperplasia, or because of an error in testosterone synthesis in the male. In females with congenital adrenal hyperplasia caused by 21-hydroxylase deficiency, the degree of virilization correlates poorly with the presence or absence of clinical signs of salt loss.

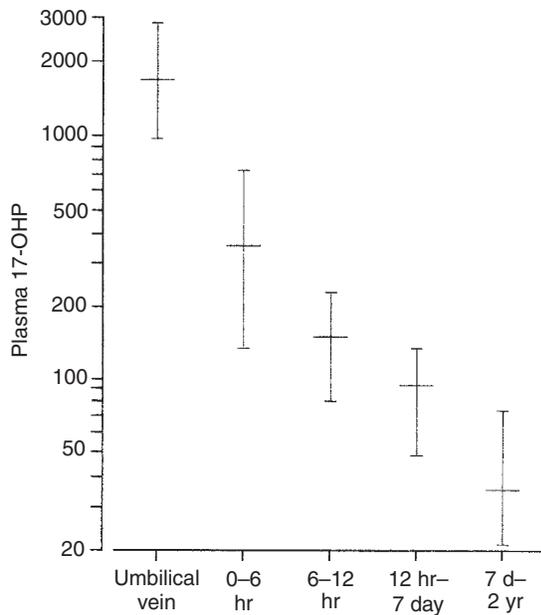


Fig. 14.15 Means and ranges of 17 α -Hydroxyprogesterone in normal newborns (data are in ng/100 mL). Note that values can be very high and quite variable for the first 24 hours of life.

P450c11 β deficiency (because of end-product inhibition of P450c21).²⁰³ 17OHP is normally high in cord blood, but falls to normal newborn levels after about 24 h (Fig. 14.15), so that assessment of 17OHP levels should not be made in the first day of life. Premature infants and term infants under severe stress (e.g., with cardiac or pulmonary disease) may have persistently elevated 17OHP concentrations with normal 21-hydroxylase. Newborn screening programs for CAH, based on 17OHP measurements, are used throughout the industrialized world. The technologies used and “cut-off” values used vary in different healthcare systems. When testing is done on full-term infants more than 24 hours after birth, the screening is reliable.⁴⁰ Endocrinologists and neonatologists must become familiar with local assays and the values found in the extremely premature, which may be read as false positives for CAH requiring repeat testing. Also negative neonatal screening does not exclude milder 21OHD causing nonclassic 21OHD beyond the newborn period.

Clinical Forms of 21-Hydroxylase Deficiency

There is a broad spectrum of clinical manifestations of 21OHD, depending on the particular gene mutation. These different forms of 21OHD are not different diseases, as there is a continuous spectrum of manifestations, ranging from the severe “salt-wasting” form to clinically inapparent forms that may be normal variants. Thus the typical disease forms discussed later are mainly a clinical convenience.⁴⁰

Salt-wasting 21-hydroxylase deficiency. Salt-wasting (SW) 21OHD is caused by a nearly complete lack of P450c21 activity, effectively eliminating both glucocorticoid and mineralocorticoid synthesis. Females with SW 21OHD are frequently diagnosed at birth because of their DSD. After appropriate resuscitation of the cardiovascular collapse, acidosis, and electrolyte disorders, the mineralocorticoids and glucocorticoids can be replaced orally and the DSD may be corrected with plastic surgical procedures. The steroidal replacement management is difficult because of the rapidly changing needs of a growing infant or child (see section on “Treatment”). Drug doses must

be adjusted frequently, and there is individual variability in what constitutes “physiologic” replacement. As an underdose of glucocorticoids can be life-threatening, especially during illness, most pediatricians have tended to “err on the safe side,” so that these children often receive inappropriately large doses of glucocorticoids. It is not possible to compensate for the growth lost during the first 2 years of life, when growth is fastest, so these children typically end up shorter than predicted from their genetic potential. Adult females may have sexual dysfunction, marry with a low frequency, are more reluctant to form intimate relationships, and have decreased fertility. Prenatal androgens appear to affect behavior, but not sexual identity.⁴⁰ Males with SW 21OHD are generally undiagnosed at birth and come to medical attention through the neonatal screening or during the salt-losing crisis that follows at day 5 to 15 of life; untreated infants typically die. Adult males appear to have less steady heterosexual relationships and decreased fertility, especially with insufficient treatment, which promotes the occurrence of testicular adrenal rest tumors. The most common CYP21A2 mutations causing around 50% of SW 21OHD are large gene deletions, large gene conversions, and a C to G substitution 13 bases from the splice acceptor site in intron 2.^{246,247}

Simple virilizing 21-hydroxylase deficiency. Virilized females who have elevated concentrations of 17OHP, but who do not suffer a salt-losing crisis, have long been recognized as having the SV form of 21OHD. Unless detected by newborn screening, males with SV 21OHD may escape diagnosis until age 3 to 7 years, when they come to medical attention because of early development of pubic, axillary and facial hair, acne, and phallic growth. These signs may be mistaken for premature puberty, but the testes remain of prepubertal size because there is no gonadotropic stimulation, whereas boys with true central precocious puberty have pubertal-sized testes (≥ 4 mL). These children grow rapidly and are tall for age when diagnosed, but their epiphyseal maturation (bone age) advances disproportionately rapidly, so that ultimate adult height is compromised. Untreated or poorly treated children with 21OHD may fail to undergo normal puberty, and boys may have small testes and azoospermia because of the feedback of the adrenally produced testosterone on pituitary gonadotropins. When treatment is begun at several years of age, suppression of adrenal testosterone secretion may remove tonic inhibition of the hypothalamus, occasionally resulting in true central precocious puberty, requiring treatment with a gonadotropin-releasing hormone (GnRH) agonist. High concentrations of ACTH may stimulate the enlargement of testicular adrenal rest tumors (TARTs). Because of the high (~25%) incidence of TARTs,²⁴⁸ the testes of postpubertal males with 21OHD should be examined every 1 to 2 years by ultrasound.⁴⁰ Because the adrenal normally produces 100 to 1000 times as much cortisol as aldosterone, mild defects (amino acid replacement mutations) in P450c21 are less likely to affect mineralocorticoid secretion than cortisol secretion. This is reflected physiologically by the increased plasma renin activity seen in patients with SV 21OHD, after moderate salt restriction. The most common mutation causing SV 21OHD is I172N,^{249,250} which retains about 5% of normal activity.²⁵¹

Nonclassic 21-hydroxylase deficiency. Very mild, nonclassic (NC) forms of 21OHD may be evidenced by mild to moderate hirsutism, virilism, acne, menstrual irregularities, and decreased fertility in adult women (sometimes called “late-onset” 21OHD), or there may be no phenotypic manifestations other than an increased response of plasma 17OHP to an intravenous ACTH test (so-called “cryptic” 21OHD).⁴⁰ These individuals may also have hormonal

evidence of a mild impairment in mineralocorticoid secretion, evidenced by an unusually greater rise in PRA when dietary sodium is restricted. *CYP21A2* genotype analyses identified a heterozygous carrier rate of 15% (95% confidence interval [CI], 10.4–20.7) among 200 Ashkenazi Jews and a carrier rate of 9.5% (95% CI, 5.8–14.4) among 200 non-Jewish Caucasians; this difference was not significant ($P = .13$), yielding an overall frequency of about 1 in 200.²⁵² The most common *CYP21A2* mutation causing NC 21OHD is V281L,^{253,254} which retains around 20% of normal activity.^{255,256}

There is some inconsistency in classifying patients with 21OHD because the three “forms” are not separate diseases but represent typical pictures in a continuous spectrum of disease, caused by a spectrum of genetic lesions. Because some mutant *CYP21A2* alleles are common in the general population, most patients are compound heterozygotes, carrying a different mutation on the allele inherited from each parent. Finally, factors other than the specific mutations found in *CYP21A2* will influence the clinical phenotype, including the presence of extraadrenal 21-hydroxylases and variations in androgen metabolism and sensitivity. Thus discordances between genotype and phenotype are to be expected.

Incidence of 21-Hydroxylase Deficiency

Perinatal screening for elevated serum 17OHP has shown that the incidence of classic SW and SV 21OHD is about 1 in 14,000,

yielding a heterozygous carrier rate of 1 in 60. Screening of 1.9 million newborns in Texas yielded an overall incidence of 1 in 16,000, including incidences of 1 in 15,600 Caucasians, 1 in 14,500 Hispanics (mostly Mexican-Americans of indigenous American ancestry), and 1 in 42,300 African-Americans.²⁵⁷ Because about 20% of the African-American gene pool is of European origin, the calculated incidence in individuals of wholly sub-Saharan African ancestry is about 1 in 250,000; the basis for this apparent ethnic difference is unknown.³⁴ NC 21OHD is much more common, with the highest incidence among Mediterranean peoples.^{10,40,258} The high incidence of NC 21OHD, its nonlethality, and its negligible impact on fertility suggest it is a polymorphic variant and not a disease in the classic sense. Nevertheless, patients with NC 21OHD may seek help for complaints of virilism, menstrual disorders, and fertility.²⁵⁸

Genetics of 21-Hydroxylase Deficiency

The *CYP21* Genes. The functional *CYP21A2* gene and the nearby nonfunctional *CYP21A1P* pseudogene consist of 10 exons, are ~3.4kb long, are duplicated in tandem with the *C4A* and *C4B* genes encoding the fourth component of serum complement, and lie in the class III region of the human major histocompatibility complex (MHC)^{10,242,243} (Fig. 14.16). *CYP21A1P* is transcribed, but the resultant RNAs do not encode protein.^{259,260} The MHC locus on chromosome 6p21 is characterized by a very high rate of genetic recombination; this high

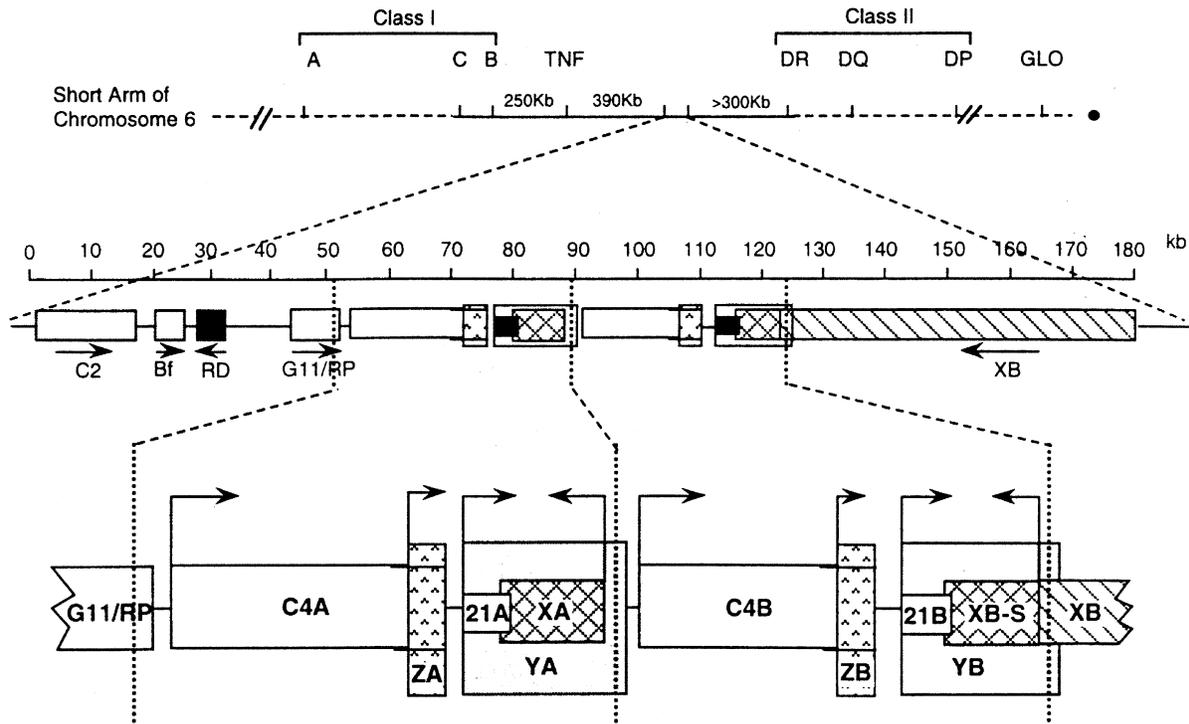


Fig. 14.16 Genetic map of the human leukocyte antigen (HLA) locus containing the genes for P450c21. The top line shows the p21.1 region of chromosome 6, with the telomere to the left and the centromere to the right. Most *HLA* genes are found in the class I and class II regions; the class III region containing the P450c21 genes lies between these two. The second line shows the scale (in kilobases) for the diagram immediately subsequent, showing (from left to right) the genes for complement factor C2, properdin factor Bf, and the RD and *G11/RP* *STK19* genes of unknown function; arrows indicate transcriptional orientation. The bottom line shows the 21-hydroxylase locus on an expanded scale, including the *C4A* and *C4B* genes for the fourth component of complement, the inactive *CYP21A1P* gene (21A) and the active *CYP21A2* gene (21B) that encodes P450c21. XA, YA, and YB are adrenal-specific transcripts that lack open reading frames. The *XB* gene encodes the extracellular matrix protein Tenascin-X; *XB-S* encodes a truncated adrenal-specific form of the Tenascin-X protein whose function is unknown. ZA and ZB are adrenal-specific transcripts that arise within the *C4* genes and have open reading frames, but it is not known if they are translated into protein; however, the promoter elements of these transcripts are essential components of the *CYP21A* and *CYP21B* promoters. The arrows indicate transcriptional orientation. The vertical dotted lines designate the boundaries of the genetic duplication event that led to the presence of A and B regions.

rate of intergenic exchange of DNA results in the *CYP21A2* and *CYP21A1P* genes differing in only 87 or 88 bases. The *cyp21* genes of mice and cattle are similarly duplicated in the MHC loci, but the gene duplication borders differ, and some mammals have only single *cyp21* genes, indicating that the gene duplication postdates mammalian speciation.^{242,261}

Human Leukocyte Antigen Linkage. Because the *CYP21* genes lie in the MHC locus, HLA typing had been used for prenatal diagnosis and to identify heterozygous family members, but this approach has now been replaced by direct genetic analysis. Statistical associations (linkage disequilibrium) are well established between 21OHD and certain specific HLA types.^{10,242,243} SW 21OHD is associated with HLA-B60 and HLA-40 in some populations, and HLA Bw47 is strongly associated with SW 21OHD. HLA-Bw51 is associated with SV 21OHD in some populations, and about 40% of haplotypes for NC 21OHD carry HLA-B14. HLA-B14 is often associated with a duplication of the *C4B* gene, but all HLA-B alleles can be found linked to 21OHD. HLA-identical individuals in a single family may have different clinical features of 21OHD, despite HLA identity, possibly representing extraadrenal 21-hydroxylation, de novo mutations, or multiple genetic crossover events.

Other Genes in the *CYP21* Locus. The *C4A* and *C4B* genes produce very similar isoforms of complement component C4; the *C4B* protein has greater hemolytic activity, despite greater than 99% sequence identity with *C4A*.^{10,242} The *C4A* gene is 22 kb long, but there are long (22 kb) and short (16 kb) forms of *C4B* caused by a variation in one intron. The 3' ends of the *C4* genes are only 2466bp upstream from the transcriptional start sites of the *CYP21* genes. Promoter sequences needed for the transcription of *CYP21A2* lie within intron 35 of *C4B*.²⁶² Several other genes also lie within about 100 kb of *CYP21A2*, including the genes for complement factor C2 and properdin factor Bf (see Fig. 14.16). Lying just 3' of the *Bf* gene, on the opposite strand of DNA from *CYP21A2*, is the *STK19* gene (formerly termed *G11*), which encodes a nuclear serine/threonine kinase.²⁶³

Tenascin-X and the congenital adrenal hyperplasia-X (CAH-X) syndrome. Tenascin-X (TNX), encoded by the *TNXB* gene, is an extracellular matrix protein whose actions are largely antiadhesive, but extend beyond cellular architecture.²⁶⁴⁻²⁶⁷ The *TNXA* and *TNXB* genes are duplicated with the *C4* and *CYP21* genes, and lie on the opposite strand of DNA from the *C4* and *CYP21* genes.^{264,266} The last exons of *TNXA* and *TNXB* lie within the 3' untranslated region of exon 10 in *CYP21A1P* and *CYP21A2*, respectively. *TNXA* was truncated during the duplication of the ancestral *C4-CYP21-TNX* genetic unit; it is transcribed in the adrenal, but has no known function.²⁶¹ The TNX protein encoded by *TNXB* is a large extracellular matrix protein that is expressed in most tissues, especially connective tissue.²⁶⁴ The *TNXB* gene spans about 65 kb and includes 43 exons encoding a 12-kb mRNA. *TNXB* also encodes a truncated 74-kDa form of TNX (XB-Short; XB-S), which is identical to the carboxy-terminal 673 amino acids of TNX, arises from an intergenic promoter, and is expressed uniquely in the adrenal.²⁶⁸ XB-S associates with mitotic motor kinesin Eg5, but its precise function remains unclear.²⁶⁹ Identification of a 21OHD patient with a "contiguous gene syndrome," comprising a deletion of both the *CYP21A2* and *TNXB* genes, demonstrated that TNX deficiency results in Ehlers-Danlos syndrome (EDS).²⁷⁰ Most forms of EDS are caused by autosomal dominant mutations in collagen genes; the recessive forms are caused by mutations in genes for collagen-modifying enzymes, including TNX, which is associated with and stabilizes collagen fibrils. Homozygous TNX deficiency causes a

severe, clinically distinct, recessive form of EDS, either with or without associated 21OHD;²⁷¹ haploinsufficiency of TNX causes the mild "hypermobility form" of EDS, manifesting with joint hypermobility, pain, multiple dislocations, and, less commonly, chronic arthralgia, hernias, and cardiac defects.²⁷² Up to 10% of patients with SW 21OHD have *CYP21A2* gene deletions on one allele that extend into the *TNXB* gene, so that they are also haploinsufficient for TNX and have a distinct form of 21OHD termed CAH-X.²⁷³

CYP21A2 Gene Lesions Causing 21-Hydroxylase Deficiency

21OHD can be caused by *CYP21A2* gene deletions, gene conversions, and apparent point mutations. The most common apparent point mutations are actually small gene conversion events,^{10,242} so that gene conversions account for about 85% of the lesions in 21OHD. Each person has two *CYP21A2* alleles, one contributed by each parent. Most patients with 21OHD are compound heterozygotes, having different lesions on their two alleles. Gene deletions and large conversions eliminate gene transcription; homozygosity for these cause salt-losing 21OHD. Some microconversions, such as those creating premature translational termination, also cause salt-losing 21OHD. Milder forms, such as SV and NC 21OHD, are associated with amino acid replacements in the P450c21 protein caused by gene microconversion events. Patients with these forms of 21OHD are usually compound heterozygotes bearing a severely disordered allele and a mildly disordered allele, so that the clinical manifestations are based on the nature of the mildly disordered allele. The *CYP21A2* and *CYP21A1P* genes differ by only 87 or 88 nucleotides. Two unusual and related features of the 21-hydroxylase locus complicate its analysis. First, the gene deletions in this locus are most unusual in that they extend 30 kb from one of several points in the middle of *CYP21A1P* to the precisely homologous point in *CYP21A2*. Second, gene conversions are extremely common.^{10,242}

Gene Conversions and Microconversions Causing Salt-Wasting 21-Hydroxylase Deficiency. If a segment of gene A replaces the corresponding segment of the related gene B, the structure of recipient gene B is said to be "converted" to that of donor gene A. The hallmark of gene conversion is that the number of closely related genes remains constant, while their diversity decreases. Two types of gene conversions commonly cause 21OHD: large gene conversions that can be mistaken for gene deletions, and small microconversions that resemble point mutations. The frequency of large gene conversions versus gene deletions was formerly controversial, principally because initial studies used relatively small groups of patients from single locations or ethnic groups. A compilation of multiple studies shows that about 19% of mutant alleles have gene deletions, 8% have large gene conversions, 67% have microconversions, and 6% had other lesions²⁴² (Fig. 14.17). Because of ascertainment bias, such numbers emphasize more severely affected patients. About 75% of mutated *CYP21A2* genes are grossly intact and appear to carry point mutations, but most apparent point mutations are also found in the *CYP21A1P* pseudogene, indicating that they result from small gene conversion events (Table 14.6). Three changes in the pseudogene (8-bp deletion, exon 3; T insertion, exon 7; Gly 318 Stop, exon 8) render its RNA product nonfunctional, because of an altered reading frame and/or premature stop codon; these have been found in *CYP21A2* alleles that cause SW 21OHD. Three closely clustered base changes in *CYP21A1P* alter the normal amino acid sequence Ile-Val-Glu-Met at codons 236 to 239 in exon 6 to Asn-Glu-Glu-Lys in a small number of genes causing SW 21OHD.

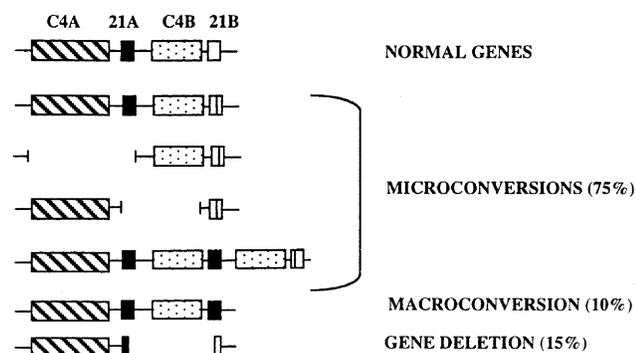


Fig. 14.17 Classes of genetic rearrangements causing 21-hydroxylase deficiency. Deletions or duplications of the *C4A* and *C4B* genes can occur with or without associated lesions in the *P450c21B* gene. Note that all “point mutations” in *P450c21B* are actually “microconversion.” Many authors combine the “gene deletion” and “macroconversion” groups because these are difficult to distinguish by Southern blotting, as both result in a loss of the *P450c21B* gene, but the genotypes are clearly distinct, as shown.

TABLE 14.6 Microconversions in the *CYP21A2* Gene That Cause 21-Hydroxylase Deficiency

Mutation	Location	Associated Phenotypes	Activity
Pro 30 → Leu	Exon 1	NC/SV	30%–60%
A → G	Intron 2	SV/SW	Minimal
8 bp deletion	Exon 3	SW	0
Ile 172 → Asn	Exon 4	SV	3%–7%
Ile 236 → Asp			
Val 237 → Glu	Exon 6	SW	0
Met 239 → Lys			
Val 281 → Leu	Exon 7	NC	18% ± 9%
Gly 292 → Ser	Exon 7	SW	
T insertion @ 306	Exon 7	SW	0
Gly 318 → Stop	Exon 8	SW	0
Arg 339 → His	Exon 8	NC	20%–50%
Arg 356 → Trp	Exon 8	SV/SW	2%
Pro 453 → Ser	Exon 10	NC	20%–50%
GG → C @ 484	Exon 10	SW	0

The most common lesion in classic 21OHD is an A → G change in the second intron, 13 bases upstream from its normal 3' splice acceptor site; this is a microconversion found in over 25% of severely affected 21OHD alleles. This intronic mutation causes abnormal splicing of the encoded RNA, which eliminates activity. However, a small portion of this mRNA may be spliced correctly in some patients so that the phenotypic presentation is variable; most such patients have SW 21OHD, but some have SV 21OHD. This intron 2 microconversion is often associated with the Ser/Thr polymorphism at codon 268, which does not alter enzymatic activity. The microconversion R356W, which is found in about 10% of severely affected alleles, may retain slight activity and has been found in both SW and SV 21OHD. A large number of rare mutations have been described in single individuals.

Mutations Causing Simple Virilizing and Nonclassic 21-Hydroxylase Deficiency. The microconversion I172N is the most common cause of SV 21OHD. When this residue is changed to Asn, Leu, Gln, or His and the resulting mutant *P450c21* protein is assayed in vitro, it retains around 3% to 7% of normal 21-hydroxylase activity. The intron 2 microconversion is occasionally seen in SV 21OHD. The microconversion P30L is generally associated with NC 21OHD, but is

found in some patients with SV 21OHD. The most common mutation causing NC 21OHD is V281L, a microconversion linked to HLA-B14 and HLA-DR1, but also found in patients with other HLA types. The microconversion P30L is found in about 15% to 20% of NC 21OHD alleles, and the mutations R339H and P453S have been associated with NC 21OHD; the P453S mutation is polymorphic in about 20% of *CYP21A1P* pseudogenes, and hence also represents a microconversion event.

Diagnosis of 21-Hydroxylase Deficiency

Prenatal Diagnosis. The diagnosis of 21OHD can be approached prenatally by measuring fetally produced steroids, such as 17OHP or Δ^4 -androstenedione in amniotic fluid, but these steroids may not be elevated above the broad range of normal in fetuses with SV or NC 21OHD. HLA typing of fetal amniocytes can be informative if there is previous linkage analysis of an affected index case and parents, but some HLA alleles are expressed weakly in cultured amniocytes, hence this procedure is rarely used. DNA sequencing is now widely used, but the complex genetics of the *CYP21* locus means this procedure is reliable only when the genetic lesion in a previously affected sibling or the parents is known.

Newborn Screening. Newborn screening programs throughout the industrialized world now incorporate measurements of 17OHP to detect 21OHD. By reducing the time to diagnosis, screening dramatically reduced mortality, morbidity from hyponatremia, length of hospitalization, learning disability, and errors of sex assignment.^{243,257,274} However, screening programs in different jurisdictions use different 17OHP assays, thresholds, and follow-up procedures, hence the physician must become familiar with local procedures. Current guidelines recommend a standardized 17OHP immunoassay from the dried blood samples used for other newborn screens.⁴⁰ Because 17OHP is elevated in premature and sick infants, and in healthy babies in the first 2 days, secondary screening by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) is recommended for all elevated results.⁴⁰ Initial screening results are improved when small babies are stratified for gestational age rather than birth weight.²⁷⁵ Because immunoassays can yield false positive results, if LC-MS/MS is not available, follow-up testing should be done with an intravenous ACTH test. About 40% of samples that are initially reported as positive will have normal 17OHP levels when measured by LC-MS/MS. Genotyping is not recommended for screening programs because of cost, high error rate, undetected mutations, and inability to distinguish mutations in *cis* versus *trans* without genotyping at least one parent.⁴⁰ Measuring additional steroids and their ratios may improve the specificity of screening by LC-MS/MS: the ratio of sum of 17OHP plus 21-deoxycortisol divided by the cortisol level identified all affected children with no false positives.²⁷⁶

Postnatal Diagnosis. When there is no newborn screening, genital ambiguity and/or a salt-losing crisis will generally alert pediatricians to most cases of severe 21OHD. Salt-losing crises generally occur in the second week of life, presenting with vomiting, diarrhea, dehydration, hyperkalemia, and hyponatremia. Occasionally, such infants are thought to have viral syndromes or gastrointestinal obstructions; such a failure to make the diagnosis can result in the infant's death. Boys with SV 21OHD may escape diagnosis until they are 3 to 7 years old, when they present with isosexual precocity, advanced bone age, and characteristically prepubertal testes. Teenage and adult females with NC 21OHD may consult an internist, obstetrician, or dermatologist for virilism, hirsutism, menstrual irregularity, infertility, or acne.

The key diagnostic maneuver is to measure 17OHP and other steroids in response to intravenous synthetic ACTH. The usual doses are 15 µg/kg in infants, 0.125 mg in children up to 2 years of age, and 0.25 mg in older children and adults. 17OHP and cortisol should be measured at 0 and 60 minutes. Individual patient responses must be compared with age- and sex-matched data from normal children. Normal responses are shown in Table 14.4 and Fig. 14.12. Both basal and stimulated levels of 17OHP are markedly elevated in patients with SW and SV 21OHD. Basal levels are usually greater than 2000 ng/dL and increase to more than 5000 to 10,000 ng/dL after ACTH. Patients with NC 21OHD typically have normal or minimally elevated basal levels, but have supranormal responses to ACTH stimulation, that is, 1500 to more than 10,000 ng/dL. The cortisol response to ACTH is absent or subnormal in patients with SW or SV 21OHD and is normal in patients with NC 21OHD. Basal plasma ACTH levels may be markedly elevated in severe forms and may be normal in patients with the milder forms who are not overtly adrenal insufficient.

Other ancillary tests are listed in Table 14.5. Urinary excretion of 17-ketosteroids is generally elevated, but this test is no longer in general use and is more useful for monitoring suppressive therapy than for initial diagnosis. When urinary steroids are measured, a complete 24-hour sample must be obtained, and a concomitant measurement of creatinine excretion is required to monitor the completeness of the collection. Less than 24-hour urine collections are not quantitatively accurate because of diurnal variations in steroid excretion. Urine steroid profiling with mass-spectrometric methods (usually GC/MS-MS) typically shows elevated metabolites of 17OHP: pregnanetriol, 17-OH-pregnanolone and pregnanetriolone.

Plasma renin, measured as renin activity or total content, and its response to salt restriction can be especially useful. Most patients with SV 21OHD have high plasma renin, which increases further on sodium restriction, confirming that these patients are partially mineralocorticoid deficient and can maintain a normal serum sodium only by hyperstimulation of the zona glomerulosa.

Treatment

The management of 21OHD remains difficult.⁴⁰ Overtreatment with glucocorticoids causes delayed growth, even when the degree of overtreatment is insufficient to produce signs of Cushing syndrome. Undertreatment results in continued overproduction of adrenal androgens, which hastens epiphyseal maturation and closure, again resulting in compromised growth and other manifestations of androgen excess.

Doses of glucocorticoids should be based on the expected normal cortisol secretory rate, which is about 6 to 8 mg/m² per day.^{145,277,278} However, effective suppression of ACTH and adrenal androgen production requires somewhat higher doses of about 10 to 15 mg/m² per day.⁴⁰ Newly diagnosed patients, especially newborns, require higher initial dosages to suppress their hyperactive CRH-ACTH-adrenal axis. The glucocorticoid used is important. Most tables of glucocorticoid dose equivalencies are based on their equivalence in antiinflammatory assays; however, growth-suppressant equivalences do not parallel antiinflammatory equivalencies: long-acting synthetic steroids, such as dexamethasone, have a disproportionately greater growth-suppressant effect and hence must be avoided in treating growing children and adolescents (Table 14.7). Most authorities favor the use of oral hydrocortisone in three divided daily doses in growing children. However, adults and older teenagers who already have fused their epiphyses may be managed with prednisone.⁴⁰

Mineralocorticoid therapy in 21OHD returns plasma volume to normal and eliminates the hypovolemic drive to ACTH secretion. Thus mineralocorticoid therapy often permits the use of lower doses of glucocorticoids in patients with SV 21OHD, optimizing growth in children and diminishing unwanted weight gain in adults. The only oral mineralocorticoid preparation generally available is fludrocortisone (9α-fluorocortisol). When the oral route is not available in severely ill patients, mineralocorticoid replacement is achieved with intravenous hydrocortisone plus sodium chloride. About 20 mg of hydrocortisone has a mineralocorticoid effect of about 0.1 mg of 9α-fluorocortisol (see Table 14.7). Mineralocorticoid dosing is not based on body mass or surface area: newborns are quite insensitive to mineralocorticoids as reflected by their high serum aldosterone concentrations (see Fig. 14.17), and often require larger doses than do adults (0.15–0.30 mg/day, depending on the sodium supplementation). In older children, the replacement dose of 9α-fluorocortisol is 0.05 to 0.15 mg daily. Mineralocorticoids are ineffective unless adequate sodium is presented to the renal tubules, hence additional salt supplementation, usually 1 to 2 g NaCl/day in the newborn, is typically needed. Some adult patients with SW 21OHD can discontinue mineralocorticoid replacement and salt supplementation; this probably reflects the increased mineralocorticoid sensitivity in adults, their free access to salty foods, and the induction of hepatic extraadrenal 21-hydroxylating enzymes.⁴¹

Long-term management requires careful clinical and laboratory monitoring. Growth should be monitored at 3- to 4-month

TABLE 14.7 Potency of Various Therapeutic Steroids (Set Relative to the Potency of Cortisol)

Steroid	Antiinflammatory Glucocorticoid Effect	Growth-Retarding Glucocorticoid Effect	Salt-Retaining Mineralocorticoid Effect	Plasma Half-Life (min)	Biologic Half-Life (h)
Cortisol (hydrocortisone)	1.0	1.0	1.0	80–120	8
Cortisone acetate (oral)	0.8	0.8	0.8	80–120	8
Cortisone acetate (IM)	0.8	1.3	0.8		18
Prednisone	4	5	0.25	200	16–36
Prednisolone	4		0.25	120–300	16–36
Methyl prednisolone	5	7.5	0.4		
Betamethasone	25		0	130–330	
Triamcinolone	5		0		
Dexamethasone	30	80	0	150–300	36–54
9α-fluorocortisol	15		200		
DOC acetate	0		20		
Aldosterone	0.3		200–1000		

DOC, Deoxycorticosterone; IM, intramuscular.

intervals in children; bone age should be assessed annually. Each visit should be accompanied by measurement of blood pressure, plasma renin and serum androstenedione, DHEA, DHEAS, and testosterone. Plasma 17OHP is generally measured but may be difficult to interpret because of its variation as a function of the timing of glucocorticoid doses, its diurnal variation, and its hyperresponsiveness to stress (e.g., clinic visits).

Experimental Prenatal Treatment of 21-Hydroxylase Deficiency

Because treatment of 21OHD centers on glucocorticoid suppression of the HPA axis, suppressing the fetal HPA axis has been proposed by administering transplacental glucocorticoids to the mother. Female fetuses affected with CAH begin to become virilized at around 6 to 8 weeks' gestation, the time at which the testes of normal male fetuses produce testosterone, causing fusion of the labioscrotal folds, enlargement of the genital tubercle into a phallus, and the formation of the phallic urethra. The adrenals of female fetuses with 21OHD may produce androgen concentrations that approach those of a normal male, resulting in varying degrees of masculinization of the external genitalia; suppressing fetal adrenal steroidogenesis in a female fetus with 21OHD can theoretically ameliorate the virilization. Dexamethasone has been used for this purpose, but such treatment remains experimental and highly controversial.⁴⁰ This approach has been suggested when the parents are known to be heterozygotes by having previously had an affected child. However, even in such pregnancies, only one in four fetuses will have 21OHD, and, as prenatal treatment is not needed for male fetuses with 21OHD, only one in eight pregnancies of heterozygous parents would harbor an affected female fetus that might potentially benefit from prenatal treatment. Treatment must be started at about 6 weeks postconception (8 weeks of amenorrhea); fetal sexing by fetal DNA in the mother's blood may be done at about this time, allowing non-treatment or early cessation of dexamethasone treatment of male fetuses. However, in female fetuses, chorionic biopsy for DNA diagnostics cannot be done until 12 to 13 weeks, and turnaround times in DNA laboratories are slow if the mutations carried by both parents are not established previously. Thus seven of eight pregnancies may be treated needlessly to treat one affected female fetus.⁴⁰ Some advanced centers might avoid treating male fetuses by identifying Y-chromosome DNA in maternal circulation by 6 weeks; however, this only reduces, but does not eliminate, the number of fetuses needlessly exposed to dexamethasone, and its general applicability is not yet established.

The ethics of such prenatal treatment remains highly controversial.^{40,279,280} The rationale is that dexamethasone, which is not metabolized by placental 11 β HSD2, will cross the placenta, suppress fetal ACTH, and consequently suppress adrenal steroidogenesis. However, it is not known precisely when the fetal hypothalamus begins to produce CRH, when the fetal pituitary begins to produce ACTH, whether all fetal ACTH production is regulated by CRH, or whether these hormones are suppressible by dexamethasone in the early fetus. Although there is evidence that pharmacologic doses of glucocorticoids do not harm pregnant women, few data exist for the fetus. Pregnant women with diseases such as nephrotic syndrome and systemic lupus erythematosus are generally treated with prednisone, which does not reach the fetus because it is inactivated by placental 11 β HSD. Treatment of a fetus with 21OHD requires the use of fluorinated steroids that escape metabolism by 11 β HSD, and few data are available about the long-term use of such agents throughout gestation.

The usual protocol uses dexamethasone doses of 20 μ g/kg of maternal body weight (max 1.5 mg/day); for a 60-kg woman, this is 1.2 mg, which is about 6 times physiologic replacement. However, the fetus normally develops in the presence of very low cortisol concentrations of only about 20 to 60 nmol/L (0.7–2.0 μ g/dL),^{281,282} which is only about 10% of the corresponding maternal level. Thus the doses used in prenatal treatment appear to achieve effective glucocorticoid concentrations that are around 60 times physiologic for the fetus. The potential benefits of prenatal treatment are reduction or elimination of virilization of external genitalia and brain, reducing the risk of gender confusion and the need for surgery. Animal studies indicate prenatally administered dexamethasone increases risks of palatal clefting; impairs brain, kidney; and islet cell development; decreases birthweight; and increases risks of hypertension.^{40,283} Human studies have been limited in methodology or size, but studies in Sweden found that prenatally treated children without 21OHD had poorer working memory and poorer self-perception of scholastic competence, and increased self-rated social anxiety²⁸⁴; there were also mild effects on sociability²⁸⁵ and gender role behavior in boys,²⁸⁶ leading the Swedish investigators to discontinue further research on this treatment.²⁸⁷ Alternative approaches, such as preimplantation genetic diagnosis, also carry some risks. Thus the risks of prenatal treatment appear to outweigh the benefits,²⁸⁰ and all medical societies that have examined this issue as of 2018 recommend that such treatment only be done with fully informed parental consent under Institutional Review Board (IRB)-approved research protocols, in centers that see enough such patients to achieve meaningful results.⁴⁰

Experimental Postnatal Treatment of 21-Hydroxylase Deficiency

Growth is compromised in most children with 21OHD. The use of short-acting glucocorticoids and mineralocorticoid supplementation help, but adult heights are typically 1 SD or more below predicted heights.⁴⁰ The loss of height in 21OHD is partially caused by the effect of sex steroids on epiphyseal closure and partially caused by glucocorticoid-induced resistance to the action of growth hormone; the most crucial times when height is lost are during the first 2 years of life and during the pubertal growth spurt. Consequently, several research studies have addressed optimizing the final height of children with CAH.⁴⁰

Antiandrogens and Aromatase Inhibitors. These drugs have been tried in the presence of low-dose mineralocorticoids and lower doses of glucocorticoids.⁴⁰ Because estrogen is the key hormone in promoting epiphyseal fusion, inhibiting the conversion of androgen to estrogen with an aromatase inhibitor (testolactone) promotes growth, whereas the antiandrogen (flutamide) ameliorates virilization. This approach permits use of physiologic replacement doses of hydrocortisone (8 mg/m²/day), rather than the usual supraphysiologic doses of 12 to 15 mg/m²/day, favoring normal growth. Similarly, trials are underway with abiraterone to inhibit P450c17, thus interfering with the synthesis of all sex steroids. The drugs are expensive and not approved for this use, long-term safety and efficacy are not established, and antiandrogens are potentially hepatotoxic. Thus like all experimental therapies, these approaches should only be pursued in controlled, prospective trials approved by Institutional Review Boards.⁴⁰

Growth Hormone and Gonadotropin-Releasing Hormone Agonist Therapy. Growth-promoting drugs have been proposed in children near the age of puberty as pharmacologic growth hormone therapy may partially overcome the effects of higher doses of glucocorticoids, and GnRH agonist will delay the progression of puberty, permitting more time to grow.

Small preliminary studies with these agents are promising, but both agents are expensive and neither is approved for this use; controlled prospective trials are needed.⁴⁰

Adrenalectomy. Because the adrenal carrying severe *CYP21A2* mutations cannot produce aldosterone or cortisol, it has been argued that these affected glands do more harm than good, and should be removed. Among 18 adrenalectomized patients, five had adrenal crises when therapy was suboptimal and two became hypoglycemic during intercurrent illnesses.⁴⁰ As a result of the high risk of postoperative morbidity and mortality, this approach is not recommended.⁴⁰

Lesions in Isozymes of P450c11

11 β -Hydroxylase Deficiency

11 β -Hydroxylase deficiency (11OHD) is the hyperandrogenic form of CAH. There are two isozymes of 11-hydroxylase. P450c11 β , encoded by the *CYP11B1* gene, is expressed in the zonae fasciculata and glomerulosa, where it catalyzes the 11 β -hydroxylation of 11-deoxycortisol to cortisol and that of DOC to corticosterone; it also has some 18-hydroxylase activity, but has no 18-methyl oxidase activity.^{10,42} P450c11 α S (aldosterone synthase) encoded by the *CYP11B2* gene, is found only in the zona glomerulosa and catalyzes 11 β -hydroxylation, 18-hydroxylation, and 18-oxidation; thus it is the sole enzyme required to convert DOC to aldosterone.²⁸⁸ The *CYP11B1* and *CYP11B2* genes are 93% identical and closely linked on chromosome 8q22, but, unlike the *CYP21A1P* and *CYP21A2* genes in the HLA locus, their genetic recombination is rare.^{10,42} Severe deficiency of P450c11 β decreases the secretion of cortisol, causing CAH and virilization of affected females. The defect in the pathway to cortisol results in accumulation of 11-deoxycortisol and the defect in the 17-deoxy pathway in the synthesis of corticosterone in the fasciculata may lead to overproduction of DOC; elevated concentrations of 11-deoxycortisol that are hyperresponsive to ACTH establish the diagnosis. Because DOC is a mineralocorticoid, these patients can retain sodium. Although DOC is less potent than aldosterone, it is secreted at high levels in 11OHD, so that salt is retained and the serum sodium remains normal. Overproduction of DOC frequently leads to hypertension; as a result, 11OHD is often termed the *hypertensive form of CAH* when detected in older children. However, newborns often manifest mild, transient salt loss,²⁸⁹ as a result of the normal newborn resistance to mineralocorticoids (Fig. 14.18); this may lead to incorrect diagnosis and treatment. Thus there may be a poor correlation between DOC concentrations, serum potassium, and blood pressure or between the degree of virilization in affected females and the electrolyte and cardiovascular manifestations. Newborns may also have elevated concentrations of 17OHP, presumably as a “back-up” phenomenon of high concentrations of 11-deoxycortisol inhibiting P450c21, so that 11OHD may be detected in newborn screening for 21OHD.²⁵⁷ The diagnosis is established by demonstrating elevated basal concentrations of DOC and 11-deoxycortisol, which hyperrespond to ACTH; a normal or suppressed plasma renin activity is also a hallmark of this disease.

11OHD appears to cause about 5% of CAH in persons of European ancestry, but is more common in both Moslem and Jewish Middle Eastern populations,^{290–292} and accounts for up to 13.5% of CAH in Turkey,²⁹³ where 13 different *CYP11B1* mutations have been found.²⁹⁴ Over 100 *CYP11B1* mutations have been reported.^{291,295} Common *CYP11B1* mutations include R448H among Moroccan Jews, and Q356X and G379V in Tunisian Arabs.²⁹¹ A milder, nonclassic form of 11OHD, analogous to nonclassic 21OHD, has been reported

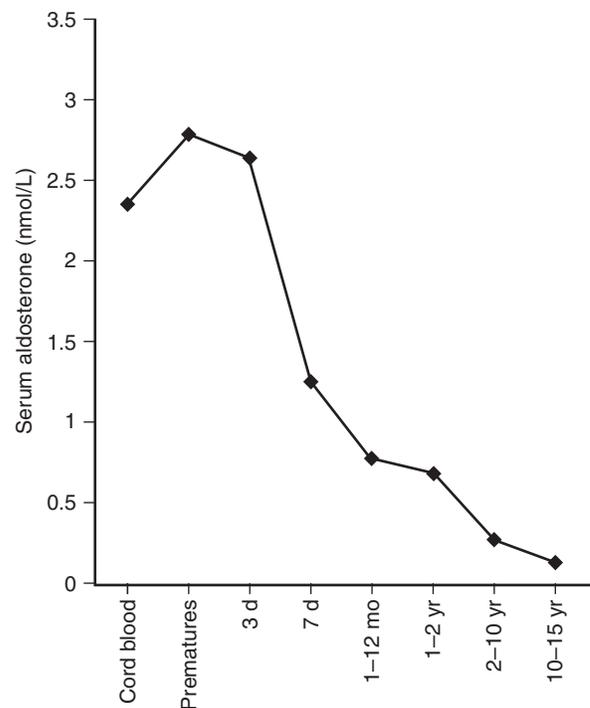


Fig. 14.18 Concentrations of aldosterone as a function of age.

in otherwise asymptomatic women with hirsutism, virilism, and menstrual irregularities, and in men with sexual precocity.^{291,296,297} However, true nonclassic 11OHD is rare; only two of five hyperandrogenic women who had 11-deoxycortisol values more than 3 times higher than the 95th percentile, in response to stimulation with ACTH, had mutations of P450c11 β , all of which retained 15% to 37% of normal activity.²⁹⁸ Repeated ACTH testing in two of the three women who lacked mutations showed much lower (but still elevated) 11-deoxycortisol values. Thus just as in the case of nonclassic 3 β HSD deficiency, an abnormal steroid response to ACTH is not sufficient to diagnose a genetic lesion. Treatment of 11OHD entails glucocorticoid replacement with doses similar to those used in 21OHD, but without mineralocorticoid replacement.

Corticosterone Methyl Oxidase Deficiencies

P450c11 α S is expressed exclusively in the zona glomerulosa where it catalyzes 11 β -hydroxylase, 18-hydroxylase, and 18 methyl oxidase activities. Disorders of P450c11 α S cause the so-called corticosterone methyl oxidase (CMO) deficiencies, wherein aldosterone biosynthesis is impaired, whereas the zona fasciculata continues to produce corticosterone and DOC.^{42,299} The absence of aldosterone biosynthesis will generally result in a salt-wasting crisis in infancy, at which time the normal secretory rate of DOC is insufficient to meet the newborn's mineralocorticoid requirements (similarly to the newborn with 11OHD). These infants typically present with hyponatremia, hyperkalemia, and metabolic acidosis, but the salt-wasting syndrome is typically less severe than in patients with 21OHD or lipid CAH because of the persistent secretion of DOC. These patients may recover spontaneously and grow to adulthood without therapy,³⁰⁰ probably reflecting the increasing sensitivity to mineralocorticoid action with advancing development, shown by the age-related decrease in serum aldosterone (see Fig. 14.18). Consistent with this, plasma renin activity is markedly elevated in affected children but may be normal in affected adults.

CMOI deficiency results from a complete loss of P450c11AS activity so that no 18-hydroxylase or 18 methyl oxidase activity persists, eliminating the biosynthesis of 18OH-corticosterone and aldosterone, while preserving the biosynthesis of corticosterone by P450c11 β . Thus the diagnosis for CMOI deficiency is usually based on an increased ratio of corticosterone to 18OH-corticosterone.⁴² CMOII deficiency results from amino acid replacement mutations in P450c11AS that selectively delete the 18-methyl oxidase activity, while preserving the 18-hydroxylase activity: serum 18OH-corticosterone is high and aldosterone is low.^{42,299} The diagnosis of CMOII deficiency requires an increased 18OH-corticosterone and very low aldosterone concentration. CMOII deficiency is common in Sephardic Jews of Iranian origin, where all affected individuals appear to be homozygous for two different mutations, R181W and V385A.³⁰¹ Family members who were homozygous for only one of these mutations were clinically unaffected; both mutations are required to cause disease. The clinical and hormonal findings in CMOI and CMOII overlap, and these syndromes should be regarded as different degrees of severity on a continuous clinical spectrum,^{302,303} similar to the overlapping clinical spectrum of 21OHD.

Glucocorticoid-Suppressible Hyperaldosteronism

Although *CYP11B* gene conversions are rare, an unusual gene duplication causes glucocorticoid-suppressible hyperaldosteronism. A homologous recombination event creates a third *CYP11B* gene that fuses the 5' flanking DNA of the *CYP11B1* gene for P450c11 β onto the *CYP11B2* gene for P450c11AS, thus placing the regulation of P450c11AS under the control of ACTH rather than the renin-angiotensin system, so that these patients make P450c11AS in response to physiology that should stimulate P450c11 β .^{304–306} The excess P450c11AS causes hyperaldosteronism and hypertension; this is then suppressible by glucocorticoid suppression of ACTH, which normally suppresses P450c11 β , hence the name "glucocorticoid-remediable hypertension"; this disorder appears to account for about 2% of hypertension.³⁰⁷

Lesions in Isozymes of 11 β -Hydroxysteroid Dehydrogenase

Cortisone and prednisone are inactive prohormones that must be reduced to cortisol or prednisolone to bind to and activate the GR. The interconversion of these keto- and hydroxysteroids is catalyzed by the two isozymes of 11 β -hydroxysteroid dehydrogenase, 11 β HSD1 (encoded by *HSD11B1*) and 11 β HSD2 (encoded by *HSD11B2*). Both enzymes are reversible in vitro, hence both can act as either an oxidase or reductase, depending on the availability of cofactors, but under physiologic situations, 11 β HSD1 generally acts to activate cortisone to cortisol, whereas 11 β HSD2 reverses this activation.^{10,16,71,73} 11 β HSD1 is primarily expressed in liver and fat, and 11 β HSD2 is expressed in mineralocorticoid-responsive tissues where it inactivates cortisol, permitting low concentrations of aldosterone to activate MRs (which also bind cortisol); 11 β HSD2 is inactive against aldosterone, DOC, and fludrocortisol. Interest in these enzymes extends far beyond their deficiency states, as they play central roles in metabolism;^{70,71} this has stimulated interest in using inhibitors of 11 β HSD1 to treat the metabolic syndrome, but such agents are not yet clinically available.³⁰⁸

Lesions in 11 β HSD1—(Apparent) Cortisone Reductase Deficiency

Defective 11 β HSD1 activity, diagnosed by reduced ratios of urinary metabolites of cortisol to those of cortisone, impairs cortisol feedback at the hypothalamic/pituitary axis, increasing the

secretion of ACTH and consequently increasing adrenal C19 steroid secretion, resulting in hyperandrogenism, sexual precocity, and polycystic ovaries. These patients may have heterozygous mutations (K187N, R137C) in the *HSD11B1* gene encoding 11 β HSD1; because 11 β HSD1 normally functions as a dimer, the presence of some mutant protein can exert dominant negative effects.³⁰⁹ Alternatively, a genetic lesion may be found in the *H6PDH* gene,³¹⁰ which encodes H6PDH, the enzyme that generates the NADPH used by 11 β HSD1 in the lumen of the endoplasmic reticulum. Although it was initially thought that mutations in both 11 β HSD1 and H6PDH interacted to cause this disease,³¹¹ mutations of H6PDH in patients appear to manifest with a more severe phenotype and are sufficient to cause this disorder, and only a few cases are caused by mutations in *HSD11B1*.^{73,309,310,312–314}

Lesions in 11 β HSD2—Apparent Mineralocorticoid Excess

Patients with apparent mineralocorticoid excess (AME) have hypervolemic hypertension, salt retention, and hypokalemic alkalosis—the classic picture of hyperaldosteronism—but with suppressed plasma renin activity and without measurable serum mineralocorticoids because of recessive mutations of 11 β HSD2.⁶⁸ About 30 different mutations in 11 β HSD2 have been described in about 60 patients with AME, and heterozygous carriers may have an increased risk of hypertension.³¹⁵ Typical features of children with AME include failure to thrive, delayed puberty, polydipsia, polyuria, muscle weakness, and hypertension. The hypertension is severe, often causing end-organ damage at an early age. Diagnosis is made from the high ratio of urinary metabolites of cortisol to cortisone. Treatment includes antagonism of the MR with spironolactone, correction of the hypokalemia, low-salt diets, and diuretics, but is only partially successful, and 10% of patients die from cerebrovascular accidents.³¹⁶

PAPSS2 Deficiency

The adrenal, gonad, and brain make DHEA, but only the adrenal effectively sulfates DHEA to DHEAS, catalyzed by SULT2A1; DHEAS may be desulfated back to DHEA by steroid sulfatase. SULT2A1 uses PAPS as its sulfate donor, which is in turn synthesized by PAPSS2 (see Section II). Sulfation by SULT2A1 predominates, especially in the zona reticularis; steroid sulfatase activity plays a relatively minor role in the adrenal, and its deficiency causes X-linked ichthyosis. SULT2A1 deficiency was suspected in a girl with mild vertebral deformities, premature pubarche, advanced bone age, and hirsutism; her DHEA, androstenedione, and testosterone were elevated but DHEAS was absent. This patient's *SULT2A1* gene was normal, but there was compound heterozygosity for PAPSS2 mutations.^{56,317,318} PAPSS2 deficiency depletes the adrenal of PAPS, increasing production of unconjugated DHEA, which is acted on by hepatic 3 β HSD1, yielding androgens. PAPSS2 deficiency had been reported in a Pakistani family with a rare skeletal defect termed "spondyloepimetaphyseal dysplasia (SEMD)/brachyolmia type 4," but endocrine studies had not been done in those individuals;^{319,320} these and other similar patients^{321–323} had complete loss of PAPSS2 activity.

ADRENAL INSUFFICIENCY

Many conditions will cause adrenal insufficiency, including CAH, hypopituitarism with ACTH deficiency, and primary adrenal disorders.³²⁴ Primary adrenal insufficiency is commonly termed *Addison disease*, but this is a vague term that encompasses many disorders. Up to World War II, most patients with "Addison disease" had tuberculosis of the adrenal, but over 80% of contemporary adult patients have autoimmune adrenalitis,

TABLE 14.8 Causes of Adrenal Insufficiency**PRIMARY ADRENAL INSUFFICIENCY**

Congenital adrenal hyperplasia
 Autoimmune disorders
 Autoimmune adrenalitis
 Autoimmune polyglandular syndromes
 Adrenal hypoplasia congenita
 X-linked adrenal hypoplasia
 Other (*SF1*, IMAGE syndrome)
 ACTH resistance syndromes
 Familial glucocorticoid deficiencies, types 1 and 2
 Triple A (Allgrove) syndrome
 Metabolic disorders
 Adrenoleukodystrophy
 Peroxisome biogenesis disorders (e.g., Zellweger)
 Cholesterol metabolism (Smith-Lemli-Opitz, Wolman)
 Mitochondrial (Kearn-Sayres, mitochondrial deletions)
 Infectious disorders
 Sepsis
 Tuberculosis
 Fungal infections
 Viral
 Infiltrative/destructive causes
 Hemorrhage
 Amyloidosis, sarcoidosis, metastases
 Drugs inhibiting steroid biosynthesis

SECONDARY ADRENAL INSUFFICIENCY

Hypothalamic tumors, radiation or surgery
 Hypopituitarism
 Isolated ACTH insufficiency
 Defects in POMC synthesis and processing
 Withdrawal from glucocorticoid therapy

ACTH, Adrenocorticotropic hormone; IMAGEe, intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia, genitourinary anomalies; POMC, proopiomelanocortin; SF1, steroidogenic factor 1.

therefore the term Addison disease is now widely used to indicate an autoimmune or idiopathic cause.

The spectrum of adrenal disorders presenting in infants, children, and adolescents differs from that presenting in adulthood (Table 14.8). CAH and autoimmune adrenal disease represent the largest proportions of cases, but some of the inherited developmental and metabolic causes of adrenal failure are also fairly common.^{160,325} Diagnosing some of these disorders is important for assessing potential associated features, initiating long-term management, and instituting genetic counseling.^{324,326} Adrenal disorders are typically divided into chronic and acute causes, but many acute presentations reflect an undiagnosed underlying chronic or developmental process (Table 14.9). Acute presentations may be triggered by intercurrent illness, trauma, or surgery, with poor fluid and sodium intake. Another way to group adrenal disorders consists of syndromic versus nonsyndromic forms, with syndromic forms being associated with additional signs and symptoms of other organ systems.

Acute Primary Adrenal Insufficiency

Acute adrenal crisis occurs most commonly in the child with undiagnosed chronic adrenal insufficiency who is subjected to an additional severe stress, such as major illness, trauma, or surgery. The major presenting symptoms and signs include abdominal pain, fever, hypoglycemia with seizures, weakness, apathy, nausea, vomiting, anorexia, hyponatremia, hypochloremia, acidemia, hyperkalemia, hypotension, shock, cardiovascular collapse, and death.³²⁴ Treatment consists of fluid and electrolyte resuscitation, ample doses of glucocorticoids, chronic glucocorticoid and mineralocorticoid replacement, and treatment of the precipitating illness.

Massive adrenal hemorrhage, with shock caused by blood loss, can occur in large infants who have had a traumatic delivery. A

TABLE 14.9 Signs and Symptoms of Adrenal Insufficiency**FEATURES SHARED BY ACUTE AND CHRONIC INSUFFICIENCY**

Anorexia
 Apathy and confusion
 Dehydration
 Fatigue
 Hyperkalemia
 Hypoglycemia
 Hyponatremia
 Hypovolemia and tachycardia
 Nausea and vomiting
 Postural hypotension
 Prolonged neonatal jaundice
 Salt craving
 Weakness

FEATURES OF ACUTE INSUFFICIENCY (ADRENAL CRISIS)

Abdominal pain
 Fever

FEATURES OF CHRONIC INSUFFICIENCY (ADDISON DISEASE)

Decreased pubic and axillary hair
 Diarrhea
 Hyperpigmentation
 Low-voltage electrocardiogram
 Small heart on x-ray
 Weight loss

flank mass is usually palpable and can be distinguished from renal vein thrombosis by microscopic rather than gross hematuria; the diagnosis is then confirmed by CT or ultrasonography. Massive adrenal hemorrhage is more commonly associated with meningococemia (Waterhouse-Friederichsen syndrome). Meningitis is often, but not always, present. The characteristic petechial rash of meningococemia can progress rapidly to large ecchymoses; the blood pressure drops and respirations become labored, frequently leading rapidly to coma and death. Immediate intervention with intravenous fluids, antibiotics, and glucocorticoids is not always successful. A similar adrenal crisis may also occur rarely with septicemia from *Streptococcus*, *Pneumococcus*, *Pseudomonas*, diphtheria, and methicillin-sensitive and resistant isolates of *Staphylococcus aureus*.³²⁷ Adrenal hemorrhage has also been reported with the antiphospholipid syndrome and in patients on anticoagulant therapy.

Chronic Primary Adrenal Insufficiency

Autoimmune Disorders

Autoimmune adrenalitis is most commonly seen in adults 25 to 45 years old, about 60% to 70% of whom are women, with an adult prevalence of about 1 in 25,000.³²⁸ Autoimmune destruction of other endocrine tissues is frequently associated with autoimmune adrenalitis. Chronic adrenal insufficiency is suggested by poor weight gain or weight loss, weakness, fatigue, anorexia, hypotension, hyponatremia, hypochloremia, hyperkalemia, frequent illnesses, nausea, and vague gastrointestinal complaints (see Table 14.9), reflecting chronic deficiency of both glucocorticoids and mineralocorticoids. Early in the course of autoimmune adrenalitis, one may see signs of glucocorticoid deficiency (weakness, fatigue, weight loss, hypoglycemia, anorexia) without signs of mineralocorticoid deficiency (hyponatremia, hyperkalemia, acidosis, tachycardia, hypotension, low voltage on electrocardiogram, small heart on chest x-ray), or evidence of mineralocorticoid deficiency without glucocorticoid deficiency. Thus an initial clinical presentation that spares one category of adrenal steroids does not mean it will be spared in the long run. The symptoms listed in Table 14.9 can be seen in chronic adrenal insufficiency that is either primary or secondary. In primary chronic adrenal insufficiency, the low concentrations of plasma cortisol stimulate

the hypersecretion of ACTH and other POMC peptides, including the various forms of MSH; consequently, chronic primary adrenal insufficiency is also characterized by hyperpigmentation of the skin and mucous membranes, whereas secondary adrenal insufficiency is not. Such hyperpigmentation is most prominent in skin exposed to sun and in flexor surfaces, such as knees, elbows, and knuckles. The diagnosis is suggested by the aforementioned signs and symptoms, verified by a low morning cortisol level with a high ACTH, and confirmed by a minimal response of cortisol to a 60-minute intravenous ACTH test. Hyponatremia, hyperkalemia, low aldosterone, and elevated PRA suggest a disturbance in mineralocorticoid production. Associated findings may include the appearance of a small heart on chest x-ray, anemia, azotemia, eosinophilia, lymphocytosis, and hypoglycemia during fasting. Treatment of chronic primary adrenal insufficiency consists of physiologic glucocorticoid and mineralocorticoid replacement therapy.

Autoimmune adrenalitis is strongly associated with specific HLA haplotypes and with polymorphisms in the gene for cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), which may be broadly involved in susceptibility to autoimmune disease.^{329–331} The diagnosis of autoimmune chronic adrenal insufficiency is based largely on finding circulating antibodies directed against adrenal cells or adrenal cellular contents. In many cases, the adrenal antigens are steroidogenic cytochrome P450 enzymes, especially P450_{sc}, P450_{c17}, and P450_{c21}.³²⁸ It is not clear how these enzymes reach immune cells to elicit an antibody response, but autopsy studies show infiltration of the adrenal cortex.¹⁶⁰ Thus it is likely that cell-mediated immunity is responsible for destruction of adrenocortical cells, resulting in a secondary discharge of cellular contents (including P450 enzymes) into the circulation, with subsequent development of secondary “marker” antibodies against these P450s. About half of adult patients with lymphocytic adrenalitis will also have autoimmune disease of another endocrine tissue with high titers of antibodies directed against specific contents of the affected tissue. This finding has led to the definition of specific autoimmune polyendocrine syndromes (APSs), some of which are more prevalent in childhood.³³¹

Type 1 Autoimmune Polyendocrine Syndrome (APS1). Also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (APECED), APS1 is characterized by chronic mucocutaneous candidiasis, autoimmune Addison disease, and hypoparathyroidism. At least two of these features must be present to make the diagnosis, and their age of onset can be highly variable. In general, chronic mucocutaneous candidiasis appears in early childhood and affects the mouth and nails. Acquired hypoparathyroidism can present with clinical hypocalcemia during mid- or late childhood, although in some cases hypocalcemia may be masked by untreated adrenal insufficiency. The adrenal disorder usually presents in childhood or adolescence;³³² autoimmune adrenal disease may be a presenting feature in about 5% of cases³³³ and is found in more than 60%, predominantly in males (~2m:1f).³³² Additional autoimmune features of this condition include alopecia and vitiligo; gastritis, chronic diarrhea, and malabsorption, with or without pernicious anemia; hypergonadotropic hypogonadism, especially in women; and less commonly hepatitis, thyroiditis, interstitial nephritis, myositis, dental enamel hypoplasia, acquired asplenia, keratoconjunctivitis, and type 1 diabetes mellitus. Keratoconjunctivitis is an important associated feature that requires careful monitoring and treatment to prevent blindness. Oral or esophageal squamous cell carcinoma occurs in 10% of individuals as adults.³³³ APS1 is rare in most populations, but is common among people of Finnish (1:25,000), Sardinian (1:14,000), and Iranian Jewish (1:9,000) ancestry.³³¹ APS1 is caused by recessively inherited mutations in a 58-kDa transcription factor called *autoimmune regulator* (AIRE).^{334,335} More than 100 different mutations in the AIRE

gene have been described, although the homozygous or compound heterozygous R257X change is especially common in the Finnish population, whereas a 13-bp deletion (p.C322del13) is prevalent in persons from Norway.³³¹ The AIRE gene is expressed in thymic epithelial cells and rare peripheral dendritic cells that mediate the ectopic presentation of otherwise tissue-restricted peptides and promote self-tolerance of autoreactive thymocytes. In addition, AIRE seems to induce specific regulatory T cells in the thymus that are able to suppress autoimmunity. Deletion of AIRE in mice results in ectopic expression of peripheral tissue antigens in thymic medullary epithelial cells, resulting in the development of an autoimmune disorder similar to APS1/APECED.^{331,336} In general, autoimmune polyendocrinopathies are treated by hormone replacement therapy. Follow-up visits are recommended annually and screening of family members is advised. APS1 is also discussed in detail in Chapter 22.

Type 2 Autoimmune Polyendocrine Syndrome (APS2). Also known as Schmidt’s syndrome, APS2 refers to the relatively common association of autoimmune adrenalitis with thyroiditis and/or type 1 diabetes.³²⁸ APS2 is more common in females (3:1 ratio), is HLA-linked, and is generally seen in young or middle-aged adults, but can present at almost any age. Primary (hypergonadotropic) ovarian failure is seen in up to one-quarter of postpubertal females with APS2, but primary testicular failure is rare.¹⁶⁰ Pernicious anemia, hepatitis, vitiligo, and alopecia may also be seen, but the hypoparathyroidism and mucocutaneous candidiasis typical of APS1 are not seen in APS2. APS2 is associated with the same HLA markers as idiopathic autoimmune adrenalitis, which may simply be a form of APS2. But the heritability of APS2 is complex. APS2 and the subsequent X-linked immunodysregulation, polyendocrinopathy and enteropathy (IPEX) syndrome are discussed in detail in Chapter 22.

X-Linked Immunodysregulation, Polyendocrinopathy, and Enteropathy (IPEX). IPEX is a rare syndrome caused by mutations in the FOXP3 gene; more than 70 mutations have been described.³³¹ IPEX comprises of early-onset type 1 diabetes, autoimmune enteropathy manifesting with diarrhea and malabsorption, and dermatitis.³³⁷ Eosinophilia and elevated immunoglobulin (Ig)E levels may be seen. Kidney disease, autoimmune thyroiditis, alopecia, as well as autoimmune hepatitis, and exocrine pancreatitis, hemolytic anemia, and thrombocytopenia may be late manifestations. The disease is mostly lethal in infancy because of infections, and allogeneic bone marrow transplantation may offer the only cure.³³⁸

Immunotherapy-Related Autoimmune Disease. Immunotherapy has been highly successful with many cancers, but immunotherapy may be associated with immunotoxicity and autoimmunity.³³⁹ Autoimmune manifestations caused by immune checkpoint inhibitors (ICIs) depend on the pathway(s) targeted. ICIs disrupt the maintenance of immunological tolerance to self-antigens. Adverse events have been reported against multiple organ systems, including the gastrointestinal tract, skin, and all endocrine glands. Frequently observed endocrine manifestations are hypo- and hyperthyroidism, hypophysitis, autoimmune diabetes mellitus, and primary adrenal insufficiency.³⁴⁰ Use of the monoclonal antibody, ipilimumab, which blocks CTLA-4, has been associated with hypophysitis, whereas programmed cell death protein-1–antibody therapy is often associated with hypothyroidism.³³⁹ ICI-related hypophysitis often leads to life-threatening secondary adrenal insufficiency, necessitating prompt glucocorticoid replacement therapy, and should be considered or treated before starting any therapy for concomitant hypothyroidism. Primary adrenal insufficiency is rare with single-agent ICI treatment, but risk may exceed 4% with combination therapies.³⁴⁰

Adrenal Hypoplasia Congenita

Adrenal hypoplasia congenita, (AHC) also known as *congenital adrenal hypoplasia* or *adrenal dysgenesis*, is a disorder of adrenal development resulting in primary adrenal insufficiency. This condition can occur with several different inheritance patterns, and with a variety of associated or syndromic features.

X-Linked Adrenal Hypoplasia Congenita. This most common form of AHC is caused by mutations of the *NR0B1* gene encoding DAX1 on chromosome Xp21. In this most common form of primary adrenal hypoplasia, the definitive zone of the fetal adrenal does not develop, and the fetal zone is vacuolated and cytomegalic. About half of boys with AHC present with salt-loss and glucocorticoid insufficiency in early infancy; the rest present more insidiously with chronic adrenal insufficiency throughout childhood.³²⁵ Hypogonadotropic hypogonadism and incomplete pubertal development are associated features, although early puberty, with subsequent pubertal arrest, has been reported in rare cases. An underlying defect in spermatogenesis may also be present.

DAX1 is a nuclear transcription factor involved in adrenal and testicular development, as well as being expressed in pituitary gonadotropes. About two-thirds of boys with AHC have point mutations,³²⁵ the other one-third have *NR0B1* gene deletions either in isolation or as part of a contiguous gene deletion syndrome involving a telomeric X-linked mental retardation locus (*ILIRAPL1*) and/or centromeric genes for glycerol kinase deficiency (*GKD*) and sometimes ornithine transcarbamylase (*OTC*) and Duchenne muscular dystrophy (*DMD*). An adult-onset form of AHC caused by point mutations has also been described.³⁴¹

Boys with AHC respond well to glucocorticoid and mineralocorticoid replacement therapy. Testosterone treatment to induce secondary sexual characteristics is needed in adolescence; spontaneous fertility is rare and attempts to induce spermatogenesis with gonadotropins are rarely successful. Female carriers are unaffected, but half of their sons will be affected. Close monitoring and genetic counseling can help to prevent life-threatening adrenal crises in other family members or future pregnancies. Thus a family history of adrenal failure, unexplained death, or pubertal abnormalities in the male relatives of a boy with adrenal insufficiency should suggest AHC; indeed, a substantial proportion of boys with (sporadic) adrenal hypoplasia have DAX1 mutations.^{195,325,342}

Autosomal Forms of Adrenal Hypoplasia. In addition to X-linked AHC, autosomal recessive forms of adrenal hypoplasia have been reported and are yielding to genetic analysis. Mutations in SF1 (*NR5A1*), which were discussed earlier as a disorder of steroidogenesis that resembles lipoid CAH, are sometimes classified as a form of adrenal hypoplasia.^{342,343} However, SF1 mutations affect the gonad more severely than the adrenal, and have not been reported in phenotypic males with adrenal hypoplasia.³²⁵ Primary adrenal failure has also been associated with Pena-Shoekir syndrome type I (*DOK7*, *RAPSN*), pseudotrisomy 13, Meckel syndrome (*MKS1*), hydrolethalus syndrome (*HYLS1*), Galloway-Mowat syndrome (*WDR73*), the Pallister-Hall syndrome (*GLI3*), and with defects in *WNT3*.^{4,344}

Triple A (Allgrove) Syndrome. This disorder consists of (1) ACTH-resistant adrenal (glucocorticoid) deficiency (80% of individuals), (2) achalasia of the cardia (85%) and (3) alacrima (90%). The alacrima is the earliest and most consistent symptom, and the achalasia and adrenal insufficiency develop over the first 2 decades, although the achalasia is usually noted first. Mineralocorticoid insufficiency is reported in about 15% of cases, and up to 60% of patients develop progressive

neurological symptoms, such as intellectual impairment, sensorineural deafness, peripheral and cranial neuropathies, optic atrophy, parkinsonism, and autonomic dysfunction.¹⁴⁶ About 80% of affected patients have autosomal recessive mutations in *AAAS*, which encodes a WD-repeat protein termed ALADIN^{345,346}; the basis of the disease in patients lacking *AAAS* mutations is not completely solved yet. ALADIN colocalizes to the cytoplasmic side of the nuclear pore, where it interacts with and participates in the nuclear translocation of ferritin heavy chain protein, FTH1, thus rendering cells progressively susceptible to oxidative damage.³⁴⁷ Deficiency of ALADIN impairs the redox potential of adrenal cells and inhibits steroidogenesis.³⁴⁸ ALADIN is also involved in the regulation of mitotic controllers, essential for the formation of the mitotic spindle.³⁴⁹ Clinical findings of the *AAAS* can be quite variable even within the same family. Adrenal insufficiency is rarely the presenting feature. Thus a detailed family history of achalasia, alacrima, or neurological disorders is important when evaluating a patient with primary adrenal failure and may guide directly to the correct underlying disorder.

IMAGE Syndrome. Primary adrenal hypoplasia and neonatal adrenal crisis are also part of the IMAGE syndrome (intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia, genitourinary anomalies).³⁵⁰ A large, multigenerational family, with multiple, well-characterized individuals,³⁵¹ has been studied by linkage analysis, identifying a dominant missense mutation in the *CDKN1C* gene as the cause of this disorder.³⁵² In addition, four other, unrelated patients had four different missense mutations in *CDKN1C*.³⁵² The *CDKN1C* gene encodes the Wilms' tumor suppressor p57KIP2, which inhibits several cyclin-dependent kinases. This gene lies in the imprinted region of chromosome 11p15.5, so that only the maternal allele is expressed. Different mutations in this same gene cause Beckwith-Wiedemann overgrowth syndrome.³⁵³ The Beckwith mutations inhibit cell cycle, whereas the IMAGE mutations do not, and, when expressed in *Drosophila*, the Beckwith mutations have no effect on eye development, but the IMAGE mutations cause restricted growth.³⁵² Thus mutations in the same gene cause both the IMAGE adrenal hypoplasia syndrome and the Beckwith-Wiedemann adrenal hyperplasia syndrome.³⁵⁴ A *CDKN1C* missense mutation has also been described in a patient with Silver Russell syndrome sharing growth restriction but no other features with IMAGE syndrome.³⁵⁵

MIRAGE Syndrome. MIRAGE syndrome³⁵⁶ is characterized by myelodysplasia, infections, restricted growth, adrenal hypoplasia, genital anomalies, and enteropathy, and is associated with poor prognosis. Heterozygous *SAMD9* mutations (on chromosome 7q) have been identified in affected individuals, of which some survived longer, but developed a myelodysplastic syndrome (MDS). These mutations cause gain function of the growth repressor *SAMD9*. In some patients, progressive loss of mutated *SAMD9* by somatic adaptive changes in the bone marrow led to monosomy 7 and thereby rescued the negative effect on growth resulting in prolonged survival; but at the price of MDS.³⁵⁷

MCM4 Mutations. In the Irish Traveler population, the *MCM4* gene encoding minichromosome maintenance complex component 4 has also been found in patients presenting with primary adrenal insufficiency. These patients found in a genetically isolated population present with isolated cortisol deficiency, growth failure, increased chromosomal breakage, and natural killer cell deficiency.³⁵⁸ They may be more susceptible to infections and have an increased risk for neoplasias, but longer follow-up and better understanding of the underlying pathomechanism is needed.

Adrenocorticotrophic Hormone Resistance Syndromes

Hereditary unresponsiveness to ACTH, also termed familial glucocorticoid deficiency (FGD), can present as an acute adrenal crisis, precipitated by an intercurrent illness in an infant, or with the signs and symptoms of chronic adrenal insufficiency in childhood. Several different recessively inherited causes of FGD have been identified so far; these include *MC2R*, *MRAP1*, *StAR*, *CYP11A1*, *NNT*, *MCM4*, and *TXNRD2*. Unlike individuals with autoimmune adrenalitis, adrenal hypoplasia, or other forms of destruction of adrenal tissue, patients with hereditary unresponsiveness to ACTH typically continue to produce mineralocorticoids, because production of aldosterone by the adrenal zona glomerulosa is regulated principally by the renin-angiotensin system. Thus the presenting picture consists of failure to thrive, lethargy, pallor, hyperpigmentation, and hypoglycemia, often associated with seizures. Rare cases may also entail electrolyte abnormalities or increased plasma renin activity, leading to misdiagnosis as a different form of adrenal insufficiency.

MC2R Mutations: Familial Glucocorticoid Deficiency

Type 1. The ACTH receptor is a 7-transmembrane, G protein-coupled member of the family of melanocortin receptors termed MC2R.^{359,360} MC2R mutations appear to be the most common cause of ACTH resistance (25%), with several dozen reported cases, but the statistical distribution of these cases is unclear, as many different clinical criteria have been applied.¹⁸⁵

Most MC2R mutations are missense mutations, which lead to a retention of the receptor in the endoplasmic reticulum. Interestingly, one constitutively active mutation (F278C) of the MC2R protein has been described in a patient with Cushing syndrome.³⁶¹ However, all other patients harboring MC2R mutations present with isolated glucocorticoid deficiency and hyperpigmentation, often early after birth to up to 2 years of age; hypoglycemia is common and ACTH levels are grossly elevated, accounting for the hyperpigmentation. Mineralocorticoid and sex steroid production are usually not affected. However, absent adrenarche caused by low DHEAS is observed in many and establishes a role for ACTH in adrenarche.³⁶² Tall stature, increased head circumference, and advanced bone age have been reported in some cases; these might be secondary effects of grossly elevated ACTH levels affecting MC1R in the growth plate or stimulating estradiol synthesis. Alternatively, stimulated growth hormone axis before treatment could also be responsible.³⁶⁰ Treatment with replacement doses of glucocorticoids typically prevents adrenal crises, but may not suppress elevated ACTH levels completely; nevertheless, the use of supraphysiologic doses of steroids to suppress ACTH should be avoided.

MRAP Mutations: Familial Glucocorticoid Deficiency

Type 2. The MC2R accessory protein, MRAP1, is a small transmembrane protein that forms unusual antiparallel homodimers and serves two functions: it facilitates trafficking of MC2R from the endoplasmic reticulum to the cell membrane, and it interacts with MC2R on the cell surface to facilitate receptor action.³⁶³ Both MRAP1 and the related MRAP2 protein serve similar functions with other members of the MCR family.³⁶⁴ Whereas MRAP1 collaborates with MC2R, MRAP2, which is abundantly expressed in the hypothalamus, collaborates with MC4R and prokineticin receptors (PKRs), and is important in energy homeostasis and thus severe obesity. MRAP1 mutations are a common cause of FGD accounting for about 10% of cases, but MRAP1 mutations are clinically indistinguishable from MC2R mutations.^{195,365} In contrast, human MRAP2 mutations have been described in nonsyndromic severe obesity.

Nicotinamide Nucleotide Transhydrogenase Mutations.

Nicotinamide nucleotide transhydrogenase (NNT) is a protein of the inner mitochondrial membrane that generates NADPH from the energy of the mitochondrial proton gradient. Mitochondrial steroidogenic enzymes (e.g., CYP11A1 or CYP11B2/1) require NADPH for their activity. In addition, NNT and NADPH are important for the cellular balance of reactive oxygen species (ROS). First described in 2012,³⁶⁶ mutations in NNT have now been found in about 10% of FGD patients.^{195,367} Similarly to MC2R and MRAP1 deficiency, they cause isolated cortisol deficiency and may only be distinguished by genetic testing.³⁶⁷

Other Forms of Familial Glucocorticoid Deficiency.

FGD caused by mutations in other genes and proteins disrupting the oxidative stress system in mitochondria include *TXNRD2*,³⁶⁸ *GPX1*, and *PRDX3*.³⁶⁷ Furthermore, mild mutations in STAR that cause nonclassic lipoid CAH^{184,187} or in CYP11A1 may be mistaken for FGD.^{185,367} Overall, high-throughput sequencing of patients and families presenting with an FGD phenotype have revealed disease-causing mutations in about two-thirds of the patients, whereas the rest still remains without genetic diagnosis.

Metabolic Disorders

Metabolic disorders can also cause chronic primary adrenal insufficiency, including adrenoleukodystrophy (Schilder disease), peroxisome biosynthesis disorders (e.g., Zellweger syndrome spectrum), disorders of cholesterol synthesis and metabolism (e.g., Wolman disease/CESD (discussed in Section VI), SLOS), and mitochondrial disorders (e.g., Kearns-Sayre syndrome).

Adrenoleukodystrophy. Adrenoleukodystrophy (ALD) is a peroxisomal disease and is the most common metabolic cause of adrenal failure. Most cases are caused by mutations in the peroxisomal membrane protein ALDP encoded by the *ABCD1* gene on chromosome Xq28^{369,370}; ALDP belongs to the superfamily of ATP-binding cassette transporters. There are many clinical forms of ALD, including the childhood cerebral form (CCALD) with cerebral demyelination, the adolescent cerebral form, the adult cerebral form, adrenomyeloneuropathy, with axonopathy of the pyramidal and somatosensory tracts, and peripheral neuropathy, the olivo-ponto-cerebellar form, and a form presenting only with Addison disease.³⁷¹ CCALD is the most common phenotype. The prevalence of this condition is generally reported to be between 1:20,000 to 100,000, although the overall frequency may be as high as 1:17,000.³⁷² A rare autosomal recessive form of this condition also exists, which usually presents in infancy (see later).

ALDP imports activated acyl-CoA derivatives of very long chain fatty acids (VLCFA) into peroxisomes where they are shortened by β -oxidation.^{373,374} Consequently, ALD is characterized by high ratios of C26 to C22 VLCFA in plasma and tissues, permitting the diagnosis of individual patients and affected fetuses.³⁷⁵ Carriers can usually be detected by VLCFA screening, although genetic analysis may be necessary in some cases. Symptoms of X-linked CCALD commonly develop in mid-childhood, adrenomyeloneuropathy presents in adulthood.³⁷⁶ The same ALDP mutation can cause both ALD and adrenomyeloneuropathy, hence it is likely that other genetic loci are also involved.³⁷⁷ The earliest findings in CCALD are associated with the central nervous system leukodystrophy and include behavioral changes, poor school performance, dysarthria, and poor memory progressing to severe dementia. Symptoms of adrenal insufficiency usually appear after symptoms of white matter disease, but adrenal insufficiency may be the initial finding in up to 20% of children or young

adults.^{372,378,379} In contrast, adrenomyeloneuropathy typically begins with adrenal insufficiency in childhood and adolescence, and signs of neurologic disease follow 10 to 15 years later. About 1% to 3% of female carriers of X-linked ALD may develop neurological symptoms or adrenal dysfunction. Because screening is accurate and the diagnosis has long-term implications, VLCFA should be analyzed in all boys presenting with adrenal failure where the diagnosis is not clear. In fact, some countries, like the United States, have recently started including ALD in their newborn screening program as not to miss the window of opportunity for allogeneic hematopoietic stem cell transplantation for treating CCALD (<https://adrenoleukodystrophy.info/clinical-diagnosis/newborn-screening>). Lifetime prevalence of adrenal insufficiency in ALD males is about 80%, risk is highest in the first 10 years (almost 50%) and decreases to 5% after the age of 40 years.³⁸⁰ At onset of hypocortisolism mineralocorticoid production often is still intact and therefore does not require replacement therapy until showing deficiency.

Dietary therapy with so-called *Lorenzo's oil* (a 4:1 mixture of glyceryl-trioleate and glyceryl-trierucate) improves VLCFA levels, but it has been ineffective for the treatment of established cerebral disease, although a role in preventing the onset of cerebral disease has not been evaluated.^{372,377} Other therapeutic options include hematopoietic stem cell transplantation,^{372,374} which is most successful for early cerebral disease with a reported 5- to 8-year survival rate of 56%.³⁸¹ Lovastatin treatment decreases plasma C24:0 and C26:0, but is no longer recommended because it has no effect on C26:0 in cells or on VLCFAs.³⁸²

Peroxisome Biogenesis Disorders. Peroxisome biogenesis disorders (PBDs) are a group of autosomal recessive conditions caused by mutations in PEX proteins. Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease form the "Zellweger spectrum" and are clinically distinct from rhizomelic chondrodysplasia punctata caused by *PEX7* gene mutations.^{383,384} These disorders are characterized by developmental delay, hypotonia, neurosensory deafness, optic atrophy, and dysmorphic facial development. Patients often develop seizures, especially those with neonatal adrenoleukodystrophy. The diagnosis can be confirmed by the presence of C26:1, increased C26:0, and an increased ratio of C26:C22 and C24:C22 VLCFA. Most children with the severe forms of Zellweger syndrome and neonatal ALD do not survive past 2 years, although other variants of the Zellweger spectrum are associated with longer survival.³⁸⁵

Smith-Lemli-Opitz Syndrome. SLOS is an autosomal recessive defect in cholesterol biosynthesis resulting from abnormalities in the sterol Δ -7-reductase gene, *DHCR7*.³⁸⁶ The incidence of SLOS is estimated from 1:13,000 to 1:80,000. More than 190 mutations have been described, but two predominate: the c.964G>C mutation is mostly found in North America and Western Europe, whereas the W151X mutation is frequently found in Central and Eastern Europe, suggesting founder effects for these two mutations.³⁸⁷ The clinical features of SLOS include microcephaly, developmental delay, a typical facial appearance (short nose with broad nasal bridge and anteverted nares, long philtrum, microretrognathia, blepharoptosis, low-set, posterior-rotated ears, cleft and/or high-arched palate), proximal thumbs, and syndactyly of the second and third toes (>97%). Cardiac, renal, lung, and gastrointestinal abnormalities are also common. Genital anomalies include hypospadias, cryptorchidism, and even ambiguous genitalia in males. Adrenal insufficiency is present in some cases, especially during times of stress or when LDL-derived cholesterol sources are inadequate (e.g., dietary insufficiency/bile salt depletion).³⁸⁸ The clinical spectrum of SLOS is extremely broad, likely

reflecting differences in residual intrauterine cholesterol biosynthesis and/or differences in the transplacental supply of cholesterol supporting fetal development.³⁸⁷ SLOS may be suspected in utero by low maternal estriol and ultrasound findings. Postnatal biochemical analysis of sterol Δ -7-reductase activity, coupled with genetic analysis, can confirm the diagnosis. Alternatively, LC-MS/MS is a good method for sterol detection that can be used for diagnostic confirmation of SLOS. This approach may be adopted for newborn screening for SLOS via dried blood samples in the future. Life expectancy varies with disease severity. Dietary cholesterol supplementation, ranging from 20 to 300 mg/kg/day, has become an established therapeutic intervention. Beneficial effects include providing cholesterol to tissues outside the central nervous system (CNS) and downregulating HMG-CoA reductase, probably suppressing 7-DHC synthesis. Although this may result in amelioration of the extra CNS phenotype, a beneficial effect on brain is unlikely as plasma cholesterol does not cross the blood-brain barrier. Adding simvastatin to cholesterol supplementation did not really improve outcome.³⁸⁷

Mitochondrial Disorders. Disordered mitochondrial function can be associated with primary adrenal dysfunction.^{389,390} Typical clinical features include lactic acidosis, cataracts, sensorineuronal deafness, and myopathy/ophthalmoplegia. Adrenal failure is rare in childhood, but subclinical adrenocortical insufficiency may be found in adults with multisystem disease, and is a poor prognostic factor. The Kearns-Sayre syndrome results from large-scale deletions of mitochondrial DNA and can be associated with additional endocrinopathies, such as hypothyroidism, hypogonadism, diabetes, growth failure, and hypoparathyroidism.

Other metabolic disorders have also been reported to cause adrenal insufficiency, including Niemann-Pick disease type B (not to be confused with the type C disease, discussed in Section VI), a lysosomal lipid storage disease caused by mutations in the sphingomyelin phosphodiesterase 1 (*SMPD1*) gene, manifesting with hepatosplenomegaly and pulmonary disease.

Sphingosine-1-Phosphate Lyase. Sphingosine-1-phosphate lyase (SGPL1) deficiency is a recently described syndromic form of autosomal recessive adrenal insufficiency that manifests in combination with steroid-resistant nephrotic syndrome.^{391,392} Other associated features are ichthyosis, hypothyroidism, cryptorchidism, neurological symptoms, bone malformation, and immunodeficiency. SGPL1 is the enzyme responsible for the final step in the metabolism of sphingolipids. Mutations in upstream components of this pathway lead to sphingolipidoses, such as Gaucher, Niemann-Pick, Fabry, Krabbe, Sandhoff, and Tay-Sachs diseases, and GM1-gangliosidosis, which are lysosomal storage disorders resulting in the accumulation of sphingolipids in organs. Sphingolipidoses are generally progressive, multisystem disorders, some of which have a renal phenotype, but adrenal phenotypes have not been described. With SGPL1 deficiency sphingosine-1-phosphate and other precursors accumulate, leading to adrenal dysfunction and disrupted steroidogenesis.³⁹² The clinical phenotype with SGPL1 mutations has been broader than expected,³⁹¹⁻³⁹⁶ possibly caused by differences among genetic mutations and residual enzyme activity.

Other Causes

Hemorrhage and infections, discussed earlier as causes of acute primary adrenal insufficiency, may spare some adrenal tissue, leaving severely compromised, rather than totally absent, adrenal function. The result, as with autoimmune adrenalitis, is a chronic disorder with insidious onset of the broad range of nonspecific findings described earlier. Tuberculosis, fungal

infections (e.g., histoplasmosis, coccidiomycosis), viral infections (e.g., human immunodeficiency virus [HIV], cytomegalovirus), metastases, amyloidosis, and sarcoidosis may cause a similar clinical picture. Cortisol biosynthesis can also be inhibited by drugs, such as aminoglutethimide, etomidate, suramin, ketoconazole, or anti-HIV treatments; these drugs can cause adrenal insufficiency, and must be used with caution.

Secondary Adrenal Insufficiency

ACTH is required for adrenal cellular growth and for transcription of genes for steroidogenic factors, hence any impairment of ACTH synthesis, release, or action can cause secondary adrenal insufficiency. Examples include hypothalamic defects, hypopituitarism, disorders of POMC synthesis and processing, and suppression of the hypothalamic-pituitary axis, following exogenous steroid treatment.

Most forms of secondary adrenal insufficiency affect glucocorticoid and androgen synthesis rather than mineralocorticoid release, as angiotensin II is the primary drive to the zona glomerulosa. However, the clinical and biochemical assessment may be complicated, as glucocorticoids are necessary for renal free water clearance and concomitant vasopressin (AVP, ADH) insufficiency may be present. In fact, treatment of secondary adrenal insufficiency can unmask a previously inapparent deficiency of ADH and thus precipitate clinical diabetes insipidus, so close attention must be given to fluid and electrolyte balance, when steroid replacement is introduced. Conversely, the hypothyroidism resulting from thyroid-stimulating hormone (TSH) deficiency will result in slowed metabolism of the small amount of cortisol produced, and therefore protects the patient from the symptoms of adrenal insufficiency. Treatment of hypothyroidism with thyroxine will accelerate metabolism of these small amounts of cortisol, thus unmasking adrenal insufficiency caused by ACTH deficiency and, on occasion, can precipitate an acute adrenal crisis. Therefore careful evaluation of the pituitary-adrenal axis is required in hypopituitarism with secondary hypothyroidism. Many clinicians will choose to “cover” a patient with small doses of glucocorticoids (one-fourth to one-half of physiologic replacement) during initial treatment of such secondary hypothyroidism. Finally, it is important to appreciate that combined deficiency of growth hormone and ACTH will strongly predispose the patient to hypoglycemia, as both hormones act to raise plasma glucose. This effect is especially important in infancy and early childhood, when children are vulnerable to hypoglycemia during periods of prolonged fasting.

Hypothalamic Causes

Hypothalamic causes of ACTH insufficiency include tumors and radiotherapy. Tumors, such as craniopharyngioma, are associated with ACTH deficiency in about 25% of patients.^{397,398} The frequency may be higher in tumors, such as germinoma and astrocytoma. Adrenal insufficiency is rarely the presenting complaint but may contribute to the clinical picture. After surgery and/or radiation therapy, the great majority of patients with hypothalamic tumors will have ACTH deficiency as part of their surgical or radiation-induced hypothalamic-pituitary damage. Therefore all such patients should receive glucocorticoid coverage during treatment, irrespective of the status of the HPA axis at the time the tumor is identified. Because treatment of secondary adrenal insufficiency can precipitate diabetes insipidus, close attention to water balance is essential. ACTH insufficiency can also result following whole-brain irradiation for brain tumors and other central malignancies. This may involve both hypothalamic and pituitary mechanisms. The frequency of ACTH

insufficiency in such cases is much lower than following the treatment of hypothalamic tumors but may only manifest some years after treatment.³⁹⁹

Adrenocorticotrophic Hormone Deficiency

ACTH may be deficient as part of a multiple pituitary hormone deficiency (MPHD, panhypopituitarism) or as isolated ACTH insufficiency. MPHD can result from pituitary surgery or radiotherapy, from an infiltrative process (e.g., Langerhans cell histiocytosis), or from a poorly understood form of hypothalamic dysfunction. In most cases, growth hormone secretion is lost first, followed in order by gonadotropins, TSH, and ACTH, so ongoing vigilance and assessment of these patients is required for many years. MPHD can also result from disorders of hypothalamic-pituitary development involving transcription factors, such as HESX1, LHX4, and SOX3. Associated features, such as optic nerve hypoplasia (HESX1) and cerebellar abnormalities (LHX4), may help to focus the diagnosis. Impaired ACTH secretion has also been described in individuals with PROP1 mutations, one of the most frequent genetic causes of MPHD. This finding can occur many years after presentation with growth hormone, TSH, and gonadotropin deficiency, highlighting the importance of long-term follow-up of patients with pituitary disorders.^{400,401}

Patients with MPHD often have a relatively mild form of adrenal insufficiency. Mineralocorticoid secretion is normal, whereas cortisol secretion is reduced but not absent. However, adrenal reserve is severely compromised by the chronic under-stimulation of steroidogenic enzyme biosynthesis. Because some cortisol synthesis continues, the diagnosis may not be apparent unless a CRF or metyrapone test of pituitary ACTH production capacity and an intravenous ACTH test of adrenal reserve are performed. This can be especially true when TSH deficiency is a component of hypopituitarism, as outlined earlier; treatment of secondary hypothyroidism with thyroxine will accelerate metabolism of these small amounts of cortisol, thus unmasking adrenal insufficiency caused by ACTH deficiency and, on occasion, can precipitate an acute adrenal crisis.

Isolated ACTH insufficiency is a rare condition that can be caused by recessively inherited mutations in the *TPIT* gene. *TPIT* encodes a T-box factor (TBX19) that regulates transcription of the POMC promoter in corticotropes.⁴⁰² These patients usually present with severe, early-onset ACTH insufficiency.^{403,404} Hypoglycemia and prolonged jaundice are frequently present, and neonatal death may result.⁴⁰⁵ As this defect is confined to POMC synthesis in the corticotropes, the additional features of generalized POMC deficiency (outlined later) are not present. *TPIT* mutations are not found in approximately half of patients with isolated ACTH insufficiency, suggesting that other causes are yet to be found.⁴⁰⁶ Hypocortisolemia caused by isolated ACTH insufficiency has also been described with hippocampal (memory) defects and hair abnormalities (alopecia) as part of the “Triple H syndrome,” a possible autoimmune association.⁴⁰⁷

Disorders of Proopiomelanocortin

Defects in POMC synthesis and processing can also cause abnormal ACTH production and action. In patients with POMC mutations, plasma ACTH and cortisol concentrations are usually extremely low, but depending on the genetic defect, plasma ACTH concentrations may also be elevated reflecting the production of an immunoreactive but bioinactive protein.⁴⁰⁸ Recessively inherited mutations or deletion of the *POMC* gene will affect multiple POMC peptides, including MSH and β -endorphin. Thus red hair, pale skin, and obesity are associated features of this form of secondary adrenal insufficiency.⁴⁰⁹ These clinical signs may be more subtle in

individuals with naturally dark hair and pigmented skin, or red hair may darken in adulthood.⁴¹⁰ Mutations in prohormone convertase 1 (PC1, *PCKS1*), which is required for the processing of POMC to ACTH, cause abnormal cleavage and processing of several hormone systems, including the generation of bioactive ACTH from POMC.⁴¹¹ Patients with this rare recessive disorder can have hypocortisolemia together with abnormal glucose metabolism, obesity, hypogonadotropic hypogonads, and persistent malabsorptive diarrhea.^{411,412} These patients need glucocorticoid replacement therapy for adrenal insufficiency and their severe obesity and hyperphagia may be successfully treated by setmelanotide, an MC4R agonist.⁴¹³

Long-Term Steroid Therapy

Long-term glucocorticoid therapy can suppress *POMC* gene transcription and the synthesis and storage of ACTH. Furthermore, long-term therapy apparently decreases the synthesis and storage of CRF and diminishes the abundance of receptors for CRF in the pituitary. Therefore recovery of the HPA axis from long-term glucocorticoid therapy entails recovery of multiple components in a sequential cascade, and hence often requires considerable time (see section on Glucocorticoid Therapy and Withdrawal). Patients successfully withdrawn from glucocorticoid therapy or successfully treated for Cushing disease may exhibit a fairly rapid normalization of plasma cortisol values, while continuing to have diminished adrenal reserve for over 12 months. Inhaled steroids, nasal sprays, and even steroid eye drops can cause suppression of the adrenal axis, so vigilance may be needed following their withdrawal or at time of additional stress (e.g., surgery, intercurrent illness).^{414–416} Treatment with cortisone and prednisone during pregnancy will only result in minimal suppression of the fetal adrenal, because of the protective effects of placental 11 β HSD; dexamethasone treatment in pregnancy can affect fetal adrenal steroidogenesis.

ADRENAL EXCESS

Cushing Syndrome

The term “Cushing syndrome” describes any form of glucocorticoid excess; “Cushing disease” designates hypercortisolism caused by pituitary overproduction of ACTH. The related disorder caused by ACTH of nonpituitary origin is termed the “ectopic ACTH syndrome.” The term “Cushing syndrome” is sometimes used to refer specifically to hypersecretion of cortisol from adrenal tumors, but this is ambiguous and should be avoided. Other causes of Cushing syndrome include adrenal adenoma, adrenal carcinoma, and multinodular adrenal hyperplasia. All of the aforementioned are distinct from iatrogenic Cushing syndrome, which is the similar clinical constellation resulting from the chronic administration of suprphysiologic quantities of ACTH or glucocorticoids.

Clinical Findings

The physical features of Cushing syndrome result from chronic hypercortisolism with or without hyperandrogenism. Central obesity, “moon facies,” hirsutism, and facial flushing are seen in over 80% of adults with Cushing syndrome. Striae, hypertension, muscular weakness, back pain, “buffalo hump” fat distribution, psychological disturbances, acne, and easy bruising are commonly described (35%–80%). However, these are the signs and features of advanced Cushing syndrome. When annual photographs of such patients are available, it is often apparent that these features can take 5 years or longer to develop. Thus the classic “Cushingoid appearance” will usually not be the initial picture seen in the child with Cushing syndrome. The earliest, most reliable indicators of hypercortisolism in children

TABLE 14.10 Findings in 39 Children With Cushing Disease

Sign/Symptom	# of Patients	%
Weight gain	36/39	92
Growth failure	31/37	84
Osteopenia	14/19	74
Fatigue	26/39	67
Hypertension	22/35	63
Delayed or Arrested puberty	21/35	60
Plethora	18/39	46
Acne	18/39	46
Hirsutism	18/39	46
Compulsive behavior	17/39	44
Striae	14/39	36
Bruising	11/39	28
Buffalo hump	11/39	28
Headache	10/39	26
Delayed bone age	2/23	13
Nocturia	3/39	8

(From Devoe, D. J., Miller, W. L., Conte, F. A., et al. (1997). Long-term outcome in children and adolescents after transsphenoidal surgery for Cushing's disease. *J Clin Endocrinol Metab*, 82, 3196–3202.)

are weight gain and growth arrest⁴¹⁷ (Table 14.10). Cumulative data from three large studies of pediatric Cushing disease identified weight gain at presentation in 91/97 (94%) cases and growth failure in 82/95 (86%) cases.^{417–419} Thus any overweight child who stops growing should be evaluated for Cushing syndrome. Glucocorticoids suppress growth by increasing hypothalamic secretion of somatostatin, suppressing growth hormone secretion and IGF-1 production, and by acting directly on the epiphyses to inhibit sulfation of cartilage, inhibit mineralization, and inhibit cell proliferation. In contrast, children with simple dietary obesity often grow more rapidly and are tall for their age (presumably because of chronic secondary hyperinsulinism). The obesity of Cushing disease in children is initially generalized rather than centripetal, and a buffalo hump is evidence of long-standing disease. Psychological disturbances, especially compulsive overachieving behavior, are seen in about 40% of children and adolescents with Cushing disease,⁴¹⁷ and are distinctly different from the depression typically seen in adults.⁴²⁰ Emotional lability has been described in approximately 30% of cases.⁴¹⁸ An underappreciated aspect of pediatric Cushing is the substantial degree of bone loss and undermineralization in these patients.^{417,421,422} Thus Cushing disease is generally diagnosed in young adults because the diagnosis was missed, rather than absent, during adolescence. Rarely, Cushing syndrome caused by adrenal carcinoma or the ectopic ACTH syndrome can produce a rapid fulminant course.

Cushing Disease

Although generally described in great detail and illustrated with striking photographs in endocrine texts, Cushing disease is fairly rare in adults.⁴²³ Furthermore, about 25% of patients referred to large centers for Cushing disease are children, and many patients first seen as adults actually experience the onset of symptoms in childhood or adolescence. For example, Harvey Cushing's original patient was a young woman of 23 years whose clinical features indicated long-standing disease, and who was only 144 cm tall,⁴²⁴ suggesting disease onset around age 11 years. In adults and children over 7 years of age, the most common cause of Cushing syndrome is true Cushing disease (adrenal hyperplasia caused by hypersecretion of pituitary ACTH).⁴²⁵ Boys are more frequently affected than girls in the prepubertal period, although the sex ratios are equal during adolescence and women have a higher incidence of Cushing

TABLE 14.11 Etiology of Cushing Syndrome in Infancy

	Males	Females
ADRENAL TUMORS (n = 48)		
Carcinoma	5	20
Adenoma	4	16
Not defined	2	1
Ectopic ACTH syndrome	1	1
Nodular adrenal hyperplasia	1	4
Undefined adrenal hyperplasia	2	2
ACTH-producing tumor	1	0
TOTAL	16	44

ACTH, Adrenocorticotropic hormone.

(From Miller, W. L., Townsend, J. J., Grumbach, M. M., & Kaplan, S. L. (1979). An infant with Cushing's disease due to an adrenocorticotropic-producing pituitary adenoma. *J Clin Endocrinol Metab*, 48, 1017–1025.)

disease in adulthood.⁴²⁶ In infants and children under 7 years, adrenal tumors predominate. Among 60 infants under 1 year of age with Cushing syndrome, 48 had adrenal tumors⁴²⁷ (Table 14.11).

Among adults, over 90% of patients with Cushing disease have identifiable pituitary microadenomas.^{420,428} These tumors are generally 2 to 10 mm in diameter, are not encapsulated, have ill-defined boundaries, and are frequently detectable with a contrast-enhanced pituitary MRI. These tumors are often identifiable only by minor differences in their appearance and texture from surrounding tissue, thus the frequency of surgical cure is correlated with the technical skill of the surgeon. Among children and adolescents, about 80% to 85% of those with Cushing disease have surgically identifiable microadenomas.^{417,429,430} Although removal of the tumor usually appears curative, 20% of such “cured” patients suffer relapse and again manifest Cushing disease within about 5 years so that the net cure rate is 65% to 75%.^{417–419,431,432}

The high cure rate of transsphenoidal microadenomectomy in Cushing disease indicates that the majority of such patients have primary disease of the pituitary itself, rather than secondary hyperpituitarism resulting from hyperstimulation of the pituitary by CRF or other agents. Careful follow-up studies of these patients confirm this.^{417,430,431} In most postoperative patients, the circadian rhythms of ACTH and cortisol return to normal, ACTH and cortisol respond appropriately to hypoglycemia, cortisol is easily suppressed by low doses of dexamethasone, and the other hypothalamic-pituitary systems return to normal.

However, some patients with Cushing disease have no identifiable microadenoma, and some “cured” patients relapse. This suggests that this smaller population of patients may have a primary hypothalamic disorder, or that some of the pituitary tissue responsible for ACTH-hypersecretion was not excised.⁴³³ Present clinical investigation suggests that Cushing disease is usually caused by a primary pituitary adenoma, but that sometimes it may be caused by hypothalamic dysfunction. Microsurgery can be curative in the former, but not the latter. Unfortunately, no diagnostic maneuver is available to distinguish the two possibilities; thus transsphenoidal exploration remains the preferred initial therapeutic approach to the patient with Cushing disease.

Whole-exome sequencing of ACTH-secreting microadenomas has identified recurrent gain-of-function mutations in the gene coding ubiquitin-specific protease 8 (*USP8*), a deubiquitinase that regulates the turnover of EGF receptors and other membrane proteins.⁴³⁴ To date, these somatic mutations have been found mostly in adult female patients. This and similar advances may lead to more targeted medical therapies.

Cushing Disease: Treatment. Transsphenoidal surgery offers the best initial approach for rapid and complete cure of most patients, but alternative approaches may be necessary in younger children if the sphenoid sinus is not yet aerated. Short-term consequences of transsphenoidal surgery include transient diabetes insipidus and cerebrospinal fluid rhinorrhea.^{418,419,435} Persistent panhypopituitarism is rare, but the effects of hypercortisolism on growth hormone secretion may remain for 1 to 2 years after treatment, and growth hormone deficiency can occur even in those children who have not received irradiation.^{418,419,435} Final height may be reduced by 1.5 to 2.0 SD by the long-term hypercortisolism;^{417,436} treatment with growth hormone may ameliorate this growth loss in patients with growth hormone insufficiency.^{437,438} Management of hypocortisolemia during the postoperative period is important, because the HPA axis will be suppressed. Careful monitoring for recovery of the axis is necessary over several months, until stress responsiveness returns.

The management of nonresponsive or relapsed Cushing disease is challenging. Repeat transsphenoidal surgery is typically the first approach, especially if there is evidence of a distinct lesion or lateral hypersecretion of ACTH. Second-line approaches include subtotal hypophysectomy, Gamma Knife irradiation, dopamine agonists (cabergoline), somatostatin analogs (pasireotide), adrenalectomy, and drugs that target the adrenal (mitotane), steroidogenesis (ketoconazole, metyrapone, etomidate), or GRs (mifepristone). All have significant disadvantages, especially in children. Subtotal hypophysectomy has the potential to eliminate pituitary secretion of growth hormone, TSH, and gonadotropins, causing growth failure, hypothyroidism, and failure to progress in puberty, respectively. Although hypothyroidism is easily treated with oral thyroxine replacement, growth hormone deficiency requires very expensive replacement therapy. Sex steroid replacement can be used to achieve secondary sexual characteristics at the age of puberty; however, gonadotropin replacement will be needed to achieve fertility. Pituitary irradiation has been touted to avoid many of these problems and is effective in treating Cushing disease, but growth-hormone deficiency occurs in most cases and additional endocrinopathies can occur with time.⁴³⁹ The interval from radiotherapy to cure can be more than 1 year, during which time therapeutic blockade of hypercortisolemia is necessary to prevent the ongoing effects of Cushing disease on growth, weight, and bone mineralization. Furthermore, large doses of radiation increase the risk of cerebral arteritis, leukoencephalopathy, leukemia, glial neoplasms, and bone tumors involving the skull; stereotactic radiotherapy may reduce these potential effects, but few data exist yet in children.

Bilateral laparoscopic adrenalectomy represents definitive therapy for patients with persistent Cushing disease; pituitary irradiation may be used when future fertility is not an issue. In addition to the obvious effects of eliminating normal production of glucocorticoids and mineralocorticoids, removal of the adrenal also eliminates the physiologic feedback inhibition of the pituitary. In some adults with residual pituitary adenomas, this results in the development of pituitary macroadenomas, producing very large quantities of ACTH. These can expand and impinge on the optic nerves and can produce sufficient POMC to yield enough MSH to produce profound darkening of the skin (Nelson syndrome), but this is rarely seen in children. There is relatively little pediatric experience with ketoconazole and other drugs that inhibit steroidogenesis, but these may provide a useful form of therapy for selected patients or for controlling hypercortisolemia in the short-term.⁴⁴⁰ Metyrapone is not useful for long-term therapy; mitotane, an adrenolytic agent, may be used to effect a “chemical adrenalectomy,” but its side effects of nausea, anorexia, and

vomiting are severe. Etomidate may be useful in the acute setting for severe or life-threatening Cushing disease before surgery.^{441,442} Mifepristone has been reported to successfully treat the symptoms associated with hypercortisolism in Cushing syndrome in both adult and pediatric patients.^{443,444}

Other Endogenous Causes of Cushing Syndrome

Adrenal Tumors. Adrenal carcinomas are the typical cause of Cushing syndrome in infants and small children (see Table 14.11); the first report of a pediatric adrenocortical carcinoma is of a 6-year-old girl who died in 1688.⁴⁴⁵ These tend to occur more frequently in girls, for unknown reasons. Adrenal adenomas almost always secrete cortisol with minimal secretion of mineralocorticoids or sex steroids. In contrast, adrenal carcinomas tend to secrete both cortisol and androgens, and are often associated with progressive virilization.^{446,447} Adrenal adenoma or carcinoma may be associated with hemihypertrophy, sometimes as part of the Beckwith-Wiedemann syndrome, with overproduction of IGF-2. Additional genetic associations include germline mutations or loss heterozygosity of the tumor suppressor gene *p53*, sometimes as part of the Li-Fraumeni syndrome, as well as mutations in the canonical Wnt/ β -catenin pathway.^{448–450} DNA sequencing of adrenal adenomas causing ACTH-independent Cushing syndrome in adults has also revealed sporadic gain-of-function mutations in *PRKACA*, the gene encoding the catalytic subunit of cAMP-dependent protein kinase (PKA), as well as in the phosphodiesterases that normally inactivate cAMP, *PDE11A*, and *PDE8B*.⁴⁵¹

CT and MRI are useful in the diagnosis of adrenal tumors, and steroid analysis can be informative at the time of presentation and for monitoring for potential relapse. The treatment for both adenoma and carcinoma is surgical, and complete resection is needed for cure. In some cases, the histologic differentiation of adrenal adenomas and carcinomas is difficult, but a worse prognosis is associated with increased tumor size, capsular and/or vascular invasion, retroperitoneal lymph nodes, metastases, or failure to normalize hormonal values postoperatively.^{447,452–454} Although a few patients with residual or metastatic disease have done well with adjunctive therapy with mitotane or other chemotherapeutic strategies, the general prognosis remains poor. To improve outcome for such rare diseases, patients should be enrolled in clinical trials and international registries.

Adrenocorticotrophic Hormone-Independent Multinodular Adrenal Hyperplasias. This group of generally rare and benign adrenal hyperplasias includes ACTH-independent macronodular adrenocortical hyperplasia (also known as massive macronodular adrenocortical disease) and micronodular hyperplasias, mostly primary pigmented nodular adrenocortical disease (PPNAD). Traditionally, macronodular disorders have been associated with nodules greater than 1 cm and micronodular disorders encompass nodules that are less than 1 cm; however, in the genomic era it is increasingly recognized that such designations can be arbitrary.

In childhood, bilateral macroadenomatous hyperplasia is mostly seen with McCune-Albright syndrome caused by somatic mutations in the guanine nucleotide-binding protein, alpha-stimulating polypeptide (*GNAS*) 1 gene. Patients present typically with the triad of polyostotic fibrous dysplasia, café-au-lait skin spots, with irregular margins, and gonadotropin-independent sexual precocity. However, as the disorder is caused by somatic cell mutations, rather than germ line mutations, its manifestations are clinically heterogeneous and may include other endocrine disorders, such as thyrotoxicosis, hyperparathyroidism, pituitary gigantism, and hyperprolactinemia. Cushing syndrome is rare in McCune-Albright syndrome and usually presents before 6 months of age. Most

cases of macronodular adrenocortical hyperplasia resulting in Cushing syndrome are sporadic, isolated, and occur in middle age. The etiology of such lesions has been linked to the ectopic formation of hormone receptors, such as vasopressin, serotonin, and LH/hCG, as well as to the local production of ACTH from the adrenal cortex itself.^{455–457} Another cause is germline mutations in tumor suppressor genes, including armadillo repeat containing 5 gene (*ARMC5*), multiple endocrine neoplasia type 1 (*MEN1*), adenomatous polyposis coli (*APC*), and fumarate hydratase (*FH*).^{458–461}

The group of micronodular benign adrenal hyperplasias includes PPNAD and isolated micronodular adrenocortical disease. PPNAD, characterized by the secretion of both cortisol and adrenal androgens,^{462,463} is a rare entity seen in infants, children, and young adults, with females affected more frequently. It is usually seen as part of the “Carney complex,” which is a form of multiple endocrine neoplasia (MEN), consisting of pigmented lentigines and blue nevi on the face, lips, and conjunctiva, and a variety of tumors, including schwannomas and atrial myxomas, and occasionally growth hormone-secreting pituitary adenomas, Leydig cell tumors, calcifying Sertoli cell tumors (which may secrete estrogens), and medullary carcinoma of the thyroid.^{463,464} Typical features of Cushing syndrome are often seen in the pediatric population.⁴⁶⁵ The adrenals are not truly hyperplastic but consist of discrete pigmented nodules surrounded by atrophic tissue, which permits their identification by MRI or CT. Because the hypercortisolism is resistant to suppression with high doses of dexamethasone and because both glucocorticoids and sex steroids are produced, PPNAD is clinically difficult to distinguish from the ectopic ACTH syndrome, but plasma ACTH assays are usually diagnostic. Complete adrenalectomy is usually indicated, although some successes have been reported with subtotal resections.

Carney complex, like other MEN disorders, is typically autosomal dominant. Loss of heterozygosity and mutations in a regulatory subunit of protein kinase A (*PRKARIA*) have been found in 73% of patients with Carney complex,^{466–470} or as sporadic germline or somatic events in isolated adrenal tumors (isolated PPNAD).⁴⁷¹ In general, patients with Carney Complex harboring mutations in *PRKARIA* manifest at a younger age and have more myxomas, schwannomas, thyroid tumors, and gonadal tumors than patients without mutations in *PRKARIA*. Patients with isolated PPNAD typically carry the *PRKARIA* mutations c.709-7del6 or c.1A>G/p.M1V, which is important for genetic counseling and screening.⁴⁷⁰

Patients with micronodular benign adrenal hyperplasia without Carney Complex and *PRKARIA* mutations may harbor mutations in other cAMP signaling proteins. Mutations in the gene encoding phosphodiesterase 11A4 (*PDE11A*) have been reported in individuals with isolated micronodular adrenocortical disease and PPNAD and mutations in phosphodiesterase 8B (*PDE8B*) in isolated micronodular adrenocortical disease.^{470,472} Both *PDE11A* and *PDE8B* catalyze the hydrolysis of cAMP and cyclic guanosine monophosphate and are expressed in several endocrine tissues, including the adrenals. As noted earlier, sporadic mutations in *PRKARIA*, *PDE11A*, and *PDE8B* have also been associated with isolated adrenal adenomas causing with ACTH-independent Cushing syndrome. Thus abnormalities in signaling pathways play an important role in adrenal hyperplasia and tumorigenesis.⁴⁷³

The Ectopic Adrenocorticotrophic Hormone Syndrome. The ectopic ACTH syndrome is most commonly seen in adults with oat cell carcinoma of the lung, carcinoid tumors, pancreatic islet cell carcinoma, and thymoma, but it may also be seen in children. Ectopically produced POMC and ACTH are derived from the same gene that produces pituitary

POMC but is not sensitive to glucocorticoid feedback in the malignant cells. This phenomenon permits distinction between pituitary and ectopic ACTH by suppressibility of the former by high doses of dexamethasone. Although the ectopic ACTH syndrome is rare in children, it has been described in infants younger than 1 year of age. Associated tumors have included neuroblastoma, pheochromocytoma, islet cell carcinoma of the pancreas, and neuroendocrine tumors of the thymus.⁴⁷⁴ The ectopic ACTH syndrome is typically associated with ACTH concentrations 10 times higher than those seen in Cushing disease. However, both adults and children with this disorder may show little or no clinical evidence of hypercortisolism, probably caused by the typically rapid onset of the disease and to the general catabolism associated with malignancy. Unlike patients with Cushing disease, adults and children with the ectopic ACTH syndrome frequently have hyporeninemic hypokalemic alkalosis, presumably because the extremely high levels of ACTH stimulate the production of DOC by the adrenal fasciculata, and/or stimulate the production of aldosterone by adrenal glomerulosa. Rare intracranial, nonpituitary tumors may produce ectopic ACTH but be mistaken for pituitary Cushing disease because of their location.^{475,476}

DICER1 Syndrome and Infant Cushing Disease. Mutations in *DICER1*, the gene encoding the DICER1 ribonuclease that participates in the generation of small interfering RNAs (siRNAs) and microRNAs,⁴⁷⁷ were first discovered in pleuropulmonary blastoma (PPB),⁴⁷⁸ and subsequently reported in pituitary blastoma,⁴⁷⁹ and other embryonal tumors.⁴⁸⁰ The PPB familial tumor and dysplasia syndrome [OMIM #601200] is a pleiotropic tumor predisposition syndrome. Pituitary blastoma has been described in over a dozen cases of infants with Cushing disease,^{481,482} and may be autosomal recessive or associated with an inherited germ line mutation plus a somatic mutation, both in *DICER1*; among the few cases reported, the prognosis has been poor.

Approach to the Child With Endogenous Cushing Syndrome

Cushing syndrome in children is usually suggested by weight gain, growth arrest, mood change, and change in facial appearance (plethora, acne, hirsutism). The diagnosis in children may be subtle and difficult when it is sought at a relatively early point in the natural history of the disease. For initial screening, three laboratory investigations are recommended: (1) diurnal ACTH and cortisol profiles from blood (the latter may be performed on saliva), (2) 24-hour UFC measurements, and (3) the overnight 1 mg dexamethasone suppression test.⁴⁶³ Absolute elevations above the "upper limits of normal" for concentrations of plasma ACTH and cortisol are often absent. Rather than finding morning concentrations of cortisol greater than 20 µg/dL or of ACTH greater than 50 pg/mL, it is more typical to find mild, often equivocal elevations in the afternoon and evening values. This loss of the diurnal rhythm, evidenced by continued secretion of ACTH and cortisol throughout the afternoon, evening, and nighttime, is usually the earliest reliable laboratory index of Cushing disease. A single plasma cortisol measurement obtained at midnight from an indwelling venous catheter, while the patient remains asleep, should be less than 2 µg/dL in normal individuals, and more than 2 µg/dL in Cushing disease.⁴⁸³ Similarly, late-night free cortisol values, measured in saliva, should normally be less than around 1 ng/mL (2.8 nmol/L) at 11 PM.⁴⁸⁴ Interpretation of late-night salivary samples can be influenced by factors ranging from a disordered sleep pattern to gingival disease. Thus samples should be collected under optimal conditions (e.g., after rinsing one's mouth but before brushing one's teeth) to avoid false positive results. Salivary samples are stable at room temperature for several days but can be frozen if longer term storage is required.

The values for ACTH and cortisol are typically extremely high in the ectopic ACTH syndrome, whereas cortisol is elevated, but ACTH is suppressed in adrenal tumors and in multinodular adrenal hyperplasia (Table 14.12). In all forms of Cushing syndrome, monitoring of the 24-hour UFC assists in

TABLE 14.12 Diagnostic Values in Various Causes of Cushing Syndrome

Test		Normal Values	Adrenal Carcinoma	Adrenal Adenoma	Nodular Adrenal Hyperplasia	Cushing Disease	Ectopic ACTH Syndrome
Plasma Cortisol concentration	AM	>14	↑	↑	↑	±	↑↑
	PM	<8	↑	↑	↑	↑	↑↑
Plasma ACTH concentration	AM	<100	↓	↓	↓	↑	↑↑
	PM	<50	↓	↓	↓	↑	↑↑
Low-Dose Dex Suppression	Cortisol	<3	No Δ	No Δ	No Δ	^a	No Δ
	ACTH	<30	No Δ	No Δ	No Δ	^a	No Δ
	17OHCS	<2	No Δ	No Δ	No Δ	^a	No Δ
High-Dose Dex Suppression	Cortisol	↓↓	No Δ	No Δ	^b	↓	No Δ
	ACTH	↓↓	No Δ	No Δ	^b	↓	No Δ
	17OHCS	↓↓	No Δ	No Δ	^b	↓	No Δ
IV ACTH Test	Cortisol	>20	No Δ	± ↑	± ↑	↑	No Δ
Metyrapone Test	Cortisol	↓	± ↓	No Δ	± ↓	↓	± ↓
11 Deoxycortisol		↑	± ↑	No Δ	± ↑	↑	± ↑
	ACTH	↑	No Δ	No Δ	± ↑	↑	No Δ
	17OHCS	↑	No Δ	No Δ	±	↑	No Δ
24-hour urinary excretion (Basal)	17OHCS		↑↑	↑	↑	↑	↑
	17KS		↑↑	± ↑	↑	↑	↑
Plasma concentration DHEA or DHEA-S			↑↑	↓	± ↑	↑	↑

^aIncomplete response, i.e., ±. Cortisol concentration in µg/dL.

^bUsually no Δ.

ACTH concentration in pg/mL.

17OHCS in mg/24h.

AM typically refers to 8 AM; PM to 4 PM

17OHCS, 17-hydroxycorticosteroids; 17KS, 17-ketosteroid; ACTH, adrenocorticotropic hormone; Dex, dexamethasone, DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; IV, intravenous.

deciding whether further investigations are warranted. A normal 24-hour UFC value is generally less than 70 $\mu\text{g}/\text{m}^2/\text{day}$ (with radioimmunoassays). Several repeat collections are advisable, and it is important to use normal ranges adjusted for size, as well as age, as children with simple obesity have higher cortisol secretion rates. Another baseline test is the low-dose (1 mg) dexamethasone overnight suppression test with a cut-off for cortisol lower than 1.8 $\mu\text{g}/\text{dL}$ (50 nmol/L). If both the 24-hour UFC and the low-dose dexamethasone overnight suppression test are normal, the diagnosis of Cushing syndrome is usually excluded. An exception is patients with intermittent or periodic cortisol hypersecretion who need longer follow-up and repeat testing for diagnosis of Cushing syndrome.

Once the diagnosis of Cushing syndrome is established, further investigations may be needed to find the origin of disease. Low- and high-dose dexamethasone suppression tests can be useful when done with care. To achieve reliable results in pediatric patients, children should be hospitalized, preferably on a pediatric clinical research ward. Two days of baseline (control) data should be obtained. Low-dose dexamethasone (20 $\mu\text{g}/\text{kg}/\text{day}$, up to a maximum of 2.0 mg) should be given, divided into equal doses given every 6 hours for 2 days, followed by high-dose dexamethasone (80 $\mu\text{g}/\text{kg}/\text{day}$) given in the same fashion. Eight AM and 8 PM (or midnight) values for ACTH and cortisol and 24-hour urine collections for 17OHCS, 17-ketosteroid (17KS), free cortisol, and creatinine (to monitor the completeness of the collection) should be obtained on each of the 6 days of the test. Measurements of either UFC or 17OHCS are probably equally reliable if the laboratory has established good pediatric standards. Because of variations caused by episodic secretion of ACTH, the 8 AM and 8 PM blood values should be drawn in triplicate at 8:00, 8:15, and 8:30.

In patients with exogenous obesity or other non-Cushing disorders, cortisol, ACTH, and urinary steroids will be suppressed readily by low-dose dexamethasone. Plasma cortisol should be less than 1.8 $\mu\text{g}/\text{dL}$, ACTH less than 20 pg/mL, and 24-hour urinary 17OHS less than 1 mg/g of creatinine. Patients with adrenal adenoma, adrenal carcinoma, or the ectopic ACTH syndrome will have values relatively insensitive to both low- and high-dose dexamethasone, although some patients with multinodular adrenal hyperplasia may respond to high-dose suppression with a paradoxical rise in cortisol following dexamethasone, as has been reported with the Carney complex.^{485,486} Patients with Cushing disease classically respond with suppression of ACTH, cortisol, and urinary steroids during the high-dose treatment, but not during the low-dose treatment. However, some children, especially those early in the course of their illness, may exhibit partial suppression in response to low-dose dexamethasone. Thus if the low dose that is given exceeds 20 $\mu\text{g}/\text{kg}/\text{day}$ or if the assays used are insufficiently sensitive to distinguish partial from complete suppression, false-negative tests may result.

Petrosal sinus sampling is widely used in adults with Cushing disease, to distinguish pituitary Cushing disease from the ectopic ACTH syndrome. The smaller vascular bed in children increases the risk of this procedure, but inferior petrosal venous sampling has been used with some success in adolescents in an attempt to localize pituitary adenomas before surgery.⁴⁸⁷ Such approaches should only be undertaken in highly specialized centers; jugular venous sampling may provide an alternative approach, although extensive data in the pediatric population are not available.⁴⁸⁸ In general, the diagnosis of Cushing disease is considerably more difficult to establish in children than in adults. In addition to laboratory tests, imaging studies may help in establishing the exact diagnosis of Cushing syndrome. An adrenal CT scan or MRI may visualize an adrenal

cortex tumor or a macro-/micronodular adrenal hyperplasia, whereas adrenal ultrasound often fails and therefore should not be trusted as a diagnostic tool for adrenal disorders. MRI is also the preferred method for visualizing the hypothalamus and the anterior pituitary for lesions.

Primary Hyperaldosteronism

Aldosterone is normally produced in glomerulosa cells in response to depleted intravascular volume via the renin-angiotensin system and/or high plasma potassium. In primary aldosteronism, the adrenal constitutively produces aldosterone in the absence of angiotensin II or hyperkalemia. Primary aldosteronism is the most common form of secondary hypertension, affecting around 10% of all hypertensive patients. In addition to the adverse cardiovascular outcomes associated with hypertension, excess aldosterone is an independent risk factor for cardiovascular disease and renal damage, likely owing to the direct effects of aldosterone on tissues other than the kidney.⁴⁸⁹

Clinical Findings

Primary aldosteronism is classically characterized as presenting with hypertension, polyuria, hypokalemic alkalosis, and low plasma renin activity because of autonomous production of aldosterone. Aldosterone-producing adenomas are well described in adults but are rare in children. Familial cases of primary aldosteronism have also been described, including glucocorticoid-remediable aldosteronism (GRA) (see Section VI). Primary aldosteronism exists across a continuum of disease severity, ranging from mild to severe, suggesting current estimates of disease prevalence may underrepresent the true burden of disease. Early in the course of disease, primary aldosteronism may simply present with increased blood pressure without corresponding hypokalemia or low renin activity.⁴⁹⁰

Differential Diagnosis

Since the initial description of primary aldosteronism, a number of distinct etiologies have been described, including (1) unilateral aldosterone-producing adenomas (APAs, Conn syndrome), accounting for 30% to 40% of cases; (2) bilateral idiopathic hyperaldosteronism, accounting for 60% to 70% of cases; and rarer forms, such as (3) familial hyperaldosteronism (FH), and (4) primary nodular adrenal hyperplasias, as described earlier.

Aldosterone-Producing Adenomas (Conn syndrome).

Recurrent somatic mutations causing APAs, most commonly in the gene encoding the K^+ channel *KCNJ5* (also known as *Kir3.4*) have been identified recently.^{491–494} Multicenter studies have identified two somatic mutations in *KCNJ5* (G151R or L168R) in 38% of all APAs.⁴⁹⁵ Such mutations are more prevalent in females and at a younger age, and manifest with higher preoperative aldosterone levels.⁴⁹⁶ These *KCNJ5* mutations affect the ion selectivity filter of the K^+ channel, causing an increase in Na^+ influx and permanent depolarization of the zona glomerulosa cell membrane.⁴⁹² Membrane depolarization leads to activation of voltage-gated Ca^{2+} channels, which increases intracellular Ca^{2+} , thus providing the normal signal for aldosterone production and glomerulosa cell proliferation. Recently, exome sequencing studies have identified additional recurrent somatic mutations that arise independently of mutations in *KCNJ5*, including in *ATP1A1*, *ATP2B3*, *CACNA1D*, and *CTNNA1*.^{497–499} *ATP1A1* encodes the alpha subunit of the Na^+/K^+ ATPase and accounts for ~5% of APAs. *ATP2B3* encodes a Ca^{2+} ATPase and accounts for around 2% of APAs. *CACNA1D* encodes a voltage-gated calcium channel and may

account for 11% of APAs. Each of these channel-associated mutations have been functionally linked to depolarization of zona glomerulosa cells and aldosterone hypersecretion. It remains unclear, however, whether they are sufficient to drive the hyperplastic response leading to the APA. *CTNNB1* is the gene encoding β -catenin, a canonical member of the Wnt signaling pathway. In addition to APAs, activating mutations in exon 3 of *CTNNB1* have been associated with both nonsecreting adrenal adenomas and adrenocortical carcinomas.⁵⁰⁰

Bilateral Idiopathic Hyperaldosteronism. Bilateral idiopathic hyperaldosteronism accounts for 60% to 70% of cases, although it can sometimes present as unilateral disease. Bilateral idiopathic hyperaldosteronism is generally considered a disease of adults, but it may initially present earlier in life. Despite its high prevalence, the underlying pathophysiology remains poorly understood. Although patients with bilateral idiopathic hyperaldosteronism have increased sensitivity to angiotensin II, it remains unknown why such lesions are unresponsive to angiotensin II receptor antagonists. Somatic mutations in *KCNJ5* are associated with APAs; however, germline mutations in *KCNJ5* are associated with bilateral adrenal hyperplasia (as in cases of FH Type III, later).

Familial Hyperaldosteronism. Four subtypes of autosomal-dominant FH have been described. FH type I, characterized by severe hypertension presenting in the first 2 decades of life is glucocorticoid-suppressible hyperaldosteronism (described in Section VI). FH type II, caused by mutations in *CLCN2*,⁵⁰¹ most closely resembles sporadic forms of primary aldosteronism, though in contrast to the familial form, no genetic mutations have yet to be linked to this sporadic condition. FH type III has been associated with germline mutations in *KCNJ5* and classically presents as severe hypertension and hypokalemia early in life. Recently, FH type IV has been associated with germline mutations in *CACNA1H* (encoding the α subunit of a T-type calcium channel, Cav3.2), presenting with primary aldosteronism in the first decade of life. Rarely, de novo germline mutations in *CACNA1H* have also been described in children presenting with the constellation of primary aldosteronism, cerebral palsy, epilepsy, and neurological abnormalities.^{500,502}

Cellular Origins of Primary Aldosteronism. Immunohistochemical analysis of normal adrenal glands for CYP11B2 (aldosterone synthase) expression has revealed the existence of aldosterone-producing cell clusters (APCCs) that arise under the adrenal capsule and extend into the zona fasciculata. APCCs were initially described adjacent to APAs, suggesting they may represent a precursor lesion. Genomic analysis of 23 APCCs and normal adjacent adrenal tissue revealed mutations previously linked to aldosterone-producing lesions in 35% of cases, with *CACNA1D* and *ATP1A1* being affected most commonly; mutations in *KCNJ5* were not found in APCC lesions.⁵⁰³ Among adrenals from autopsied normotensive Japanese subjects, the incidence of having at least one APCC was around 30%.⁵⁰⁴ Among APCCs found in normal adrenals from kidney donors, 12% were found in donors younger than 19 years old, and 40% in donors younger than 35 years old, suggesting that primary aldosteronism may often originate in adolescence and young adulthood.⁵⁰⁵

Approach to the Child With Hyperaldosteronism

The diagnostic task is to differentiate primary aldosteronism from physiologic secondary hyperaldosteronism occurring in response to another physiologic disturbance. Any loss of sodium, retention of potassium, or decrease in blood volume will result in hyperreninemic secondary hyperaldosteronism. Renal tubular acidosis, treatment with diuretics, salt-wasting

nephritis, or hypovolemia caused by nephrosis, ascites, or blood loss are typical settings for physiologic secondary hyperaldosteronism. Although the constellation of hypertension, hypokalemic alkalosis, and low plasma renin activity may point toward primary aldosteronism, patients suspected of having this condition but not meeting these criteria may benefit from careful evaluation in a clinical research setting, where the effects of sodium loading and other interventions on aldosterone levels and renin activity can be formally assessed.⁴⁹⁰

Virilizing and Feminizing Adrenal Tumors

Most virilizing adrenal tumors are adrenal carcinomas producing a mixed array of androgens and glucocorticoids; virilizing and feminizing adrenal adenomas are quite rare. Virilizing tumors in boys will have a presentation similar to that of SVCAH. There will be phallic enlargement, erections, pubic and axillary hair, acne, increased muscle mass, deepening of the voice, and scrotal thinning, but the testicular size will be prepubertal. Elevated concentrations of testosterone in young boys alter behavior, with increased irritability, rambunctiousness, hyperactivity, and rough play, but without evidence of libido. Diagnosis is based on hyperandrogenemia unsuppressible by glucocorticoids. The treatment is surgical; all such tumors should be handled as if they are malignant, with care exerted not to cut the capsule and seed cells onto the peritoneum. The histologic distinction between adrenal adenoma and carcinoma is difficult, especially in pediatric patients.

Feminizing adrenal tumors are extremely rare in either sex. P450aro, the enzyme aromatizing androgenic precursors to estrogens, is not normally found in the adrenals but is found in peripheral tissues, such as fat, and is also found in some adrenal carcinomas. It is not known whether most feminizing adrenal tumors exhibit ectopic adrenal production of this enzyme, whether some other enzyme mediates aromatization in the tumor, or whether these are truly androgen-producing, virilizing tumors occurring in a setting where there is unusually effective peripheral aromatization of adrenal androgens. Feminizing adrenal (or extraadrenal) tumors can be distinguished from true (central) precocious puberty in girls by the absence of increased circulating concentrations of gonadotropins and by a prepubertal response of LH to an intravenous challenge of GnRH (luteinizing hormone-releasing factor [LRF], GnRH). In boys, such tumors will cause gynecomastia, which will resemble the benign gynecomastia that often accompanies puberty. However, as with virilizing adrenal tumors, testicular size, and the gonadotropin response to LRF testing will be prepubertal. The diagnosis of a feminizing tumor in a pubertal boy can be extremely difficult, but it is usually suggested by an arrest in pubertal progression and can be proved by the persistence of circulating plasma estrogens after administration of testosterone.

Other Disorders

Familial Glucocorticoid Resistance

Familial glucocorticoid resistance is a rare disorder caused by mutations in the α -isoform of the GR. Decreased glucocorticoid action results in grossly increased ACTH secretion, which, in addition to stimulating the production of cortisol, also stimulates the production of other adrenal steroids. Thus these patients may present with fatigue, hypertension, and hypokalemic alkalosis, suggesting a mineralocorticoid excess syndrome, and also have symptoms of hyperandrogenism.^{506,507} In contrast, they typically lack Cushingoid features with biochemical hypercortisolism. Circadian rhythmicity of the HPA axis is

maintained and resistance to dexamethasone suppression is observed. Patients have been described who are homozygous for missense mutations,⁵⁰⁸ heterozygous for a gene deletion,⁵⁰⁹ or homozygous for a wholly null mutation.⁸⁶ Heterozygous point mutations with incomplete dominant negative activity or multiple effects on GR α action have also been described.⁵¹⁰ Point mutations may interfere with GR α -dependent transcriptional regulation through altered DNA binding, impaired ligand binding, delayed nuclear localization, abnormal nuclear aggregation, and disrupted interaction with coactivators, depending on the position of the mutation.⁵⁰⁶ Thus familial glucocorticoid resistance is usually a syndrome of partial resistance to the action of glucocorticoids. Treatment consists of supraphysiologic doses of dexamethasone that will elicit a physiologic response with the poorly functioning receptor, usually starting with 0.25 to 0.5 mg/day at bedtime and titrating to the suppression of morning ACTH and thus androgens, blood pressure, and serum potassium levels.⁵⁰⁷ The dose of dexamethasone may be reduced to a minimum according to individual needs. Hypertension may require additional treatment with aldosterone antagonists.

Pseudohypoaldosteronism

Pseudohypoaldosteronism (PHA) is a salt-wasting disorder of infancy characterized by hyponatremia, hyperkalemia, and increased plasma renin activity in the face of elevated aldosterone concentrations, reflecting aldosterone resistance.⁵¹¹ The more common, more severe, systemic, autosomal recessive form of PHA ("pseudohypoaldosteronism Type II") is caused by inactivating mutations in any of the three subunits (α , β , γ) of the amiloride-sensitive sodium channel, ENaC (encoded by the *SCNN1A*, *B* and *G* genes).⁵¹² This condition is often associated with lower respiratory tract congestion, cough and wheezing (but not pulmonary infections), and clear rhinorrhea, as ENaC mutations increase the volume of airway fluid.⁵¹³ PHA type II persists into adulthood, requiring vigorous salt-replacement therapy throughout life. Gain-of-function mutations caused by carboxy-terminal truncation of β -ENaC cause Liddle syndrome, an autosomal dominant form of salt-retaining hypertension.⁵¹²

Autosomal dominant renal Type 1 pseudohypoaldosteronism (PHA type I) is caused by inactivating mutations in the MR (encoded by the *NR3C2* gene).⁵¹⁴ More than 50 different mutations have been found in this receptor, which interfere with mineralocorticoid binding and gene transcription.⁵¹⁵ PHA type I is milder than the recessive forms of PHA caused by ENaC mutations and remits with age, but requires sodium replacement therapy in infancy and childhood. Rarely, point mutations in the MR have been found in association with an autosomal dominant form of severe hypertension, which begins in adolescence and worsens in pregnancy.⁵¹⁶ In these cases, alterations in the structure of the ligand-binding domain of the MR result in mild constitutive activation, as well as permitting binding and activation of the receptor by progesterone.

An acquired, transient form of PHA is rarely seen in infants with obstructive uropathy, especially shortly following surgical relief of the obstruction, or with urinary tract infections. The lesion is renal tubular, resembling renal tubular acidosis, type 4,⁵¹⁷ so that mineralocorticoid treatment is generally ineffective; salt replacement generally suffices, while the renal lesion resolves. The etiology of mineralocorticoid resistance in such cases is poorly understood.

GLUCOCORTICOID THERAPY AND WITHDRAWAL

Glucocorticoids are so termed because of their major actions to increase plasma concentrations of glucose. This occurs by their

diverse insulin counterregulatory actions and induction of the transcription of the genes encoding the enzymes of the Embden-Meyerhof glycolytic pathway and other hepatic enzymes that divert amino acids, such as alanine, to the production of glucose. Since their introduction into clinical medicine in the early 1950s, glucocorticoids have been used to attempt to treat a substantial number of diseases. At present, their rational use falls into two broad categories: replacement in adrenal insufficiency and pharmacotherapeutic use. The latter category is largely related to the antiinflammatory and immunosuppressive properties of glucocorticoids but also includes their actions to cause growth arrest and induce apoptosis in normal and leukemic leukocytes, lower plasma calcium concentrations, and reduce increased intracranial pressure.

Virtually all of these actions are mediated through GRs, which are found in most cells. There are three principal variants of GRs (α , β and γ) that are generated through alternative splicing patterns from the same mRNA transcript from the *GR* gene. GR α is the most abundant, correlates with the levels of GR protein, and is thought to be the primary form that mediates glucocorticoid action. However, emerging evidence indicates that the other GRs may have a more important role than originally appreciated. For example, GR β and GR γ have dominant-negative activity over GR α , and may contribute to the development of steroid resistance that may occur during therapy.^{518,519} Furthermore, despite the initial simple appearance of the hormone-receptor relationship, receptor bound glucocorticoids exert surprisingly diverse activities that can be specific to particular tissues. This diversity is mediated by a variety of mechanisms, including differences in the receptor shape in different tissues, resulting in distinct transcriptional activities.⁵²⁰ This can be seen by comparing mRNA expression profiles across different tissues and cell types in response to the same glucocorticoid signal. These molecular differences in GR across different cellular contexts are the mechanism for the different tissue responses to glucocorticoids, such as promoting visceral adipose tissue expansion, while simultaneously inhibiting growth of muscle and bone. Other features of Cushing syndrome are similarly attributable to tissue-specific differences in the transcriptional activity of specific glucocorticoid-sensitive genes. Although complicating our understanding of glucocorticoid action, this diversity may permit development of drugs that are more selective for certain GR isoforms, thus maintaining therapeutic efficacy, while mitigating adverse side effects,^{521,522} similarly to the development of selective estrogen receptor modulators (SERMs).⁵²³

Nevertheless, the only differences among the currently available glucocorticoid preparations are their ratio of glucocorticoid to mineralocorticoid activity, their capacity to bind to various binding proteins, their molar potency, and their biologic half-life. An important exception to this paradigm is the use of the potent synthetic glucocorticoid dexamethasone for reducing increased intracranial pressure and brain edema. Neurosurgical experience indicates that the optimal doses for this action are 10 to 100 times those that would thoroughly saturate all available receptors, suggesting that this action of dexamethasone may represent activity that is not mediated through the GR. However, receptor-independent glucocorticoid activity of physiologic levels of glucocorticoids has yet to be identified.

In the normal population, there is a wide interindividual variation in glucocorticoid sensitivity which is, at least in part, explained by genetic variants of the *GR* gene.⁵⁰⁷ Several single nucleotide polymorphisms have been associated with changes in glucocorticoid sensitivity. The A3669G (rs6198) polymorphism, present in about 35% of the normal population, is associated with glucocorticoid insensitivity and a more active immune system with an increased risk for autoimmune disease.^{524,525} The ER22/23EK polymorphism (rs6189) and

rs6190) present in about 7% of the population and is associated with mild glucocorticoid resistance and a healthier metabolic profile.⁵²⁶ In contrast, polymorphisms N363S (rs1695) and Bcl1 (rs41423247) present in about 8% and 45% of people, respectively, are associated with mild glucocorticoid hypersensitivity yielding a less fortunate metabolic profile (more body fat, less lean mass, increased cholesterol, and insulin resistance etc.).⁵⁰⁷ Some of the variability in glucocorticoid sensitivity is explained by GR subtypes arising from posttranslational modifications, such as phosphorylation, ubiquitination, sumoylation, and acetylation.⁵²⁰ These processes contribute to the broad range of glucocorticoid responsiveness to specifically regulate glucocorticoid-dependent genes.⁵²⁷ Thus individual glucocorticoid sensitivity plays a role in modulating risk for diseases (cardiovascular, autoimmune, metabolic) and may also affect responsiveness to glucocorticoid treatment.

Mechanisms of Glucocorticoid Action

The GR was the first nuclear hormone receptor to be cloned,⁵²⁸ revealing a series of functional domains in the protein that turned out to be prototypical for the nuclear receptor family. The ligand binding domain (LBD) is located at the C-terminus of the protein, which also interacts with coactivator proteins. The LBD of GR is highly homologous to the MR LBD. Subtle structural differences between MR and GR confer specificity for aldosterone binding to MR, but cortisol is able to bind to both receptors. Sensitive tissues, like the kidney, express HSD11B2, which inactivates cortisol to cortisone, protecting MRs from being activated by cortisol. The DNA binding domain (DBD), which is conserved among nuclear receptors, is centrally located in the GR protein. The DBD facilitates receptor dimerization and contains two zinc-finger motifs that can bind to the major groove of DNA. The N-terminal domain of GR is the least conserved and enables interactions with coregulators.

Unbound GRs exist predominantly in the cytoplasm; when intracellular glucocorticoid levels increase, more GRs become bound by hormone and translocate into the nucleus. Rather than being a passive process, the binding of glucocorticoids induces changes in the shape of GR that expose nuclear localization motifs, promoting facilitated trafficking of the hormone-receptor complex into the nucleus. Once in the nucleus, the liganded GR acts by (1) binding to defined sequences of DNA (glucocorticoid response elements; GREs), (2) interactions with cofactors and coregulators, and (3) engagement of the glucocorticoid/GR complex with transcriptional machinery. The sum of these actions regulates the expression of GR target genes. GR access to GREs in the DNA is also influenced by chromatin structure, further contributing to tissue-specific activity of glucocorticoids. GRs can bind to GREs as a GR monomer or dimer ("simple") or associated with another transcription factor ("composite"), or GR can "tether" to a GRE by binding to another protein that is bound to the GRE. Thus GR does not need to bind to DNA directly to regulate target genes. GREs can be found in the classic "promoter proximal" location in target genes and also at significant distances from the target gene, which then loop into closer proximity to the regulated gene. This can complicate the assignment of GREs to particular GR target genes, but adds to the multiple levels by which glucocorticoids can regulate gene expression. Similarly, posttranslational modifications of GR further refine glucocorticoid action on target genes. Together, these variables enable tissue and cell-specific glucocorticoid activity, as well as a capacity for fine-tuned regulation of the transcription levels of target genes in response to dynamic physiologic needs.

Replacement Therapy

Glucocorticoid replacement therapy is complicated by undesirable side effects with even minor degrees of overtreatment or undertreatment. Overtreatment can cause the signs and symptoms of Cushing syndrome; and even minimal overtreatment can impair growth. Undertreatment will cause the signs and symptoms of adrenal insufficiency (see Table 14.9) only if the extent of undertreatment (dose and duration) is considerable. However, undertreatment may impair the individual's capacity to respond to stress. Glucocorticoid replacement therapy is most commonly used in CAH caused by 21OHD; however, in this setting, undertreatment will lead to overproduction of adrenal androgens, which will promote virilization and hasten epiphyseal maturation and closure, thus compromising ultimate adult height. Therefore when formulating a program of adrenal replacement therapy for the growing child, one should use the lowest doses possible that achieve the therapeutic goal.

To optimize pediatric glucocorticoid replacement therapy, astute physicians have gauged their therapy to resemble the endogenous secretory rate of cortisol. Several studies indicate that the cortisol secretory rate is 6 to 8 mg/m²/day in children and adults,^{144,145} but data are not available for infants and children under 5 years. The range of normal values varies considerably, indicating that therapy must be tailored and individualized for each patient to achieve optimal results. The management of this delicate balance between overtreatment and undertreatment of the child, requiring replacement therapy, is thus confounded by considerable variation in the "normal" cortisol secretory rate among different children of the same size and the probability that most therapeutic guidelines err on the side of overtreatment. However, several additional factors must be considered in tailoring a specific child's glucocorticoid replacement regimen.

The specific form of adrenal insufficiency being treated significantly influences therapy. When treating autoimmune adrenalitis or any other form of "Addison disease," it is prudent to avoid overtreatment. This will eliminate the possibility of glucocorticoid-induced iatrogenic growth retardation and often permits the pituitary to continue to produce normal to slightly elevated concentrations of ACTH. This ACTH will continue to stimulate the remaining functional adrenal steroidogenic machinery and also provide a fairly convenient means of monitoring the effects of therapy. In contrast, when treating severe, virilizing CAH, the adrenal should be suppressed more completely, as essentially all adrenal steroidogenesis will result in the production of unwanted androgens, with their consequent virilization and rate of advancement of bony maturation that is more rapid than the rate of advancement of height. However, overtreatment will also compromise growth and could have long-term adverse implications, including for bone density, body composition, and metabolism.

The presence or absence of associated mineralocorticoid deficiency is an important variable. Children with mild degrees of mineralocorticoid insufficiency, such as those with SV CAH, may continue to have mildly elevated ACTH values, suggesting insufficient glucocorticoid replacement in association with elevated PRA. In some children, the ACTH is elevated in response to chronic, compromised hypovolemia, attempting to stimulate the adrenal to produce more mineralocorticoid. In such children, without overt signs and symptoms of mineralocorticoid insufficiency, mineralocorticoid replacement therapy ensures euvolemia, permitting one to decrease the amount of glucocorticoid replacement needed to suppress plasma ACTH and urinary 17KS. This reduction in glucocorticoid therapy reduces the likelihood that adult height will be compromised

and mitigates against other potential adverse effects of excess glucocorticoids.

The specific formulation of glucocorticoid used is also of great importance. Extremely potent, long-acting glucocorticoids, such as dexamethasone or prednisone, may be used in the treatment of adults but are rarely appropriate for replacement therapy in children. As children are continually growing and changing their weights and body surface areas, it is necessary to adjust their dose frequently; small, incremental changes are more easily done with relatively weaker glucocorticoids. The use of short-acting steroids permits the patient to have physiologically low nighttime glucocorticoid activity, facilitating rest, growth, and pituitary stimulation, as well as mimicking the natural circadian oscillations in cortisol levels. As ACTH and cortisol concentrations are high in the morning and low in the evening, it is intellectually and logically appealing to attempt to duplicate this circadian rhythm in replacement therapy. Such circadian oscillations in glucocorticoid secretion are seen in most species, including those that do not have prolonged periods of fasting, suggesting that other physiologic processes are affected by this rhythmicity. A circadian molecular clock is embedded in several important glucocorticoid signaling pathways.^{529–532} However, the clinical data are insufficient to recommend diurnal variation in steroid dosing, although most clinicians chose to mimic the diurnal rhythm by giving higher doses in the morning.⁵³³

Pharmacologic Therapy

Pharmacologic doses of glucocorticoids are used in a variety of clinical situations, including immune suppression in organ transplantation, tumor chemotherapy, treatment of “autoimmune” collagen, vascular and nephrotic syndromes, regional enteritis, and ulcerative colitis. Asthma, pseudotumor cerebri, dermatitis, certain infections, neuritis, and certain anemias are also often treated with glucocorticoids. The choice of glucocorticoid preparation to be used is guided by the pharmacologic parameters described earlier and in Table 14.7, and by custom (e.g., the use of betamethasone rather than dexamethasone to induce fetal lung maturation in impending premature deliveries). There is substantial variation in the relative glucocorticoid and mineralocorticoid activities of each steroid, depending on the assay used,⁵³⁴ hence Table 14.7 is an integration of multiple studies, and can only be taken as a rough guide. Furthermore, dosage equivalents among various glucocorticoids can be misleading. Virtually all handbooks of therapy publish tables of equivalency for the most commonly used pharmaceutical preparations of glucocorticoids; a similar set of equivalencies is shown in Table 14.7. Because most preparations of glucocorticoids are intended for pharmacotherapeutic use rather than replacement therapy, and because the most common indication for pharmacologic doses of glucocorticoids is for their antiinflammatory properties, virtually all tables of glucocorticoid equivalencies are based on antiinflammatory, immunosuppressive equivalencies. However, the differences in the plasma half-life and ability to bind to plasma proteins result in different biologic equivalencies when one assesses, for example, antiinflammatory versus growth-suppressant equivalencies. Thus dexamethasone is widely reported as being about 30 times more potent than cortisol when its antiinflammatory capacities are measured, but the growth suppressant activity of dexamethasone is about 80 times that of cortisol (see Table 14.7). The variables discussed earlier explain why there is little unanimity in recommendations for designing a glucocorticoid treatment regimen. However, an understanding of these variables will permit appropriate monitoring of the patient and encourage the

TABLE 14.13 Complications of High-Dose Glucocorticoid Therapy

Short-Term Therapy	Long-Term Therapy
Gastritis	Gastric ulcers
Growth arrest	Short stature
↑ Appetite	Weight gain
Hypercalciuria	Osteoporosis, fractures
Glycosuria	Slipped epiphyses
Immune suppression	Ischemic bone necrosis
Masked symptoms of infection, esp. fever and inflammation	Poor wound healing
Toxic psychoses	Catabolism
	Cataracts
	Bruising (capillary fragility)
	Adrenal/pituitary suppression
	Toxic psychosis

physician to vary the treatment according to the responses and needs of the individual child.

Pharmacologic doses of glucocorticoids administered for more than 1 or 2 weeks will cause the signs and symptoms of iatrogenic Cushing syndrome. These are similar to the glucocorticoid-induced findings in Cushing disease, but may be more severe (but lack adrenal androgen effects) because of the high doses involved (Table 14.13). The frequency of these adverse effects has stimulated broad interest in the development of more selective GR analogues, discussed earlier, but none have yet reached clinical use. Until more targeted approaches become available, alternate-day therapy can often be tried to decrease the toxicity of pharmacologic glucocorticoid therapy, especially suppression of the HPA axis and suppression of growth. The basic premise of alternate-day therapy is that the disease state can be suppressed with intermittent therapy, while there is significant recovery of the HPA axis during the “off” day. Thus alternate-day therapy requires the use of a relatively short-acting glucocorticoid administered only once in the morning of each therapeutic day to ensure that the off day is truly off. Long-acting glucocorticoids, such as dexamethasone, should not be used for alternate-day therapy; results are best with oral prednisone or methyl prednisolone.

Commonly Used Glucocorticoid Preparations

Numerous chemical derivatives and variants of the naturally occurring steroids are commercially available in a huge array of dosage forms, vehicles, and concentrations. Choosing the appropriate product can be simplified by considering only the most widely used steroids listed in Table 14.7.

There are four relevant considerations in the choice of which drug to use. First, the glucocorticoid potency of the various drugs is generally calculated and described according to the antiinflammatory potency, but this may not be the appropriate consideration in many cases. Second, the growth-suppressant effect of a glucocorticoid preparation may be significantly different from its antiinflammatory effect. This is because of differences in half-life, metabolism and protein binding, and receptor affinity (potency). Third, the mineralocorticoid activity of various glucocorticoid preparations varies widely. Glucocorticoids can bind to both GR and MR. Mineralocorticoid activity is intimately related to the activity of 11 β HSD2, which metabolizes glucocorticoids, but not mineralocorticoids, to forms that cannot bind the receptor. Thus the relative mineralocorticoid potency of various steroids is determined by both their affinity for MR and their resistance to the activity of 11 β HSD2. An understanding that some commonly used glucocorticoids, such as cortisol, cortisone, prednisolone, and prednisone, have significant mineralocorticoid activity is especially important when large doses of glucocorticoids are used as “stress doses” in a patient on replacement therapy. Such stress

doses of the glucocorticoid preparation may provide sufficient mineralocorticoid activity to meet physiologic needs; therefore mineralocorticoid supplementation is not needed and sustained administration of very high doses of these preparations have the potential to result in signs of mineralocorticoid excess. Fourth, the plasma half-life and biologic half-life of the various preparations may be discordant and will vary widely depending on binding to plasma proteins, hepatic metabolism, and hepatic activation. The plasma half-life of intravenously administered cortisol (hydrocortisone) is variable (mean of 75–100 min) and more than 90% of orally administered hydrocortisone is absorbed within an hour.^{535,536} Cortisone and prednisone are biologically inactive (and even have mild steroid antagonist actions), until they are metabolized by hepatic 11 β HSD1 to their active forms, cortisol, and prednisolone. Thus the glucocorticoid potency of these preparations is affected by hepatic function. Cortisone and prednisone are cleared more rapidly in patients receiving drugs, such as phenobarbital or phenytoin, that induce hepatic enzymes and are cleared more slowly in patients with liver failure.

In addition to these chemical considerations in the choice of glucocorticoid, the route of administration is important. Glucocorticoids are available for oral, intramuscular, intravenous, intrathecal, intraarticular, inhalant, and topical use; topical preparations include those designed for use on skin, mucous membranes, and conjunctiva. Each preparation is designed to deliver the maximal concentration of steroid to the desired tissue, while delivering less steroid systemically. However, all such preparations are absorbed to varying extents, so that the widely used inhalant preparations used to treat asthma can, in sufficient doses, cause growth retardation and other signs of Cushing syndrome and, in rare cases, result in adrenal insufficiency when abruptly stopped. In general, and in contradistinction to many other drugs, orally administered steroids are absorbed rapidly, but incompletely, whereas intramuscularly administered steroids are absorbed slowly, but completely. Thus if the secretory rate of cortisol is 8 mg/m² of body surface area, the intramuscular or intravenous replacement dose of cortisol (hydrocortisone) would be 8 mg/m². However, the dosage equivalents listed in Table 14.7 and similar tables are only general approximations, and doses must be tailored to the clinical response.

ACTH can also be used for glucocorticoid therapy by its action to stimulate endogenous adrenal steroidogenesis. Although intravenous and intramuscular ACTH are extremely useful in diagnostic tests of adrenal function, the use of ACTH as a therapeutic agent is no longer favored, principally because it will stimulate synthesis of mineralocorticoids and adrenal androgens, as well as glucocorticoids. Furthermore, the need to administer ACTH parenterally further diminishes its usefulness. Intramuscular ACTH(1-39) in a gel form is recommended for and is the treatment of choice for infantile spasms and possibly also for other forms of epilepsy in infants resistant to conventional anticonvulsants. Whether this action is mediated by ACTH itself, by ACTH-induced adrenal steroids, or by ACTH-responsive synthesis of novel “neurosteroids”⁵³⁷ in the brain has not been determined. When pharmacologic doses of ACTH are used therapeutically, as in infantile spasms, the patient should be given a low-sodium diet to ameliorate steroid hypertension. Although greatly elevated concentrations of ACTH, as in the ectopic ACTH syndrome, will cause pituitary suppression, treatment with daily injections of ACTH results in less hypothalamic-pituitary suppression than does treatment with equivalent doses of oral glucocorticoids, presumably because the effect on the adrenal is transient. Also adrenal suppression obviously does not occur in ACTH therapy. Because the effects of ACTH on adrenal steroidogenesis are highly variable, it is even more difficult to determine dosage equivalencies for

ACTH and oral steroid preparations than it is among the various steroids, as discussed earlier. A very rough guide from studies in adults is that 40 units of ACTH(1-39) gel is approximately equivalent to 100 mg of cortisol.

Withdrawal of Glucocorticoid Therapy

Withdrawal of glucocorticoid therapy can be difficult and can lead to symptoms of glucocorticoid insufficiency. When glucocorticoid therapy has been used for 10 days or less, therapy can be discontinued abruptly, even if high doses have been used.⁵³⁸ Although only one or two doses of glucocorticoids are needed to suppress the HPA axis, this axis recovers very rapidly from short-term suppression. When therapy has persisted for 2 weeks or longer, recovery of HPA function is slower, and tapered doses of glucocorticoids are indicated. Acute discontinuation of therapy in such patients will lead to symptoms of glucocorticoid insufficiency, the so-called steroid withdrawal syndrome. This symptom complex does not include salt loss, as adrenal glomerulosa function, regulated principally by the renin-angiotensin system, remains normal. However, blood pressure can fall abruptly, as glucocorticoids are required for the action of catecholamines in maintaining vascular tone. The most prominent symptoms of the steroid withdrawal syndrome include malaise, anorexia, headache, lethargy, nausea, and fever. In reducing pharmacologic doses of glucocorticoids, it might appear logical to reduce the dosage precipitously to “physiologic” replacement doses. However, this is rarely successful and occasionally disastrous. Even when given physiologic replacement, patients who have been receiving pharmacologic doses of glucocorticoids will experience steroid withdrawal. Long-term pharmacologic glucocorticoid therapy inhibits synthesis of GR, so that physiologic concentrations of glucocorticoids will elicit subphysiologic cellular responses, resulting in the steroid withdrawal syndrome. Thus it is necessary to taper gradually from the outset. The duration of glucocorticoid therapy is a critical consideration in designing a glucocorticoid withdrawal program. Therapy for a couple of months will completely suppress the HPA axis but will not cause adrenal atrophy. Therapy of years’ duration may result in almost total atrophy of the adrenal fasciculata/reticularis, and hence may require a withdrawal regimen that takes months.

Procedures for tapering steroids are empirical. Their success is determined by the length and mode of therapy and by individual patient responses. Patients who have been on alternate-day therapy can be withdrawn more easily than those receiving daily therapy, especially daily therapy with a long-acting glucocorticoid, such as dexamethasone. In patients on long-standing therapy, a 25% reduction in the previous level of therapy is generally recommended weekly. If the patient has been on daily therapy equivalent to 100 mg of cortisol for many months, a tapering protocol over 8 to 10 weeks may be needed. A protocol of 75% of the previous week’s dose would thus be 75 mg/day for the first week, 56 mg/day for the second, then 42, 31.5, 24, 18, 13.5, 10, 7.5, 5.5 mg/day, then off treatment. A more practical regimen based on the sizes of available tablets would be 75, 50, 37.5, 25, 17.5, 12.5, 10, 7.5, and 5 mg/day. Most patients can be tapered more rapidly, but all patients need to be followed closely. When withdrawal is done with steroids other than cortisol, measurement of morning cortisol values can be a useful adjunct. Morning cortisol values of 10 μ g/dL or more indicate that the dose can be reduced safely.

Even after the successful discontinuation of therapy, the HPA axis is not wholly normal. Just as in the patient successfully treated for Cushing disease, the HPA axis may be incapable of responding to severe stress for 6 to 12 months, after successful withdrawal from long-term, high-dose glucocorticoid therapy.

Thus evaluation of the hypothalamus and pituitary by a CRF or metyrapone test (when available), and evaluation of adrenal responsiveness to pituitary stimulation with an intravenous low-dose ACTH test, should be done at the conclusion of a withdrawal program, and 6 months thereafter. The results of these tests will indicate if there is a need for “steroid coverage” in acute surgical stress or illness.

Stress Doses of Glucocorticoids

The cortisol secretory rate increases significantly during physiologic stress, such as trauma, surgery, or severe illness. Patients receiving glucocorticoid replacement therapy or those recently withdrawn from pharmacologic therapy need coverage with “stress doses” of steroids in such situations. However, the specific indications for this coverage and the appropriate dosage are controversial and difficult to establish; most practitioners prefer to err on the “safe” side of steroid overdosage. This is the safest tactic in the short term but can have a significant effect on growth over a period of years and, if frequently used, may prolong the time needed for the HPA axis to recover.

It is generally said that doses 3 times physiologic replacement are needed for “the stress of surgery,” but this stress varies greatly. In the past, much “surgical stress” had to do with pain, fluid loss, hypovolemia, and fever, but these are managed better in contemporary pediatric services. Modern techniques of anesthesiology; better anesthetic, analgesic, and muscle-relaxing drugs; and increased awareness of the particular needs of children in managing intraoperative fluids and electrolytes have greatly reduced surgical stress. Serum cortisol does not exceed 10 ug/dL (276 nmol/L) in adrenally intact children undergoing anesthesia and minor surgery.⁹⁴ When used, stress dosing should be given in constant doses at 6-hour intervals.⁵²⁹ Although it remains appropriate and necessary to give about 3 times physiologic requirements during such periods of stress, it is probably not necessary to give higher doses. Similarly, it is not necessary to triple a child’s physiologic replacement regimen during simple colds, upper respiratory infection, otitis media, or after immunizations.

The preparation of the hypoadrenal patient on replacement therapy for surgery requires coordination with the anesthesia team. The best approach is to place a stress dose of 25 mg hydrocortisone per square meter in the intravenous fluid, so that the glucocorticoid is delivered continuously during surgery, rather than being given a single bolus at the beginning of the procedure. It appears that the greatest stress is at the time of anesthesia reversal, rather than at the time of anesthesia induction.⁹² Regular therapy at 2 to 3 times physiologic requirements can then be reinstated on the day after the surgical procedure.

Mineralocorticoid Replacement

Replacement therapy with mineralocorticoids is indicated in salt-losing CAH and in syndromes of adrenal insufficiency that affect the zona glomerulosa. Only one mineralocorticoid, oral 9 α -fluorocortisol (Fluorinef), is currently available. There is no parenteral mineralocorticoid preparation so that hydrocortisone plus salt must be used.

Because of increasing mineralocorticoid sensitivity with age (see Fig. 14.18), mineralocorticoid doses are similar in children and adults. Newborns are quite insensitive to mineralocorticoids and often require substantially larger doses than adults. The adult replacement dose of 9 α -fluorocortisol is usually 0.05 to 0.10 mg daily, but newborns with CAH may require up to 0.4 mg. Sodium must be available to the nephrons for mineralocorticoids to promote reabsorption of sodium, thus

the newborn with salt-losing CAH must be treated with both mineralocorticoids and sodium chloride. Similarly, mineralocorticoids will cause hypertension only by retaining sodium.

Cortisol has mineralocorticoid activity: approximately 20 mg of intravenous cortisol has a mineralocorticoid action equivalent to 0.1 mg of 9 α -fluorocortisol. Thus stress doses of cortisol provide adequate mineralocorticoid activity, and mineralocorticoid replacement can be interrupted. Additional 9 α -fluorocortisol is not needed until the supraphysiologic stress doses of cortisol are decreased. Because 9 α -fluorocortisol can be administered only orally and because this may not be possible in the postoperative period, the appropriate drug for glucocorticoid replacement is cortisol, which has mineralocorticoid activity, rather than synthetic steroids, such as prednisone or dexamethasone, which have little mineralocorticoid activity.

CONCLUDING REMARKS

Because the adrenal cortex is principally concerned with steroid synthesis, most of its disorders reflect genetic lesions in adrenal development and steroidogenesis. Overproduction and underproduction of steroids, having varied and complex physiologic actions, lead to complex phenotypes and clinical presentations. These primary, genetic disorders typically present themselves in infancy and childhood. In contrast, secondary disorders, such as Cushing disease (usually a disorder of the pituitary) and Addison disease (usually a disorder of cellular immunity), may be seen at any age. Thus the pediatric endocrinologist must have a detailed understanding of the cell biology, genetics, and biochemistry of steroid hormone biosynthesis. Improvements in the speed, accuracy, and economy of DNA sequencing now permits direct genetic diagnosis of many genetic diseases. However, it is unlikely that measurements of steroid hormones will become obsolete in the foreseeable future, and an understanding of steroid physiology will always be needed to understand clinical presentations, formulate differential diagnoses, choose genes for study, and monitor therapy. Thus genetics will continue to enhance clinical physiology, but will not replace it.

REFERENCES

1. Miller WL. A brief history of adrenal research: Steroidogenesis - The soul of the adrenal. *Mol Cell Endocrinol.* 2013;37:5-14.
2. Miller WL. Molecular biology of steroid hormone synthesis. *Endocr Rev.* 1988;9:295-318.
3. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science.* 1988;240:889-895.
4. Else T, Hammer GD. Genetic analysis of adrenal absence: agenesis and aplasia. *Trends Endocrinol Metab.* 2005;16:458-468.
5. Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev.* 1997;18:378-403.
6. Hammer GD, Parker KL, Schimmer BP. Transcriptional regulation of adrenocortical development. *Endocrinology.* 2005;146:1018-1024.
7. Kim AC, Barlaskar FM, Heaton JH, Else T, Kelly VR, Krill KT, et al. In search of adrenocortical stem and progenitor cells. *Endocr Rev.* 2009;30:241-263.
8. Axelrod J, Reisine TD. Stress hormones: their interaction and regulation. *Science.* 1984;224:452-459.
9. Freedman BD, Kempna PB, Carlone DL, Shah M, Guagliardo NA, Barrett PQ, et al. Adrenocortical zonation results from lineage conversion of differentiated zona glomerulosa cells. *Dev Cell.* 2013;26:666-673.
10. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;32:81-151.
11. Miller WL, Bose HS. Early steps in steroidogenesis: intracellular cholesterol trafficking. *J Lipid Res.* 2011;52:2111-2135.
12. Miller WL. Disorders in the initial steps of steroid hormone synthesis. *J Steroid Biochem Mol Biol.* 2017;165:18-37.

13. Nebert DW, Wikvall K, Miller WL. Human cytochromes P450 in health and disease. *Philos Trans R Soc Lond B Biol Sci.* 2013;368:20120431.
14. Mizrahi D, Auchus RJ. Androgens, estrogens, and hydroxysteroid dehydrogenases. *Mol Cell Endocrinol.* 2009;301:37–42.
15. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. *Endocr Rev.* 1997;18:281–305.
16. Agarwal AK, Auchus RJ. Cellular redox state regulates hydroxysteroid dehydrogenase activity and intracellular hormone potency. *Endocrinology.* 2005;146:2531–2538.
17. Jin Y, Penning TM. Aldo-keto reductases and bioactivation/detoxication. *Annu Rev Pharmacol Toxicol.* 2007;47:263–292.
18. Miller WL. Regulation of steroidogenesis by electron transfer. *Endocrinology.* 2005;146:2544–2550.
19. Brentano ST, Black SM, Lin D, Miller WL. cAMP post-transcriptionally diminishes the abundance of adrenodoxin reductase mRNA. *Proc Natl Acad Sci U S A.* 1992;89:4099–4103.
20. Solish SB, Picado-Leonard J, Morel Y, et al. Human adrenodoxin reductase: two mRNAs encoded by a single gene on chromosome 17cen—q25 are expressed in steroidogenic tissues. *Proc Natl Acad Sci U S A.* 1988;85:7104–7108.
21. Brandt ME, Vickery LE. Expression and characterization of human mitochondrial ferredoxin reductase in *Escherichia coli*. *Arch Biochem Biophys.* 1992;294:735–740.
22. Sheffel AD, Stehling O, Pierik AJ, Elsasser HP, Muhlenhoff U, Webert H, et al. Humans possess two mitochondrial ferredoxins, Fdx1 and Fdx2, with distinct roles in steroidogenesis, heme, and Fe/S cluster biosynthesis. *Proc Natl Acad Sci U S A.* 2010;107:11775–11780.
23. Shi Y, Ghosh M, Kovtunovych G, Crooks DR, Rouault TA. Both human ferredoxins 1 and 2 and ferredoxin reductase are important for iron-sulfur cluster biogenesis. *Biochim Biophys Acta.* 2012;1823:484–492.
24. Paul A, Drecourt A, Petit F, Deguine DD, Vasnier C, Oufadem M, et al. FXR mutations cause sensorial neuropathies and expand the spectrum of mitochondrial Fe-S-synthesis diseases. *Am J Hum Genet.* 2017;101:630–637.
25. Peng Y, Shinde DN, Valencia CA, Mo JS, Rosenfeld J, Truitt M, Cho, et al. Biallelic mutations in the ferredoxin reductase gene cause novel mitochondrial neuropathy with optic atrophy. *Hum Mol Genet.* 2017;26:4937–4950.
26. Slone J, Peng Y, Chamberlin A, Harris B, Kaylor J, McDonald MT, et al. Biallelic mutations in FXR cause neurodegeneration associated with inflammation. *J Hum Genet.* 2018;63:1211–1222.
27. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev.* 1996;17:221–244.
28. Miller WL. StAR search—what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. *Mol Endocrinol.* 2007;21:589–601.
29. Stocco DM, Zhao AH, Tu LN, Morohaku K, Selvaraj V. A brief history of the search for the protein(s) involved in the acute regulation of steroidogenesis. *Mol Cell Endocrinol.* 2017;441:7–16.
30. Lin D, Sugawara T, Strauss 3rd JF, Clark BJ, Stocco DM, Saenger P, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science.* 1995;267:1828–1831.
31. Bose HS, Sugawara T, Strauss 3rd JF, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med.* 1996;335:1870–1878.
32. Bose HS, Lingappa VR, Miller WL. Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature.* 2002;417:87–91.
33. Papadopoulos V, Miller WL. Role of mitochondria in steroidogenesis. *Clin Pract Res Clin Endocrinol Metab.* 2012;26:771–790.
34. Miller WL. Steroidogenesis: unanswered questions. *Trends Endocrinol Metab.* 2017;28:771–793.
35. Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the β -hydroxysteroid dehydrogenase/D5-D4 isomerase gene family. *Endocr Rev.* 2005;26:525–582.
36. Cherradi N, Rossier MF, Vallotton MB, Timberg R, Friedberg I, Orly J, et al. Submitochondrial distribution of three key steroidogenic proteins (steroidogenic acute regulatory protein and cytochrome P450_{scc} and β -hydroxysteroid dehydrogenase isomerase enzymes) upon stimulation by intracellular calcium in adrenal glomerulosa cells. *J Biol Chem.* 1997;272:7899–7907.
37. Auchus RJ, Lee TC, Miller WL. Cytochrome b5 augments the 17,20-lyase activity of human P450_{c17} without direct electron transfer. *J Biol Chem.* 1998;273:3158–3165.
38. Miller WL. The syndrome of 17,20 lyase deficiency. *J Clin Endocrinol Metab.* 2012;97:59–67.
39. Tee MK, Miller WL. Phosphorylation of human cytochrome P450_{c17} by p38 α selectively increases 17,20 lyase activity and androgen biosynthesis. *J Biol Chem.* 2013;288:23903–23913.
40. Speiser, P.W., Arlt, W., Auchus, R.J., Baskin, L.S., Conway, G.S., Merke, D.P., et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103:4043–4088.
41. Gomes LG, Huang N, Agrawal V, Mendonca BB, Bachege TA, Miller WL. Extraadrenal 21-hydroxylation by CYP2C19 and CYP3A4: effect on 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2009;94:89–95.
42. White PC, Curnow KM, Pascoe L. Disorders of steroid 11 β -hydroxylase isozymes. *Endocr Rev.* 1994;15:421–438.
43. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, Belanger A. The key role of 17 β -hydroxysteroid dehydrogenases in sex steroid biology. *Steroids.* 1997;62:148–158.
44. Moghrabi N, Andersson S. 17 β -hydroxysteroid dehydrogenases: physiological roles in health and disease. *Trends Endocrinol Metab.* 1998;9:265–270.
45. Marchais-Oberwinkler S, Henn C, Moller G, Klein T, Negri M, Oster A, et al. 17 β -Hydroxysteroid dehydrogenases (17 β -HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *J Steroid Biochem Mol Biol.* 2011;125:66–82.
46. Moeller G, Adamski J. Integrated view on 17 β -hydroxysteroid dehydrogenases. *Mol Cell Endocrinol.* 2009;301:7–19.
47. Tremblay Y, Ringler GE, Morel Y, Mohandas TK, Labrie F, Strauss 3rd JF, Miller WL. Regulation of the gene for estrogenic 17-ketosteroid reductase lying on chromosome 17cen—q25. *J Biol Chem.* 1989;264:20458–20462.
48. Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, et al. Human 3α -hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochem J.* 2000;351:67–77.
49. Nakamura Y, Hornsby PJ, Casson P, Morimoto R, Satoh F, Xing Y, et al. Type 5 17 β -hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *J Clin Endocrinol Metab.* 2009;94:2192–2198.
50. Biswas MG, Russell DW. Expression cloning and characterization of oxidative 17 β - and 3α -hydroxysteroid dehydrogenases from rat and human prostate. *J Biol Chem.* 1997;272:15959–15966.
51. Fluck CE, Meyer-Boni M, Pandey AV, Kempna P, Miller WL, Schoenle EJ, Bason-Lauber A. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet.* 2011;89:201–218.
52. Falany CN. Enzymology of human cytosolic sulfotransferases. *FASEB J.* 1997;11:206–216.
53. Strott CA. Sulfonation and molecular action. *Endocr Rev.* 2002;23:703–732.
54. Mueller JW, Gilligan LC, Idkowiak J, Arlt W, Foster PA. The Regulation of steroid action by sulfation and desulfation. *Endocr Rev.* 2015;36:526–563.
55. Seely J, Amigh KS, Suzuki T, Mayhew B, Sasano H, Giguere V, et al. Transcriptional regulation of dehydroepiandrosterone sulfotransferase (SULT2A1) by estrogen-related receptor alpha. *Endocrinology.* 2005;146:3605–3613.
56. Noordam C, Dhir V, McNelis JC, Schlereth F, Hanley NA, Krone N, et al. Inactivating PAPSS2 mutations in a patient with premature pubarche. *N Engl J Med.* 2009;360:2310–2318.
57. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev.* 1994;15:342–355.
58. Grumbach MM, Auchus RJ. Estrogen: consequences and implications of human mutations in synthesis and action. *J Clin Endocrinol Metab.* 1999;84:4677–4694.
59. Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER. A syndrome of female pseudohermaphroditism, hypergonadotropic

- hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J Clin Endocrinol Metab.* 1994;78:1287–1292.
60. Lo JC, Schwitzgebel VM, Tyrrell JB, Fitzgerald PA, Kaplan SL, Conte FA, Grumbach MM. Normal female infants born of mothers with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 1999;84:930–936.
 61. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab.* 1995;80:3689–3698.
 62. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5 α -reductase isozyme expression. *J Clin Invest.* 1993;92:903–910.
 63. Russell DW, Wilson JD. Steroid 5 α -reductase: two genes/two enzymes. *Annu Rev Biochem.* 1994;63:25–61.
 64. Wilson JD. The role of androgens in male gender role behavior. *Endocr Rev.* 1999;20:726–737.
 65. Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM. Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science.* 1987;237:268–275.
 66. Edwards CRW, Stewart PM, Burt D, Brett L, McIntyre MA, Sutanto WS, et al. Localisation of 11 β -hydroxysteroid dehydrogenase–tissue specific protector of the mineralocorticoid receptor. *Lancet.* 1988;2:986–989.
 67. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science.* 1988;242:583–585.
 68. White PC, Mune T, Agarwal AK. 11 β -hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev.* 1997;18:135–156.
 69. Walker EA, Stewart PM. 11 β -hydroxysteroid dehydrogenase: unexpected connections. *Trends Endocrinol Metab.* 2003;14:334–339.
 70. Seckl JR, Morton NM, Chapman KE, Walker BR. Glucocorticoids and 11 β -hydroxysteroid dehydrogenase in adipose tissue. *Recent Prog Horm Res.* 2004;59:359–393.
 71. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, et al. 11 β -hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev.* 2004;25:831–866.
 72. Chapman K, Holmes M, Seckl J. 11 β -hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev.* 2013;93:1139–1206.
 73. Gathercole LL, Lavery GG, Morgan SA, Cooper MS, Sinclair AJ, Tomlinson JW, Stewart PM. 11 β -Hydroxysteroid dehydrogenase 1: translational and therapeutic aspects. *Endocr Rev.* 2013;34:525–555.
 74. White PC. Alterations of Cortisol Metabolism in Human Disorders. *Horm Res Paediatr.* 2018;89:320–330.
 75. Hewitt KN, Walker EA, Stewart PM. Hexose-6-phosphate dehydrogenase and redox control of 11 β -hydroxysteroid dehydrogenase type 1 activity. *Endocrinology.* 2005;146:2539–2543.
 76. Odermatt A, Arnold P, Stauffer A, Frey BM, Frey FJ. The N-terminal anchor sequences of 11 β -hydroxysteroid dehydrogenases determine their orientation in the endoplasmic reticulum membrane. *J Biol Chem.* 1999;274:28762–28770.
 77. Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab.* 2004;15:432–438.
 78. Wilson JD, Auchus RJ, Leihy MW, Guryev OL, Estabrook RW, Osborn SM, et al. 5 α -androstane-3 α ,17 β -diol is formed in tammur wallaby pouch young testes by a pathway involving 5 α -pregnane-3 α ,17 α -diol-20-one as a key intermediate. *Endocrinology.* 2003;144:575–580.
 79. Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative “backdoor” pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. *J Clin Endocrinol Metab.* 2012;97:E367–375.
 80. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2010;95:4133–4160.
 81. Napoli JL. 17 β -Hydroxysteroid dehydrogenase type 9 and other short-chain dehydrogenases/reductases that catalyze retinoid, 17 β - and 3 α -hydroxysteroid metabolism. *Mol Cell Endocrinol.* 2001;171:103–109.
 82. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol.* 2000;21:1–56.
 83. Goto M, Piper Hanley K, Marcos J, Wood PJ, Wright S, Postle AD, et al. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest.* 2006;116:953–960.
 84. Voutilainen R, Ilvesmaki V, Miettinen PJ. Low expression of 3 β -hydroxy-5-ene steroid dehydrogenase gene in human fetal adrenals in vivo; adrenocorticotropin and protein kinase C-dependent regulation in adrenocortical cultures. *J Clin Endocrinol Metab.* 1991;72:761–767.
 85. Miller WL. Steroid hormone biosynthesis and actions in the materno-feto-placental unit. *Clin Perinatol.* 1998;25:799–817.
 86. McMahon SK, Pretorius CJ, Ungerer JP, Salmon NJ, Conwell LS, Pearen MA, Batch JA. Neonatal complete generalized glucocorticoid resistance and growth hormone deficiency caused by a novel homozygous mutation in Helix 12 of the ligand binding domain of the glucocorticoid receptor gene (NR3C1). *J Clin Endocrinol Metab.* 2010;95:297–302.
 87. Miller WL. The hypothalamic-pituitary-adrenal axis: a brief history. *Horm Res Paediatr.* 2018;89:212–223.
 88. Sawchenko PE, Swanson LW, Vale WW. Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci U S A.* 1984;81:1883–1887.
 89. Lundblad JR, Roberts JL. Regulation of proopiomelanocortin gene expression in pituitary. *Endocr Rev.* 1988;9:135–158.
 90. Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev.* 1998;19:101–143.
 91. Eastman CI, Burgess HJ. How to travel the world without jet lag. *Sleep Med Clin.* 2009;4:241–255.
 92. Udelman R, Norton JA, Jelenich SE, et al. Responses of the hypothalamic-pituitary-adrenal and renin-angiotensin axes and the sympathetic system during controlled surgical and anesthetic stress. *J Clin Endocrinol Metab.* 1987;64:986–994.
 93. Rains PC, Rampersad N, De Lima J, Murrell D, Kinchington D, Lee JW, et al. Cortisol response to general anaesthesia for medical imaging in children. *Clin Endocrinol (Oxf).* 2009;71:834–839.
 94. Taylor LK, Auchus RJ, Baskin LS, Miller WL. Cortisol response to operative stress with anesthesia in healthy children. *J Clin Endocrinol Metab.* 2013;98:3687–3693.
 95. Khoo B, Boshier PR, Freethy A, Tharakan G, Saeed S, Hill N, et al. Redefining the stress cortisol response to surgery. *Clin Endocrinol (Oxf).* 2017;87:451–458.
 96. Prete A, Yan Q, Al-Tarrak K, Akturk HK, Prokop LJ, Alahdab F, et al. The cortisol stress response induced by surgery: A systematic review and meta-analysis. *Clin Endocrinol (Oxf).* 2018;89:554–567.
 97. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med.* 1995;332:1351–1362.
 98. Weber MM, Michl P, Auernhammer CJ, Engelhardt D. Interleukin-3 and interleukin-6 stimulate cortisol secretion from adult human adrenocortical cells. *Endocrinology.* 1997;138:2207–2210.
 99. Hardman JA, Hort YJ, Catanzaro DF, Tellam JT, Baxter JD, Morris BJ, Shine J. Primary structure of the human renin gene. *DNA.* 1984;3:457–468.
 100. Barrett PQ, Bollag WB, Isales CM, McCarthy RT, Rasmussen H. Role of calcium in angiotensin II-mediated aldosterone secretion. *Endocr Rev.* 1989;10:496–518.
 101. Pignatti E, Leng S, Carlone DL, Breault DT. Regulation of zonation and homeostasis in the adrenal cortex. *Mol Cell Endocrinol.* 2017;441:146–155.
 102. Orentreich N, Brind JL, Rizer RL, Vogelmann JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab.* 1984;59:551–555.
 103. Miller WL. Androgen synthesis in adrenarache. *Rev Endocr Metab Disord.* 2009;10:3–17.
 104. Hui XG, Akahira J, Suzuki T, Nio M, Nakamura Y, Suzuki H, Rainey WE, Sasano H. Development of the human adrenal zona

- reticularis: morphometric and immunohistochemical studies from birth to adolescence. *J Endocrinol.* 2009;203:241–252.
105. Alkatib AA, Cosma M, Elamin MB, Erickson D, Swiglo BA, Erwin PJ, Montori VM. A systematic review and meta-analysis of randomized placebo-controlled trials of DHEA treatment effects on quality of life in women with adrenal insufficiency. *J Clin Endocrinol Metab.* 2009;94:3676–3681.
 106. Pretorius E, Arlt W, Storbeck KH. A new dawn for androgens: Novel lessons from 11-oxygenated C19 steroids. *Mol Cell Endocrinol.* 2017;441:76–85.
 107. Turcu AF, Auchus RJ. Clinical significance of 11-oxygenated androgens. *Curr Opin Endocrinol Diabetes Obes.* 2017;24:252–259.
 108. Campana C, Rege J, Turcu AF, et al. Development of a novel cell based androgen screening model. *J Steroid Biochem Mol Biol.* 2016;156:17–22.
 109. Imamichi Y, Yuhki KI, Orisaka M, Kitano T, Mukai K, Ushikubi F, et al. 11-Ketotestosterone is a major androgen produced in human gonads. *J Clin Endocrinol Metab.* 2016;101:3582–3591.
 110. Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC, et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *J Clin Endocrinol Metab.* 2013;98:1182–1188.
 111. Storbeck KH, Bloem LM, Africander D, Schloms L, Swart P, Swart AC. 11 β -Hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: a putative role in castration resistant prostate cancer? *Mol Cell Endocrinol.* 2013;377:135–146.
 112. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano TJ, Shields JJ, et al. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. *Eur J Endocrinol.* 2016;174:601–609.
 113. Rege J, Turcu AF, Kasa-Vubu JZ, Lerario AM, Auchus GC, Auchus RJ, et al. 11-Ketotestosterone is the dominant circulating bioactive androgen during normal and premature adrenarche. *J Clin Endocrinol Metab.* 2018;103:4589–4598.
 114. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. Androgen excess is due to elevated 11-oxygenated androgens in treated children with congenital adrenal hyperplasia. *J Steroid Biochem Mol Biol.* 2018;178:221–228.
 115. Turcu AF, Mallappa A, Elman MS, Avila NA, Marko J, Rao H, et al. 11-Oxygenated androgens are biomarkers of adrenal volume and testicular adrenal rest tumors in 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2017;102:2701–2710.
 116. Hammond GL. Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. *Endocr Rev.* 1990;11:65–79.
 117. Rosner W. The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocr Rev.* 1990;11:80–91.
 118. Moore CCD, Mellon SH, Murai J, Siiteri PK, Miller WL. Structure and function of the hepatic form of 11 β -hydroxysteroid dehydrogenase in the squirrel monkey, an animal model of glucocorticoid resistance. *Endocrinology.* 1993;133:368–375.
 119. Voccia E, Saenger P, Peterson RE, Rauh W, Gottesdiener K, Levine LS, New MI. 6 β -Hydroxycortisol excretion in hypercortisolemic states. *J Clin Endocrinol Metab.* 1979;48:467–471.
 120. Falany CN, Green MD, Tephly TR. The enzymatic mechanism of glucuronidation catalyzed by two purified rat liver steroid UDP-glucuronosyltransferases. *J Biol Chem.* 1987;262:1218–1222.
 121. Duax WL, Ghosh D, Pletnev V. Steroid dehydrogenase structures, mechanism of action, and disease. *Vitam Horm.* 2000;58:121–148.
 122. Takahashi N, Inui N, Morita H, Takeuchi K, Uchida S, Watanabe H, Nakamura H. Effect of thyroid hormone on the activity of CYP3A enzyme in humans. *J Clin Pharmacol.* 2010;50:88–93.
 123. Dimaraki EV, Jaffe CA. Troglitazone induces CYP3A4 activity leading to falsely abnormal dexamethasone suppression test. *J Clin Endocrinol Metab.* 2003;88:3113–3116.
 124. Nowak SN, Edwards DJ, Clarke A, Anderson GD, Jaber LA. Pioglitazone: effect on CYP3A4 activity. *J Clin Pharmacol.* 2002;42:1299–1302.
 125. Arlt W, Auchus RJ, Miller WL. Thiazolidinediones but not metformin directly inhibit the steroidogenic enzymes P450c17 and 3 β -hydroxysteroid dehydrogenase. *J Biol Chem.* 2001;276:16767–16771.
 126. Kempna P, Hofer G, Mullis PE, Fluck CE. Pioglitazone inhibits androgen production in NCI-H295R cells by regulating gene expression of CYP17 and HSD3B2. *Mol Pharmacol.* 2007;71:787–798.
 127. Murphy PA, Kern SE, Stanczyk FZ, Westhoff CL. Interaction of St. John's Wort with oral contraceptives: effects on the pharmacokinetics of norethindrone and ethinyl estradiol, ovarian activity and breakthrough bleeding. *Contraception.* 2005;71:402–408.
 128. Pfrunder A, Schiesser M, Gerber S, Haschke M, Bitzer J, Drewe J. Interaction of St John's wort with low-dose oral contraceptive therapy: a randomized controlled trial. *Br J Clin Pharmacol.* 2003;56:683–690.
 129. Witjes FJ, Debruyne FM, Fernandez del Moral P, Geboers AD. Ketoconazole high dose in management of hormonally pretreated patients with progressive metastatic prostate cancer. Dutch south-Eastern Urological Cooperative Group. *Urology.* 1989;33:411–415.
 130. Loli P, Berselli ME, Tagliaferri M. Use of ketoconazole in the treatment of Cushing's syndrome. *J Clin Endocrinol Metab.* 1986;63:1365–1371.
 131. Boonen E, Vervenne H, Meersseman P, Andrew R, Mortier L, Declercq PE, et al. Reduced cortisol metabolism during critical illness. *N Engl J Med.* 2013;368:1477–1488.
 132. Andrew R, Phillips DI, Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. *J Clin Endocrinol Metab.* 1998;83:1806–1809.
 133. Tomlinson JW, Finney J, Gay C, Hughes BA, Hughes SV, Stewart PM. Impaired glucose tolerance and insulin resistance are associated with increased adipose 11 β -hydroxysteroid dehydrogenase type 1 expression and elevated hepatic 5 α -reductase activity. *Diabetes.* 2008;57:2652–2660.
 134. Deck KA, Fischer B, Hillen H. Studies on cortisol metabolism during haemodialysis in man. *Eur J Clin Invest.* 1979;9:203–207.
 135. Weir RA, Tzorlalis IK, Steedman T, Dargie HJ, Fraser R, McMurray JJ, Connell JM. Aldosterone and cortisol predict medium-term left ventricular remodelling following myocardial infarction. *Eur J Heart Fail.* 2011;13:1305–1313.
 136. Auchus RJ. Steroid assays and endocrinology: best practices for basic scientists. *Endocrinology.* 2014;155:2049–2051.
 137. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. *J Clin Endocrinol Metab.* 2013;98:3971–3973.
 138. Kulle A, Krone N, Holterhus PM, Schuler G, Greaves RF, Juul A, et al. Steroid hormone analysis in diagnosis and treatment of DSD: position paper of EU COST Action BM 1303 'DSDnet.' *J Clin Endocrinol.* 2017;176:P1–P9.
 139. Wudy SA, Schuler G, Sanchez-Guijo A, Hartmann MF. The art of measuring steroids: Principles and practice of current hormonal steroid analysis. *J Steroid Biochem Mol Biol.* 2018;179:88–103.
 140. Krone N, Hughes BA, Lavery GG, Stewart PM, Arlt W, Shackleton CH. Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS). *J Steroid Biochem Mol Biol.* 2010;121:496–504.
 141. Wilkes EH, Rumsby G, Woodward GM. Using machine learning to aid the interpretation of urine steroid profiles. *Clin Chem.* 2018;64(11):1586–1595.
 142. Dimitriou T, Maser-Gluth C, Remer T. Adrenocortical activity in healthy children is associated with fat mass. *Am J Clin Nutr.* 2003;77:731–736.
 143. Remer T, Maser-Gluth C, Boye KR, Hartmann MF, Heinze E, Wudy SA. Exaggerated adrenarche and altered cortisol metabolism in Type 1 diabetic children. *Steroids.* 2006;71:591–598.
 144. Kerrigan JR, Veldhuis JD, Leyo SA, Iranmanesh A, Rogol AD. Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. *J Clin Endocrinol Metab.* 1993;76:1505–1510.
 145. Linder BL, Esteban NV, Yergey AL, Winterer JC, Loriaux DL, Cassorla F. Cortisol production rate in childhood and adolescence. *J Pediatr.* 1990;117:892–896.
 146. Clark AJ, Weber A. Adrenocorticotropin insensitivity syndromes. *Endocr Rev.* 1998;19:828–843.
 147. Avgerinos PC, Yanovski JA, Oldfield EH, Nieman LK, Cutler Jr GB. The metyrapone and dexamethasone suppression

- tests for the differential diagnosis of the adrenocorticotropin-dependent Cushing syndrome: a comparison. *Ann Intern Med.* 1994;121:318–327.
148. Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res.* 2011;52:6–34.
 149. Klima H, Ullrich K, Aslanidis C, Fehringer P, Lackner KJ, Schmitz G. A splice junction mutation causes deletion of a 72-base exon from the mRNA for lysosomal acid lipase in a patient with cholesteryl ester storage disease. *J Clin Invest.* 1993;92:2713–2718.
 150. Anderson RA, Byrum RS, Coates PM, Sando GN. Mutations at the lysosomal acid cholesteryl ester hydrolase gene locus in Wolman disease. *Proc Natl Acad Sci U S A.* 1994;91:2718–2722.
 151. Pagani F, Pariyarath R, Garcia R, Stuanli C, Burlina AB, Ruotolo G, et al. New lysosomal acid lipase gene mutants explain the phenotype of Wolman disease and cholesteryl ester storage disease. *J Lipid Res.* 1998;39:1382–1388.
 152. Lohse P, Maas S, Sewell AC, van Diggelen OP, Seidel D. Molecular defects underlying Wolman disease appear to be more heterogeneous than those resulting in cholesteryl ester storage disease. *J Lipid Res.* 1999;40:221–228.
 153. Zschenker O, Jung N, Rethmeier J, Trautwein S, Hertel S, Zeigler M, Ameis D. Characterization of lysosomal acid lipase mutations in the signal peptide and mature polypeptide region causing Wolman disease. *J Lipid Res.* 2001;42:1033–1040.
 154. Pisciotto L, Fresa R, Bellocchio A, Pino E, Guido V, Cantafora A, et al. Cholesteryl Ester Storage Disease (CESD) due to novel mutations in the LIPA gene. *Mol Genet Metab.* 2009;97:143–148.
 155. Ozmen MN, Aygun N, Kilic I, Kuran L, Yalcin B, Besim A. Wolman's disease: ultrasonographic and computed tomographic findings. *Pediatr Radiol.* 1992;22:541–542.
 156. Westra SJ, Zaninovic AC, Hall TR, Kangaroo H, Boechat MI. Imaging of the adrenal gland in children. *Radiographics.* 1994;14:1323–1340.
 157. Gramatges MM, Dvorak CC, Regula DP, Enns GM, Weinberg K, Agarwal R. Pathological evidence of Wolman's disease following hematopoietic stem cell transplantation despite correction of lysosomal acid lipase activity. *Bone Marrow Transplant.* 2009;44:449–450.
 158. Tolar J, Petryk A, Khan K, Bjoraker KJ, Jessurun J, Dolan M, et al. Long-term metabolic, endocrine, and neuropsychological outcome of hematopoietic cell transplantation for Wolman disease. *Bone Marrow Transplant.* 2009;43:21–27.
 159. Valayannopoulos V, Malinova V, Honzik T, Balwani M, Breen C, Deegan PB, et al. Sebelipase alfa over 52 weeks reduces serum transaminases, liver volume and improves serum lipids in patients with lysosomal acid lipase deficiency. *J Hepatol.* 2014;61:1135–1142.
 160. Perry R, Kecha O, Paquette J, Huot C, Van Vliet G, Deal C. Primary adrenal insufficiency in children: twenty years experience at the Sainte-Justine Hospital, Montreal. *J Clin Endocrinol Metab.* 2005;90:3243–3250.
 161. Bernstein DL, Hulkova H, Bialer MG, Desnick RJ. Cholesteryl ester storage disease: review of the findings in 135 reported patients with an underdiagnosed disease. *J Hepatol.* 2013;58:1230–1243.
 162. Mukherjee S, Maxfield FR. Lipid and cholesterol trafficking in NPC. *Biochim Biophys Acta.* 2004;1685:28–37.
 163. Chang TY, Reid PC, Sugii S, Ohgami N, Cruz JC, Chang CC. Niemann-Pick type C disease and intracellular cholesterol trafficking. *J Biol Chem.* 2005;280:20917–20920.
 164. Fink JK, Filling-Katz MR, Sokol J, Cogan DG, Pikus A, Sonies B, et al. Clinical spectrum of Niemann-Pick disease type C. *Neurology.* 1989;39:1040–1049.
 165. Griffin LD, Gong W, Verot L, Mellon SH. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat Med.* 2004;10:704–711.
 166. Miller WL. Congenital lipid adrenal hyperplasia: the human gene knockout for the steroidogenic acute regulatory protein. *J Mol Endocrinol.* 1997;19:227–240.
 167. Lin D, Gitelman SE, Saenger P, Miller WL. Normal genes for the cholesterol side chain cleavage enzyme, P450_{sc}, in congenital lipid adrenal hyperplasia. *J Clin Invest.* 1991;88:1955–1962.
 168. Ogata T, Matsuo N, Saito M, Prader A. The testicular lesion and sexual differentiation in congenital lipid adrenal hyperplasia. *Helv Paediatr Acta.* 1989;43:531–538.
 169. Saenger P, Klonari Z, Black SM, Compagnone N, Mellon SH, Fleischer A, et al. Prenatal diagnosis of congenital lipid adrenal hyperplasia. *J Clin Endocrinol Metab.* 1995;80:200–205.
 170. Chen X, Baker BY, Abduljabbar MA, Miller WL. A genetic isolate of congenital lipid adrenal hyperplasia with atypical clinical findings. *J Clin Endocrinol Metab.* 2005;90:835–840.
 171. Bose HS, Pescovitz OH, Miller WL. Spontaneous feminization in a 46,XX female patient with congenital lipid adrenal hyperplasia due to a homozygous frameshift mutation in the steroidogenic acute regulatory protein. *J Clin Endocrinol Metab.* 1997;82:1511–1515.
 172. Fujieda K, Tajima T, Nakae J, Sageshima S, Tachibana K, Suwa S, et al. Spontaneous puberty in 46,XX subjects with congenital lipid adrenal hyperplasia. Ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. *J Clin Invest.* 1997;99:1265–1271.
 173. Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipid adrenal hyperplasia. *Proc Natl Acad Sci U S A.* 1997;94:11540–11545.
 174. Hasegawa T, Zhao L, Caron KM, Majdic G, Suzuki T, Shizawa S, et al. Developmental roles of the steroidogenic acute regulatory protein (STAR) as revealed by StAR knockout mice. *Mol Endocrinol.* 2000;14:1462–1471.
 175. Bose HS, Sato S, Aisenberg J, Shalev SA, Matsuo N, Miller WL. Mutations in the steroidogenic acute regulatory protein (StAR) in six patients with congenital lipid adrenal hyperplasia. *J Clin Endocrinol Metab.* 2000;85:3636–3639.
 176. Nakae J, Tajima T, Sugawara T, Arakane F, Hanaki K, Hotsubo T, et al. Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipid adrenal hyperplasia. *Hum Mol Genet.* 1997;6:571–576.
 177. Kang E, Kim YM, Kim GH, Lee BH, Yoo HW, Choi JH. Mutation spectrum of STAR and a founder effect of the p.Q258* in Korean patients with congenital lipid adrenal hyperplasia. *Mol Med.* 2017;23.
 178. Kim JM, Choi JH, Lee JH, Kim GH, Lee BH, Kim HS, et al. High allele frequency of the p.Q258X mutation and identification of a novel mis-splicing mutation in the STAR gene in Korean patients with congenital lipid adrenal hyperplasia. *Eur J Endocrinol.* 2011;165:771–778.
 179. Abdulhadi-Atwan M, Jean A, Chung WK, Meir K, Ben Neriah Z, Stratigopoulos G, et al. Role of a founder c.201_202delCT mutation and new phenotypic features of congenital lipid adrenal hyperplasia in Palestinians. *J Clin Endocrinol Metab.* 2007;92:4000–4008.
 180. Alswailem MM, Alzahrani OS, Alhomaidah DS, Alasmari R, Qasem E, Murugan AK, et al. Mutational analysis of rare subtypes of congenital adrenal hyperplasia in a highly inbred population. *Mol Cell Endocrinol.* 2018;461:105–111.
 181. Fluck CE, Maret A, Mallet D, Portrat-Doyen S, Achermann JC, Leheup B, et al. A novel mutation L260P of the steroidogenic acute regulatory protein gene in three unrelated patients of Swiss ancestry with congenital lipid adrenal hyperplasia. *J Clin Endocrinol Metab.* 2005;90:5304–5308.
 182. Miller WL. Mechanisms in endocrinology: rare defects in adrenal steroidogenesis. *Eur J Endocrinol.* 2018;179:R125–R141.
 183. Gassner HL, Toppari J, Quinteiro Gonzalez S, Miller WL. Near-miss apparent SIDS from adrenal crisis. *J Pediatr.* 2004;145:178–183.
 184. Baker BY, Lin L, Kim CJ, Raza J, Smith CP, Miller WL, Achermann JC. Nonclassic congenital lipid adrenal hyperplasia: a new disorder of the steroidogenic acute regulatory protein with very late presentation and normal male genitalia. *J Clin Endocrinol Metab.* 2006;91:4781–4785.
 185. Metherell LA, Naville D, Halaby G, Begeot M, Huebner A, Nurnberg G, et al. Nonclassic lipid congenital adrenal hyperplasia masquerading as familial glucocorticoid deficiency. *J Clin Endocrinol Metab.* 2009;94:3865–3881.
 186. Sahakitrungruang T, Soccio RE, Lang-Muritano M, Walker JM, Achermann JC, Miller WL. Clinical, genetic, and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (StAR). *J Clin Endocrinol Metab.* 2010;95:3352–3359.

187. Fluck CE, Pandey AV, Dick B, Camats N, Fernandez-Cancio M, Clemente M, et al. Characterization of novel StAR (steroidogenic acute regulatory protein) mutations causing non-classic lipid adrenal hyperplasia. *PLoS One*. 2011;6: e20178.
188. Khoury K, Barbar E, Ainmelk Y, Ouellet A, Lehoux JG. Gonadal function, first cases of pregnancy, and child delivery in a woman with lipid congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2009;94:1333–1337.
189. Sertedaki A, Pantos K, Vrettou C, et al. Conception and pregnancy outcome in a patient with 11-bp deletion of the steroidogenic acute regulatory protein gene. *Fertil Steril*. 2009;91(934): e915–938.
190. Tajima T, Fujieda K, Kouda N, Nakae J, Miller WL. Heterozygous mutation in the cholesterol side chain cleavage enzyme (P450sc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. *J Clin Endocrinol Metab*. 2001;86:3820–3825.
191. Gucev ZS, Tee MK, Chitayat D, Wherrett DK, Miller WL. Distinguishing deficiencies in the steroidogenic acute regulatory protein and the cholesterol side chain cleavage enzyme causing neonatal adrenal failure. *J Pediatr*. 2013;162:819–822.
192. Tee MK, Abramsohn M, Loewenthal N, Harris M, Siwach S, Kaplinsky A, et al. Varied clinical presentations with mutations in CYP11A1 encoding the cholesterol side chain cleavage enzyme, P450sc. *J Clin Endocrinol Metab*. 2013;713–720.
193. Kim CJ, Lin L, Huang N, Quigley CA, AvRuskin TW, Achermann JC, Miller WL. Severe combined adrenal and gonadal deficiency caused by novel mutations in the cholesterol side chain cleavage enzyme, P450sc. *J Clin Endocrinol Metab*. 2008;93:696–702.
194. Goursaud C, Mallet D, Janin A, Menassa R, Tardy-Guidollet V, Russo G, et al. Aberrant splicing is the pathogenicity mechanism of the p.Glu314Lys variant in CYP11A1 gene. *Front Endocrinol*. 2018;9:491.
195. Guran T, Buonocore F, Saka N, Ozbek MN, Aycan Z, Bereket A, et al. Rare causes of primary adrenal insufficiency: genetic and clinical characterization of a large nationwide cohort. *J Clin Endocrinol Metab*. 2016;101:284–292.
196. Rubtsov P, Karmanov M, Sverdlova P, Spirin P, Tiulpakov A. A novel homozygous mutation in CYP11A1 gene is associated with late-onset adrenal insufficiency and hypospadias in a 46,XY patient. *J Clin Endocrinol Metab*. 2009;94:936–939.
197. Sahakitrungruang T, Tee MK, Blackett PR, Miller WL. Partial defect in the cholesterol side-chain cleavage enzyme P450sc (CYP11A1) resembling nonclassic congenital lipid adrenal hyperplasia. *J Clin Endocrinol Metab*. 2011;96:792–798.
198. Achermann JC, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet*. 1999;22:125–126.
199. Camats N, Pandey AV, Fernandez-Cancio M, Andaluz P, Janner M, Toran N, et al. Ten novel mutations in the NR5A1 gene cause disordered sex development in 46,XY and ovarian insufficiency in 46,XX individuals. *J Clin Endocrinol Metab*. 2012;97: E1294–E1306.
200. Moisan AM, Ricketts ML, Tardy V, Desrochers M, Mebarki F, Chaussain JL, et al. New insight into the molecular basis of 3 β -hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the HSD3B2 gene eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. *J Clin Endocrinol Metab*. 1999;84:4410–4425.
201. Rheaume E, Simard J, Morel Y, Mebarki F, Zachmann M, Forest MG, et al. Congenital adrenal hyperplasia due to point mutations in the type II 3 β -hydroxysteroid dehydrogenase gene. *Nat Genet*. 1992;1:239–245.
202. Burckhardt MA, Udhane SS, Marti N, Schnyder I, Tapia C, Nielsen JE, et al. Human 3 β -hydroxysteroid dehydrogenase deficiency seems to affect fertility but may not harbor a tumor risk: lesson from an experiment of nature. *Eur J Endocrinol*. 2015;173:K1–K12.
203. Cara JF, Moshang Jr T, Bongiovanni AM, Marx BS. Elevated 17-hydroxyprogesterone and testosterone in a newborn with 3 β -hydroxysteroid dehydrogenase deficiency. *N Engl J Med*. 1985;313:618–621.
204. Jeandron DD, Sahakitrungruang T. A novel homozygous Q334X mutation in the HSD3B2 gene causing classic 3 β -hydroxysteroid dehydrogenase deficiency: an unexpected diagnosis after a positive newborn screen for 21-hydroxylase deficiency. *Horm Res Paediatr*. 2012;77:334–338.
205. Lee TC, Miller WL, Auchus RJ. Medroxyprogesterone acetate and dexamethasone are competitive inhibitors of different human steroidogenic enzymes. *J Clin Endocrinol Metab*. 1999;84:2104–2110.
206. Thomas JL, Mason JI, Brandt S, Spencer Jr BR, Norris W. Structure/function relationships responsible for the kinetic differences between human type 1 and type 2 3 β -hydroxysteroid dehydrogenase and for the catalysis of the type 1 activity. *J Biol Chem*. 2002;277:42795–42801.
207. Pang S, Carbanaru G, Haider A, et al. Carriers for type II 3 β -hydroxysteroid dehydrogenase (HSD3B2) deficiency can only be identified by HSD3B2 genotype study and not by hormone test. *Clin Endocrinol (Oxf)*. 2003;58:323–331.
208. Pang S. The molecular and clinical spectrum of 3 β -hydroxysteroid dehydrogenase deficiency disorder. *Trends Endocrinol Metab*. 1998;9:82–86.
209. Mermejo LM, Elias LL, Marui S, Moreira AC, Mendonca BB, de Castro M. Refining hormonal diagnosis of type II 3 β -hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on HSD3B2 genotyping. *J Clin Endocrinol Metab*. 2005;90:1287–1293.
210. Lutfallah C, Wang W, Mason JI, Chang YT, Haider A, Rich B, et al. Newly proposed hormonal criteria via genotypic proof for type II 3 β -hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab*. 2002;87:2611–2622.
211. Auchus RJ. The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinol Metab Clin North Am*. 2001;30:101–119.
212. Auchus RJ. Steroid 17-hydroxylase and 17,20-lyase deficiencies, genetic and pharmacologic. *J Steroid Biochem Mol Biol*. 2017;165:71–78.
213. Imai T, Yanase T, Waterman MR, Simpson ER, Pratt JJ. Canadian Mennonites and individuals residing in the Friesland region of The Netherlands share the same molecular basis of 17 α -hydroxylase deficiency. *Hum Genet*. 1992;89:95–96.
214. Fardella CE, Zhang LH, Mahachokertwattana P, Lin D, Miller WL. Deletion of amino acids Asp487-Ser488-Phe489 in human cytochrome P450c17 causes severe 17 α -hydroxylase deficiency. *J Clin Endocrinol Metab*. 1993;77:489–493.
215. Zhang M, Sun S, Liu Y, Zhang H, Jiao Y, Wang W, Li X. New, recurrent, and prevalent mutations: Clinical and molecular characterization of 26 Chinese patients with 17 α -hydroxylase/17,20-lyase deficiency. *J Steroid Biochem Mol Biol*. 2015;150:11–16.
216. Han B, Liu W, Zuo CL, Zhu H, Li L, Xu C, et al. Identifying a novel mutation of CYP17A1 gene from five Chinese 17 α -hydroxylase/17,20-lyase deficiency patients. *Gene*. 2013;516:345–350.
217. Han B, Xue L, Fan M, Zhao S, Liu W, Zhu H, et al. Clinical and molecular manifestation of fifteen 17OHD patients: a novel mutation and a founder effect. *Endocrine*. 2016;53:784–790.
218. Costa-Santos M, Kater CE, Auchus RJ. Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2004;89:49–60.
219. Geller DH, Auchus RJ, Mendonca BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. *Nat Genet*. 1997;17:201–205.
220. Geller DH, Auchus RJ, Miller WL. P450c17 mutations R347H and R358Q selectively disrupt 17,20-lyase activity by disrupting interactions with P450 oxidoreductase and cytochrome b5. *Mol Endocrinol*. 1999;13:167–175.
221. Hershkovitz E, Parvari R, Wudy SA, et al. Homozygous mutation G539R in the gene for P450 oxidoreductase in a family previously diagnosed as having 17,20-lyase deficiency. *J Clin Endocrinol Metab*. 2008;93:3584–3588.
222. Suzuki T, Sasano H, Takeyama J, Kaneko C, Freije WA, Carr BR, Rainey WE. Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. *Clin Endocrinol (Oxf)*. 2000;53:739–747.
223. Giordano SJ, Kaftory A, Steggle AW. A splicing mutation in the cytochrome b5 gene from a patient with congenital methemoglobinemia and pseudohermaphroditism. *Hum Genet*. 1994;93:568–570.

224. Kok RC, Timmerman MA, Wolffenbuttel KP, Drop SL, de Jong FH. Isolated 17,20-lyase deficiency due to the cytochrome b5 mutation W27X. *J Clin Endocrinol Metab.* 2010;95:994–999.
225. Idkowiak J, Randell T, Dhir V, Patel P, Shackleton CH, Taylor NF, et al. A missense mutation in the human cytochrome b5 gene causes 46,XY disorder of sex development due to true isolated 17,20 lyase deficiency. *J Clin Endocrinol Metab.* 2012;97:E465–E475.
226. Pandey AV, Miller WL. Regulation of 17,20 lyase activity by cytochrome b5 and by serine phosphorylation of P450c17. *J Biol Chem.* 2005;280:13265–13271.
227. Zhang LH, Rodriguez H, Ohno S, Miller WL. Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. *Proc Natl Acad Sci U S A.* 1995;92:10619–10623.
228. Fluck CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. *Nat Genet.* 2004;36:228–230.
229. Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, et al. Diversity and function of mutations in P450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis. *Am J Hum Genet.* 2005;76:729–749.
230. Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, et al. Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab.* 2005;90:414–426.
231. Scott RR, Miller WL. Genetic and clinical features of P450 oxidoreductase deficiency. *Horm Res.* 2008;69:266–275.
232. Krone N, Reisch N, Idkowiak J, Dhir V, Ivison HE, Hughes BA, et al. Genotype-phenotype analysis in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *J Clin Endocrinol Metab.* 2012;97: E257–267.
233. Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I, et al. Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. *J Clin Endocrinol Metab.* 2006;91:2643–2649.
234. Laue K, Pogoda HM, Daniel PB, van Haeringen A, Alanay Y, von Ameln S, et al. Craniosynostosis and multiple skeletal anomalies in humans and zebrafish result from a defect in the localized degradation of retinoic acid. *Am J Hum Genet.* 2011;89:595–606.
235. Sahakitrungruang T, Huang N, Tee MK, Agrawal V, Russell WE, Crock P, et al. Clinical, genetic, and enzymatic characterization of P450 oxidoreductase deficiency in four patients. *J Clin Endocrinol Metab.* 2009;94:4992–5000.
236. Miller WL, Agrawal V, Sandee D, Tee MK, Huang N, Choi JH, et al. Consequences of POR mutations and polymorphisms. *Mol Cell Endocrinol.* 2011;336:174–179.
237. Pandey AV, Fluck CE. NADPH P450 oxidoreductase: structure, function, and pathology of diseases. *Pharmacol Ther.* 2013;138:229–254.
238. Tomalik-Scharte D, Maiter D, Kirchheiner J, Ivison HE, Fuhr U, Arlt W. Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency. *Eur J Endocrinol.* 2010;163:919–924.
239. Dhir V, Ivison HE, Krone N, Shackleton CH, Doherty AJ, Stewart PM, Arlt W. Differential inhibition of CYP17A1 and CYP21A2 activities by the P450 oxidoreductase mutant A287P. *Mol Endocrinol.* 2007;21:1958–1968.
240. Pandey AV, Kempna P, Hofer G, Mullis PE, Fluck CE. Modulation of human CYP19A1 activity by mutant NADPH P450 oxidoreductase. *Mol Endocrinol.* 2007;21:2579–2595.
241. Huang N, Agrawal V, Giacomini KM, Miller WL. Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *Proc Natl Acad Sci U S A.* 2008;105:1733–1738.
242. Morel Y, Miller WL. Clinical and molecular genetics of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Adv Hum Genet.* 1991;20:1–68.
243. Speiser PW, White PC. Congenital adrenal hyperplasia. *N Engl J Med.* 2003;349:776–788.
244. Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, et al. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *N Engl J Med.* 2000;343:1362–1368.
245. Kamrath C, Wettstaedt L, Boettcher C, Hartmann MF, Wudy SA. The urinary steroidome of treated children with classic 21-hydroxylase deficiency. *J Steroid Biochem Mol Biol.* 2017;165:396–406.
246. Krone N, Braun A, Roscher AA, Knorr D, Schwarz HP. Predicting phenotype in steroid 21-hydroxylase deficiency? Comprehensive genotyping in 155 unrelated, well defined patients from southern Germany. *J Clin Endocrinol Metab.* 2000;85:1059–1065.
247. Speiser PW, Dupont J, Zhu D, Serrat J, Buegeleisen M, Tusie-Luna MT, et al. Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Invest.* 1992;90:584–595.
248. Martinez-Aguayo A, Rocha A, Rojas N, Garcia C, Parra R, Lagos M, et al. Testicular adrenal rest tumors and Leydig and Sertoli cell function in boys with classical congenital adrenal hyperplasia. *J Clin Endocrinol Metab.* 2007;92:4583–4589.
249. Amor M, Parker KL, Globerman H, New MI, White PC. Mutation in the CYP21B gene (Ile-172→Asn) causes steroid 21-hydroxylase deficiency. *Proc Natl Acad Sci U S A.* 1988;85:1600–1604.
250. Urabe K, Kimura A, Harada F, Iwanaga T, Sasazuki T. Gene conversion in steroid 21-hydroxylase genes. *Am J Hum Genet.* 1990;46:1178–1186.
251. Chiou SH, Hu MC, Chung BC. A missense mutation at Ile172→Asn or Arg356→Trp causes steroid 21-hydroxylase deficiency. *J Biol Chem.* 1990;265:3549–3552.
252. Hannah-Shmouni F, Morissette R, Sinaii N, Elman M, Prezant TR, et al. Revisiting the prevalence of nonclassical congenital adrenal hyperplasia in US Ashkenazi Jews and Caucasians. *Genet Med.* 2017;19:1276–1279.
253. Deneuve C, Tardy V, Dib A, Mornet E, Billaud L, Charron D, et al. Phenotype-genotype correlation in 56 women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2001;86:207–213.
254. Speiser PW, Knochenhauer ES, Dewailly D, Fruzzetti F, Marcondes JA, Azziz R. A multicenter study of women with nonclassical congenital adrenal hyperplasia: relationship between genotype and phenotype. *Mol Genet Metab.* 2000;71:527–534.
255. Tusie-Luna MT, Traktman P, White PC. Determination of functional effects of mutations in the steroid 21-hydroxylase gene (CYP21) using recombinant vaccinia virus. *J Biol Chem.* 1990;265:20916–20922.
256. Wu DA, Chung BC. Mutations of P450c21 (steroid 21-hydroxylase) at Cys428, Val281, and Ser268 result in complete, partial, or no loss of enzymatic activity, respectively. *J Clin Invest.* 1991;88:519–523.
257. Therrell Jr BL, Berenbaum SA, Manter-Kapanke V, Simmank J, Korman K, Prentice L, et al. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics.* 1998;101:583–590.
258. Witchel SF. Nonclassical congenital adrenal hyperplasia. *Curr Opin Endocrinol Diabetes Obes.* 2012;19:151–158.
259. Bristow J, Gitelman SE, Tee MK, Staels B, Miller WL. Abundant adrenal-specific transcription of the human P450c21A “pseudogene.” *J Biol Chem.* 1993;268:12919–12924.
260. Chang SF, Chung BC. Difference in transcriptional activity of two homologous CYP21A genes. *Mol Endocrinol.* 1995;9:1330–1336.
261. Gitelman SE, Bristow J, Miller WL. Mechanism and consequences of the duplication of the human C4/P450c21/gene X locus. *Mol Cell Biol.* 1992;12:2124–2134.
262. Wijesuriya SD, Zhang G, Dardis A, Miller WL. Transcriptional regulatory elements of the human gene for cytochrome P450c21 (steroid 21-hydroxylase) lie within intron 35 of the linked C4B gene. *J Biol Chem.* 1999;274:38097–38106.
263. Gomez-Escobar N, Chou CF, Lin WW, Hsieh SL, Campbell RD. The G11 gene located in the major histocompatibility complex encodes a novel nuclear serine/threonine protein kinase. *J Biol Chem.* 1998;273:30954–30960.
264. Bristow J, Tee MK, Gitelman SE, Mellon SH, Miller WL. Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. *J Cell Biol.* 1993;122:265–278.
265. Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. *Dev Dyn.* 1995;203:491–504.

266. Morel Y, Bristow J, Gitelman SE, Miller WL. Transcript encoded on the opposite strand of the human steroid 21-hydroxylase/complement component C4 gene locus. *Proc Natl Acad Sci U S A*. 1989;86:6582–6586.
267. Valcourt U, Alcaraz LB, Exposito JY, Lethias C, Bartholin L. Tenascin-X: beyond the architectural function. *Cell Adh Migr*. 2015;9:154–165.
268. Tee MK, Thomson A, Bristow J, Miller WL. Sequences promoting the transcription of the human XA gene overlapping P450c21A correctly predict the presence of a novel, adrenal-specific, truncated form of Tenascin-X. *Genomics*. 1995;28:171–178.
269. Endo T, Ariga H, Matsumoto K. Truncated form of tenascin-X, XB-S, interacts with mitotic motor kinesin Eg5. *Mol Cell Biochem*. 2009;320:53–66.
270. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, et al. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. *Nat Genet*. 1997;17:104–108.
271. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen IM, et al. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. *N Engl J Med*. 2001;345:1167–1175.
272. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, et al. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. *Am J Hum Genet*. 2003;73:214–217.
273. Miller WL, Merke DP. Tenascin-X, Congenital Adrenal Hyperplasia, and the CAH-X Syndrome. *Horm Res Paediatr*. 2018;89:352–361.
274. Grosse SD, Van Vliet G. How many deaths can be prevented by newborn screening for congenital adrenal hyperplasia? *Horm Res*. 2007;67:284–291.
275. van der Kamp HJ, Oudshoorn CG, Elvers BH, van Baarle M, Otten BJ, et al. Cutoff levels of 17 α -hydroxyprogesterone in neonatal screening for congenital adrenal hyperplasia should be based on gestational age rather than on birth weight. *J Clin Endocrinol Metab*. 2005;90:3904–3907.
276. Janzen N, Peter M, Sander S, Steuerwald U, Terhardt M, Holtkamp U, Sander J. Newborn screening for congenital adrenal hyperplasia: additional steroid profile using liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab*. 2007;92:2581–2589.
277. Esteban NV, Loughlin T, Yergey AL, Zawadzki JK, Booth JD, Winterer JC, Loriaux DL. Daily cortisol production rate in man determined by stable isotope dilution/mass spectrometry. *J Clin Endocrinol Metab*. 1991;72:39–45.
278. Zumoff B, Fukushima DK, Hellman L. Intercomparison of four methods for measuring cortisol production. *J Clin Endocrinol Metab*. 1974;38:169–175.
279. Miller WL. Dexamethasone treatment of congenital adrenal hyperplasia in utero: an experimental therapy of unproven safety. *J Urol*. 1999;162:537–540.
280. Miller WL, Witchel SF. Prenatal treatment of congenital adrenal hyperplasia: Risks outweigh benefits. *Am J Obstet Gynecol*. 2013;354–359.
281. Partsch CJ, Sippell WG, MacKenzie IZ, Aynsley-Green A. The steroid hormonal milieu of the undisturbed human fetus and mother at 16–20 weeks gestation. *J Clin Endocrinol Metab*. 1991;73:969–974.
282. Kari MA, Raivio KO, Stenman UH, Voutilainen R. Serum cortisol, dehydroepiandrosterone sulfate, and steroid-binding globulins in preterm neonates: effect of gestational age and dexamethasone therapy. *Pediatr Res*. 1996;40:319–324.
283. Witchel SF, Miller WL. Prenatal treatment of congenital adrenal hyperplasia—not standard of care. *J Genet Couns*. 2012;21:615–624.
284. Hirvikoski T, Nordenstrom A, Lindholm T, Lindblad F, Ritzen EM, Wedell A, Lajic S. Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *J Clin Endocrinol Metab*. 2007;92:542–548.
285. Hirvikoski T, Nordenstrom A, Lindholm T, Lindblad F, Ritzen EM, Lajic S. Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *Eur J Endocrinol*. 2008;159:309–316.
286. Hirvikoski T, Lindholm T, Lajic S, Nordenstrom A. Gender role behaviour in prenatally dexamethasone-treated children at risk for congenital adrenal hyperplasia—a pilot study. *Acta Paediatr*. 2011;100. e112–119.
287. Hirvikoski T, Nordenstrom A, Wedell A, Ritzen M, Lajic S. Prenatal dexamethasone treatment of children at risk for congenital adrenal hyperplasia: the Swedish experience and standpoint. *J Clin Endocrinol Metab*. 2012;97:1881–1883.
288. Mulatero P, Curnow KM, Aupetit-Faisant B, Foekling M, Gomez-Sanchez C, Veglio F, et al. Recombinant CYP11B genes encode enzymes that can catalyze conversion of 11-deoxycortisol to cortisol, 18-hydroxycortisol, and 18-oxocortisol. *J Clin Endocrinol Metab*. 1998;83:3996–4001.
289. Holcombe JH, Keenan BS, Nichols BL, Kirkland RT, Clayton GW. Neonatal salt loss in the hypertensive form of congenital adrenal hyperplasia. *Pediatrics*. 1980;65:777–781.
290. Chabraoui L, Abid F, Menassa R, Gaouzi A, El Hessni A, Morel Y. Three novel CYP11B1 mutations in congenital adrenal hyperplasia due to steroid 11 β -hydroxylase deficiency in a moroccan population. *Horm Res Paediatr*. 2010;74:182–189.
291. Parajes S, Loidi L, Reisch N, Dhir V, Rose IT, Hampel R, et al. Functional consequences of seven novel mutations in the CYP11B1 gene: four mutations associated with nonclassic and three mutations causing classic 11 β -hydroxylase deficiency. *J Clin Endocrinol Metab*. 2010;95:779–788.
292. Khattab A, Haider S, Kumar A, Dhawan S, Alam D, Romero R, et al. Clinical, genetic, and structural basis of congenital adrenal hyperplasia due to 11 β -hydroxylase deficiency. *Proc Natl Acad Sci U S A*. 2017;114:E1933–E1940.
293. Kandemir N, Yordam N. Congenital adrenal hyperplasia in Turkey: a review of 273 patients. *Acta Paediatr*. 1997;86:22–25.
294. Bas F, Toksoy G, Ergun-Longmire B, Uyguner ZO, Abali ZY, Poyrazoglu S, et al. Prevalence, clinical characteristics and long-term outcomes of classical 11 β -hydroxylase deficiency (11BOHD) in Turkish population and novel mutations in CYP11B1 gene. *J Steroid Biochem Mol Biol*. 2018;181:88–97.
295. Kandemir N, Yilmaz DY, Gonc EN, Ozon A, Alikasifoglu A, Dursun A, Ozgul RK. Novel and prevalent CYP11B1 gene mutations in Turkish patients with 11 β -hydroxylase deficiency. *J Steroid Biochem Mol Biol*. 2017;165:57–63.
296. Mooij CF, Parajes S, Rose IT, Taylor AE, Bayraktaroglu T, Wass JA, et al. Characterization of the molecular genetic pathology in patients with 11 β -hydroxylase deficiency. *Clin Endocrinol (Oxf)*. 2015;83:629–635.
297. Reisch N, Hogler W, Parajes S, Rose IT, Dhir V, Gotzinger J, et al. A diagnosis not to be missed: nonclassic steroid 11 β -hydroxylase deficiency presenting with premature adrenarche and hirsutism. *J Clin Endocrinol Metab*. 2013;98. E1620–E1625.
298. Joehrer K, Geley S, Strasser-Wozak EM, Azziz R, Wollmann HA, Schmitt K, et al. CYP11B1 mutations causing non-classic adrenal hyperplasia due to 11 β -hydroxylase deficiency. *Hum Mol Genet*. 1997;6:1829–1834.
299. Ullick S, Wang JZ, Morton DH. The biochemical phenotypes of two inborn errors in the biosynthesis of aldosterone. *J Clin Endocrinol Metab*. 1992;74:1415–1420.
300. Kayes-Wandover KM, Schindler RE, Taylor HC, White PC. Type 1 aldosterone synthase deficiency presenting in a middle-aged man. *J Clin Endocrinol Metab*. 2001;86:1008–1012.
301. Pascoe L, Curnow KM, Slutsker L, Rosler A, White PC. Mutations in the human CYP11B2 (aldosterone synthase) gene causing corticosterone methyl oxidase II deficiency. *Proc Natl Acad Sci U S A*. 1992;89:4996–5000.
302. Portrat-Doyen S, Tourniaire J, Richard O, Mulatero P, Aupetit-Faisant B, Curnow KM, et al. Isolated aldosterone synthase deficiency caused by simultaneous E198D and V386A mutations in the CYP11B2 gene. *J Clin Endocrinol Metab*. 1998;83:4156–4161.
303. Zhang GG, Rodriguez H, Fardella CE, Harris DA, Miller WL. Mutation T318M in the CYP11B2 gene encoding P450c11AS (aldosterone synthase) causes corticosterone methyl oxidase II deficiency. *Am J Hum Genet*. 1995;57:1037–1043.
304. Dluhy RG, Lifton RP. Glucocorticoid-remediable aldosteronism. *J Clin Endocrinol Metab*. 1999;84:4341–4344.
305. Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, et al. A chimaeric 11 β -hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*. 1992;355:262–265.

306. Pascoe L, Curnow KM, Slutsker L, et al. Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossovers between CYP11B1 and CYP11B2. *Proc Natl Acad Sci U S A*. 1992;89:8327–8331.
307. Fardella CE, Mosso L, Gomez-Sanchez C, Cortes P, Soto J, Gomez L, et al. Primary hyperaldosteronism in essential hypertensives: prevalence, biochemical profile, and molecular biology. *J Clin Endocrinol Metab*. 2000;85:1863–1867.
308. Furst-Recktenwald S, Dorr HG, Quinkler M, Dotsch J, Stewart PM. Is there sufficient evidence to consider the use of 11 β -hydroxysteroid dehydrogenase type 1 inhibition in children? *Clin Endocrinol (Oxf)*. 2012;77:159–168.
309. Lawson AJ, Walker EA, Lavery GG, Bujalska IJ, Hughes B, Arlt W, et al. Cortisone-reductase deficiency associated with heterozygous mutations in 11 β -hydroxysteroid dehydrogenase type 1. *Proc Natl Acad Sci U S A*. 2011;108:4111–4116.
310. Lavery GG, Walker EA, Tiganescu A, Ride JP, Shackleton CH, Tomlinson JW, et al. Steroid biomarkers and genetic studies reveal inactivating mutations in hexose-6-phosphate dehydrogenase in patients with cortisone reductase deficiency. *J Clin Endocrinol Metab*. 2008;93:3827–3832.
311. Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, et al. Mutations in the genes encoding 11 β -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat Genet*. 2003;34:434–439.
312. San Millan JL, Botella-Carretero JJ, Alvarez-Blasco F, Luque-Ramirez M, Sancho J, Moghetti P, Escobar-Morreale HF. A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11 β -hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2005;90:4157–4162.
313. White PC. Genotypes at 11 β -hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *J Clin Endocrinol Metab*. 2005;90:5880–5883.
314. Lavery GG, Idkowiak J, Sherlock M, Bujalska I, Ride JP, Saqib K, et al. Novel H6PDH mutations in two girls with premature adrenarche: 'apparent' and 'true' CRD can be differentiated by urinary steroid profiling. *Eur J Endocrinol*. 2013;168: K19–26.
315. Quinkler M, Stewart PM. Hypertension and the cortisol-cortisone shuttle. *J Clin Endocrinol Metab*. 2003;88:2384–2392.
316. Palermo M, Quinkler M, Stewart PM. Apparent mineralocorticoid excess syndrome: an overview. *Arq Bras Endocrinol Metabol*. 2004;48:687–696.
317. Baranowski ES, Arlt W, Idkowiak J. Monogenic disorders of adrenal steroidogenesis. *Horm Res Paediatr*. 2018;89:292–310.
318. Idkowiak J, Taylor AE, Subtil S, O'Neil DM, Vijzelaar R, Dias RP, et al. Steroid sulfatase deficiency and androgen activation before and after puberty. *J Clin Endocrinol Metab*. 2016;101:2545–2553.
319. Ahmad W, Brancolini V, ul Faiyaz MF, Lam H, ul Haque S, Haider M, et al. A locus for autosomal recessive hypodontia with associated dental anomalies maps to chromosome 16q12.1. *Am J Hum Genet*. 1998;62:987–991.
320. Faiyaz ul Haque M, King LM, Krakow D, Cantor RM, Rusiniak ME, Swank RT, et al. Mutations in orthologous genes in human spondyloepimetaphyseal dysplasia and the brachymorphic mouse. *Nat Genet*. 1998;20:157–162.
321. Iida A, Simsek-Kiper PO, Mizumoto S, Hoshino T, Elcioglu N, Horemuzova E, et al. Clinical and radiographic features of the autosomal recessive form of brachyolmia caused by PAPSS2 mutations. *Hum Mutat*. 2013;34:1381–1386.
322. Miyake N, Elcioglu NH, Iida A, Isguven P, Dai J, Murakami N, et al. PAPSS2 mutations cause autosomal recessive brachyolmia. *J Med Genet*. 2012;49:533–538.
323. Tuysuz B, Yilmaz S, Gul E, et al. Spondyloepimetaphyseal dysplasia Pakistani type: expansion of the phenotype. *Am J Hum Genet*. 2013;161A:1300–1308.
324. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, et al. Diagnosis and treatment of primary adrenal insufficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2016;101:364–389.
325. Lin L, Gu WX, Ozisik G, et al. Analysis of DAX1 (NR0B1) and steroidogenic factor-1 (NR5A1) in children and adults with primary adrenal failure: ten years' experience. *J Clin Endocrinol Metab*. 2006;91:3048–3054.
326. Lin L, Achermann JC. Inherited adrenal hypoplasia: not just for kids! *Clin Endocrinol (Oxf)*. 2004;60:529–537.
327. Adem PV, Montgomery CP, Husain AN, Koogler TK, Arangelovich V, Humilier M, et al. Staphylococcus aureus sepsis and the Waterhouse-Friderichsen syndrome in children. *N Engl J Med*. 2005;353:1245–1251.
328. Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev*. 2002;23:327–364.
329. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature*. 2003;423:506–511.
330. Ghaderi M, Gambelunghe G, Tortoioli C, Brozzetti A, Jatta K, Gharizadeh B, et al. MHC2TA single nucleotide polymorphism and genetic risk for autoimmune adrenal insufficiency. *J Clin Endocrinol Metab*. 2006;91:4107–4111.
331. Husebye ES, Anderson MS, Kampe O. Autoimmune polyendocrine syndromes. *N Engl J Med*. 2018;378:2543–2544.
332. Bruserud O, Oftedal BE, Landegren N, Erichsen MM, Bratland E, Lima K, et al. A Longitudinal follow-up of autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab*. 2016;101:2975–2983.
333. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*. 2006;91:2843–2850.
334. Finnish-German AC. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet*. 1997;17:399–403.
335. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, et al. Positional cloning of the APECED gene. *Nat Genet*. 1997;17:393–398.
336. Villaseñor J, Benoist C, Mathis D. AIRE and APECED: molecular insights into an autoimmune disease. *Immunol Rev*. 2005;204:156–164.
337. Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. *J Pediatr*. 1982;100:731–737.
338. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ, et al. Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: An international multicenter retrospective study. *J Allergy Clin Immunol*. 2018;141:1036–1049. e1035.
339. June CH, Warshauer JT, Bluestone JA. Is autoimmunity the Achilles' heel of cancer immunotherapy? *Nat Med*. 2017;23:540–547.
340. Barros-Sousa R, Ott PA, Hodi FS, Kaiser UB, Tolaney SM, Min L. Endocrine dysfunction induced by immune checkpoint inhibitors: Practical recommendations for diagnosis and clinical management. *Cancer*. 2018;124:1111–1121.
341. Tabarin A, Achermann JC, Recan D, Bex V, Bertagna X, Christin-Maitre S, et al. A novel mutation in DAX1 causes delayed-onset adrenal insufficiency and incomplete hypogonadotropic hypogonadism. *J Clin Invest*. 2000;105:321–328.
342. Suntharalingham JP, Buonocore F, Duncan AJ, Achermann JC. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. *Best Pract Res Clin Endocrinol Metab*. 2015;29:607–619.
343. Domenico S, Machado AZ, Ferreira FM, Ferraz-de-Souza B, Lerario AM, Lin L, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. *Birth Defects Res C Embryo Today*. 2016;108:309–320.
344. Fluck CE. Mechanisms in endocrinology: update on pathogenesis of primary adrenal insufficiency: beyond steroid enzyme deficiency and autoimmune adrenal destruction. *Eur J Endocrinol*. 2015;177:R99–R111.
345. Tullio-Pelet A, Salomon R, Hadj-Rabia S, Mugnier C, de Laet MH, Chaouachi B, et al. Mutant WD-repeat protein in triple-A syndrome. *Nat Genet*. 2000;26:332–325.
346. Handschug K, Sperling S, Yoon SJ, Hennig S, Clark AJ, Huebner A. Triple A syndrome is caused by mutations in AAAS, a new WD-repeat protein gene. *Hum Mol Genet*. 2001;10:283–290.

347. Storr, H.L., Kind, B., Parfitt, D.A., Chapple, J.P., Lorenz, M., Koehler, K., et al. Deficiency of ferritin heavy-chain nuclear import in triple A syndrome implies nuclear oxidative damage as the primary disease mechanism. *Mol Endocrinol*. 2009;23:2086–2094.
348. Prasad R, Metherell LA, Clark AJ, Storr HL. Deficiency of ALADIN impairs redox homeostasis in human adrenal cells and inhibits steroidogenesis. *Endocrinology*. 2013;154:3209–3218.
349. Carvalho S, Ribeiro SA, Arocena M, Kasciukovic T, Temme A, Koehler K, et al. The nucleoporin ALADIN regulates Aurora A localization to ensure robust mitotic spindle formation. *Mol Biol Cell*. 2015;26:3424–3438.
350. Vilain E, Le Merrer M, Lecoindre C, Desangles F, Kay MA, Maroteaux P, McCabe ER. IMAGE, a new clinical association of intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies. *J Clin Endocrinol Metab*. 1999;84:4335–4340.
351. Bergada I, Del Rey G, Lapunzina P, Bergada C, Fellous M, Copelli S. Familial occurrence of the IMAGE association: additional clinical variants and a proposed mode of inheritance. *J Clin Endocrinol Metab*. 2005;90:3186–3190.
352. Arboleda VA, Lee H, Parnaik R, Fleming A, Banerjee A, Ferraz-de-Souza B, et al. Mutations in the PCNA-binding domain of CDKN1C cause IMAGE syndrome. *Nat Genet*. 2012;44:788–792.
353. Hatada I, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, et al. An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. *Nat Genet*. 1996;14:171–173.
354. Eggermann T, Binder G, Brioude F, Maher ER, Lapunzina P, Cubellis MV, et al. CDKN1C mutations: two sides of the same coin. *Trends Mol Med*. 2014;20:614–622.
355. Brioude F, Oliver-Petit I, Blaise A, Praz F, Rossignol S, Le Jule M, et al. CDKN1C mutation affecting the PCNA-binding domain as a cause of familial Russell Silver syndrome. *J Med Genet*. 2013;50:823–830.
356. Narumi S, Amano N, Ishii T, Katsumata N, Muroya K, Adachi M, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. *Nat Genet*. 2016;48:792–797.
357. Buonocore F, Kuhnen P, Suntharalingham JP, Del Valle I, Digweed M, Stachelscheid H, et al. Somatic mutations and progressive monosomy modify SAMD9-related phenotypes in humans. *J Clin Invest*. 2017;127:1700–1713.
358. Hughes CR, Guasti L, Meimaridou E, Chuang CH, Schimenti JC, King PJ, et al. MCM4 mutation causes adrenal failure, short stature, and natural killer cell deficiency in humans. *J Clin Invest*. 2012;122:814–820.
359. Clark AJ, Metherell LA, Cheetham ME, Huebner A. Inherited ACTH insensitivity illuminates the mechanisms of ACTH action. *Trends Endocrinol Metab*. 2005;16:451–457.
360. Novoselova TV, Chan LF, Clark AJL. Pathophysiology of melanocortin receptors and their accessory proteins. *Best Pract Res Clin Endocrinol Metab*. 2018;32:93–106.
361. Swords FM, Noon LA, King PJ, Clark AJ. Constitutive activation of the human ACTH receptor resulting from a synergistic interaction between two naturally occurring missense mutations in the MC2R gene. *Mol Cell Endocrinol*. 2004;213:149–154.
362. Weber A, Clark AJ, Perry LA, Honour JW, Savage MO. Diminished adrenal androgen secretion in familial glucocorticoid deficiency implicates a significant role for ACTH in the induction of adrenarche. *Clin Endocrinol (Oxf)*. 1997;46:431–437.
363. Webb TR, Clark AJ. The melanocortin 2 receptor accessory proteins. *Mol Endocrinol*. 2010;24:475–484.
364. Rouault AAJ, Srinivasan DK, Yin TC, Lee AA, Sebarg JA. Melanocortin receptor accessory proteins (MRAPs): functions in the melanocortin system and beyond. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863:2462–2467.
365. Metherell LA, Chapple JP, Cooray S, David A, Becker C, Ruschendorf F, et al. Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2. *Nat Genet*. 2005;37:166–170.
366. Meimaridou E, Kowalczyk J, Guasti L, Hughes CR, Wagner F, Frommolt P, et al. Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. *Nat Genet*. 2012;44:740–742.
367. Chan LF, Campbell DC, Novoselova TV, Clark AJ, Metherell LA. Whole-exome sequencing in the differential diagnosis of primary adrenal insufficiency in children. *Front Endocrinol (Lausanne)*. 2015;6:113.
368. Prasad R, Chan LF, Hughes CR, Kaski JP, Kowalczyk JC, Savage MO, et al. Thioredoxin Reductase 2 (TXNRD2) mutation associated with familial glucocorticoid deficiency (FGD). *J Clin Endocrinol Metab*. 2014;99:E1556–E1563.
369. Lichtenberg MJ, Kemp S, Sarde CO, van Geel BM, Kleijer WJ, Barth PG, et al. Spectrum of mutations in the gene encoding the adrenoleukodystrophy protein. *Am J Hum Genet*. 1995;56:44–50.
370. Watkins PA, Gould SJ, Smith MA, Braiterman LT, Wei HM, Kok F, et al. Altered expression of ALDP in X-linked adrenoleukodystrophy. *Am J Hum Genet*. 1995;57:292–301.
371. Shimozawa N. Molecular and clinical findings and diagnostic flowchart of peroxisomal diseases. *Brain Dev*. 2011;33:770–776.
372. Moser HW, Raymond GV, Dubey P. Adrenoleukodystrophy: new approaches to a neurodegenerative disease. *JAMA*. 2005;294:3131–3134.
373. McGuinness MC, Lu JF, Zhang HP, Dong GX, Heinzer AK, Watkins PA, et al. Role of ALDP (ABCD1) and mitochondria in X-linked adrenoleukodystrophy. *Mol Cell Biol*. 2003;23:744–753.
374. Kemp S, Wanders RJ. X-linked adrenoleukodystrophy: very long-chain fatty acid metabolism, ABC half-transporters and the complicated route to treatment. *Mol Genet Metab*. 2007;90:268–276.
375. Watkins PA, Naidu S, Moser HW. Adrenoleukodystrophy: biochemical procedures in diagnosis, prevention and treatment. *J Inher Metab Dis*. 1987;10(Suppl 1):46–53.
376. Moser HW, Moser AE, Singh I, O'Neill BP. Adrenoleukodystrophy: survey of 303 cases: biochemistry, diagnosis, and therapy. *Ann Neurol*. 1984;16:628–641.
377. Moser HW. Adrenoleukodystrophy. *Curr Opin Neurol*. 1995;8:221–226.
378. Laureti S, Casucci G, Santeusano F, Angeletti G, Aubourg P, Brunetti P. X-linked adrenoleukodystrophy is a frequent cause of idiopathic Addison's disease in young adult male patients. *J Clin Endocrinol Metab*. 1996;81:470–474.
379. Sadeghi-Nejad A, Senior B. Adrenomyeloneuropathy presenting as Addison's disease in childhood. *N Engl J Med*. 1990;322:13–16.
380. Huffnagel IC, Laheji FK, Aziz-Bose R, Tritos NA, Marino R, Linthorst GE, et al. The natural history of adrenal insufficiency in X-linked adrenoleukodystrophy: an international collaboration. *J Clin Endocrinol Metab*. 2018;104(1):118–126.
381. Peters C, Charnas LR, Tan Y, Ziegler RS, Shapiro EG, DeFor T, et al. Cerebral X-linked adrenoleukodystrophy: the international hematopoietic cell transplantation experience from 1982 to 1999. *Blood*. 2004;104:881–888.
382. Engelen M, Ofman R, Dijkgraaf MG, Hijzen M, van der Wardt LA, van Geel BM, et al. Lovastatin in X-linked adrenoleukodystrophy. *N Engl J Med*. 2010;362:276–277.
383. Steinberg S, Chen L, Wei L, Moser A, Moser H, Cutting G, Braverman N. The PEX Gene Screen: molecular diagnosis of peroxisome biogenesis disorders in the Zellweger syndrome spectrum. *Mol Genet Metab*. 2004;83:252–263.
384. Wanders RJ, Waterham HR. Peroxisomal disorders I: biochemistry and genetics of peroxisome biogenesis disorders. *Clin Genet*. 2005;67:107–133.
385. Mandel H, Korman SH. Phenotypic variability (heterogeneity) of peroxisomal disorders. *Adv Exp Med Biol*. 2003;544:9–30.
386. Correa-Cerro LS, Porter FD. β -hydroxysterol D7-reductase and the Smith-Lemli-Opitz syndrome. *Mol Genet Metab*. 2005;84:112–126.
387. DeBarber AE, Eroglu Y, Merckens LS, Pappu AS, Steiner RD. Smith-Lemli-Opitz syndrome. *Expert Rev Mol Med*. 2011;13. e24.
388. Andersson HC, Frentz J, Martinez JE, Tuck-Muller CM, Bellizaire J. Adrenal insufficiency in Smith-Lemli-Opitz syndrome. *Am J Med Genet*. 1999;82:382–384.
389. Nicolino M, Ferlin T, Forest M, Godinot C, Carrier H, David M, et al. Identification of a large-scale mitochondrial deoxyribo-nucleic acid deletion in endocrinopathies and deafness: report of two unrelated cases with diabetes mellitus and adrenal insufficiency, respectively. *J Clin Endocrinol Metab*. 1997;82:3063–3067.

390. Chow J, Rahman J, Achermann JC, Dattani MT, Rahman S. Mitochondrial disease and endocrine dysfunction. *Nat Rev Endocrinol*. 2017;13:92–104.
391. Lovric S, Goncalves S, Gee HY, Oskouian B, Srinivas H, Choi WI, et al. Mutations in sphingosine-1-phosphate lyase cause nephrosis with ichthyosis and adrenal insufficiency. *J Clin Invest*. 2017;127:912–928.
392. Prasad R, Hadjidemetriou I, Maharaj A, Meimaridou E, Buonocore F, Saleem M, et al. Sphingosine-1-phosphate lyase mutations cause primary adrenal insufficiency and steroid-resistant nephrotic syndrome. *J Clin Invest*. 2017;127:942–953.
393. Atkinson D, Nikodinovic Glumac J, Asselbergh B, Ermanoska B, Blouquet D, Steiner R, et al. Sphingosine 1-phosphate lyase deficiency causes Charcot-Marie-Tooth neuropathy. *Neurology*. 2017;88:533–542.
394. Bamborschke D, Pergande M, Becker K, Koerber F, Dotsch J, Vierzig A, et al. A novel mutation in sphingosine-1-phosphate lyase causing congenital brain malformation. *Brain Dev*. 2018;40:480–483.
395. Janecke AR, Xu R, Steichen-Gersdorf E, Waldegger S, Entenmann A, Giner T, et al. Deficiency of the sphingosine-1-phosphate lyase SGPL1 is associated with congenital nephrotic syndrome and congenital adrenal calcifications. *Hum Mutat*. 2017;38:365–372.
396. Linhares ND, Arantes RR, Araujo SA, Pena SDJ. Nephrotic syndrome and adrenal insufficiency caused by a variant in SGPL1. *Clin Kidney J*. 2018;11:462–467.
397. DeVile CJ, Grant DB, Hayward RD, Stanhope R. Growth and endocrine sequelae of craniopharyngioma. *Arch Dis Child*. 1996;75:108–114.
398. Karavitaki N, Brufani C, Warner JT, Adams CB, Richards P, Ansoorge O, et al. Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. *Clin Endocrinol (Oxf)*. 2005;62:397–409.
399. Rose SR, Danish RK, Kearney NS, Schreiber RE, Lustig RH, Burghen GA, Hudson MM. ACTH deficiency in childhood cancer survivors. *Pediatr Blood Cancer*. 2005;45:808–813.
400. Mendonca BB, Osorio MG, Latronico AC, Estefan V, Lo LS, Arnhold JJ. Longitudinal hormonal and pituitary imaging changes in two females with combined pituitary hormone deficiency due to deletion of A301,G302 in the PROP1 gene. *J Clin Endocrinol Metab*. 1999;84:942–945.
401. Pernasetti F, Toledo SP, Vasilyev VV, Hayashida CY, Cogan JD, Ferrari C, et al. Impaired adrenocorticotropin-adrenal axis in combined pituitary hormone deficiency caused by a two-base pair deletion (301-302delAG) in the prophet of Pit-1 gene. *J Clin Endocrinol Metab*. 2000;85:390–397.
402. Lamolet B, Pulichino AM, Lamonerie T, Gauthier Y, Brue T, Enjalbert A, Drouin J. A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell*. 2001;104:849–859.
403. Metherell LA, Savage MO, Dattani M, Walker J, Clayton PE, Farooqi IS, Clark AJ. TPIT mutations are associated with early-onset, but not late-onset isolated ACTH deficiency. *Eur J Endocrinol*. 2004;151:463–465.
404. Pulichino AM, Vallette-Kasic S, Couture C, Gauthier Y, Brue T, David M, et al. Human and mouse TPIT gene mutations cause early onset pituitary ACTH deficiency. *Genes Dev*. 2003;17:711–716.
405. Vallette-Kasic S, Brue T, Pulichino A.M., Gueydan, M., Barlier A., David, M., et al. Congenital isolated adrenocorticotropin deficiency: an underestimated cause of neonatal death, explained by TPIT gene mutations. *J Clin Endocrinol Metab*. 2005;90:1323–1331.
406. Couture C, Saveanu A, Barlier A, Carel JC, Fassnacht M, Fluck CE, et al. Phenotypic homogeneity and genotypic variability in a large series of congenital isolated ACTH-deficiency patients with TPIT gene mutations. *J Clin Endocrinol Metab*. 2012;97: E486–E495.
407. Farooqi IS, Jones MK, Evans M, O'Rahilly S, Hodges JR. Triple H syndrome: a novel autoimmune endocrinopathy characterized by dysfunction of the hippocampus, hair follicle, and hypothalamic-pituitary adrenal axis. *J Clin Endocrinol Metab*. 2000;85:2644–2648.
408. Samuels ME, Gallo-Payet N, Pinard S, Hasselmann C, Magne F, Patry L, et al. Isolated glucocorticoid deficiency caused by immunoreactive but biologically inactive ACTH. *Horm Res*. 2012;78(Suppl 1):1.
409. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet*. 1998;19:155–157.
410. Krude H, Biebermann H, Schnabel D, Tansek MZ, Theunissen P, Mullis PE, Gruters A. Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4-10. *J Clin Endocrinol Metab*. 2003;88:4633–4640.
411. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. 1997;16:303–306.
412. Jackson RS, Creemers JW, Farooqi IS, Raffin-Sanson ML, Varro A, Dockray GJ, et al. Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest*. 2003;112:1550–1560.
413. Kuhnen P, Clement K, Wiegand S, Blankenstein O, Gottesdiener K, Martini LL, et al. Proopiomelanocortin deficiency treated with a melanocortin-4 receptor agonist. *N Engl J Med*. 2016;375:240–246.
414. Kannisto S, Korppi M, Remes K, Voutilainen R. Adrenal suppression, evaluated by a low dose adrenocorticotropin test, and growth in asthmatic children treated with inhaled steroids. *J Clin Endocrinol Metab*. 2000;85:652–657.
415. Todd GR, Acerini CL, Ross-Russell R, Zahra S, Warner JT, McCance D. Survey of adrenal crisis associated with inhaled corticosteroids in the United Kingdom. *Arch Dis Child*. 2002;87:457–461.
416. Paton J, Jardine E, McNeill E, Beaton S, Galloway P, Young D, Donaldson M. Adrenal responses to low dose synthetic ACTH (Synacthen) in children receiving high dose inhaled fluticasone. *Arch Dis Child*. 2006;91:808–813.
417. Devoe DJ, Miller WL, Conte FA, Kaplan SL, Grumbach MM, Rosenthal SM, et al. Long-term outcome in children and adolescents after transphenoidal surgery for Cushing's disease. *J Clin Endocrinol Metab*. 1997;82:3196–3202.
418. Joshi, S.M., Hewitt, R.J., Storr, H.L., Rezajooi, K., Ellamushi, H., Grossman, A.B., et al. Cushing's disease in children and adolescents: 20 years of experience in a single neurosurgical center. *Neurosurgery*, 57, 281–285.
419. Kanter AS, Diallo AO, Jane Jr JA, Sheehan JP, Asthagiri AR, et al. Single-center experience with pediatric Cushing's disease. *J Neurosurg*. 2005;103:413–420.
420. Tyrrell JB, Brooks RM, Fitzgerald PA, Cofoid PB, Forsham PH, Wilson CB. Cushing's disease. Selective trans-sphenoidal resection of pituitary microadenomas. *N Engl J Med*. 1978;298:753–758.
421. Hermus AR, Smals AG, Swinkels LM, Huysmans DA, Pieters GF, Sweep CF, et al. Bone mineral density and bone turnover before and after surgical cure of Cushing's syndrome. *J Clin Endocrinol Metab*. 1995;80:2859–2865.
422. Leong GM, Abad V, Charmandari E, Reynolds JC, Hill S, Chrousos GP, Nieman LK. Effects of child- and adolescent-onset endogenous Cushing syndrome on bone mass, body composition, and growth: a 7-year prospective study into young adulthood. *J Bone Miner Res*. 2007;22:110–118.
423. Carpenter PC. Diagnostic evaluation of Cushing's syndrome. *Endocrinol Metab Clin North Am*. 1988;17:445–472.
424. Cushing, H. *The Pituitary Body and Its Disorder*. Philadelphia: Lippincott; 1912.
425. McArthur RG, Cloutier MD, Hayles AB, Sprague RG. Cushing's disease in children. Findings in 13 cases. *Mayo Clin Proc*. 1972;47:318–326.
426. Storr HL, Isidori AM, Monson JP, Besser GM, Grossman AB, Savage MO. Prepubertal Cushing's disease is more common in males, but there is no increase in severity at diagnosis. *J Clin Endocrinol Metab*. 2004;89:3818–3820.
427. Miller WL, Townsend JJ, Grumbach MM, Kaplan SL. An infant with Cushing's disease due to an adrenocorticotropin-producing pituitary adenoma. *J Clin Endocrinol Metab*. 1979;48:1017–1025.
428. Boggan JE, Tyrrell JB, Wilson CB. Transsphenoidal microsurgical management of Cushing's disease. Report of 100 cases. *J Neurosurg*. 1983;59:195–200.

429. Styne DM, Grumbach MM, Kaplan SL, Wilson CB, Conte FA. Treatment of Cushing's disease in childhood and adolescence by transsphenoidal microadenectomy. *N Engl J Med.* 1984;310:889–893.
430. Magiakou MA, Mastorakos G, Oldfield EH, Gomez MT, Doppman JL, Cutler Jr GB, et al. Cushing's syndrome in children and adolescents. Presentation, diagnosis, and therapy. *N Engl J Med.* 1994;331:629–636.
431. Leinung MC, Kane LA, Scheithauer BW, Carpenter PC, Laws Jr ER, Zimmerman D. Long term follow-up of transsphenoidal surgery for the treatment of Cushing's disease in childhood. *J Clin Endocrinol Metab.* 1995;80:2475–2479.
432. Storr HL, Afshar F, Matson M, Sabin I, Davies KM, Evanson J, et al. Factors influencing cure by transsphenoidal selective adenectomy in paediatric Cushing's disease. *Eur J Endocrinol.* 2005;152:825–833.
433. Perez-Rivas LG, Reincke M. Genetics of Cushing's disease: an update. *J Endocrinol Invest.* 2016;39:29–35.
434. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, et al. Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet.* 2015;47:31–38.
435. Massoud AF, Powell M, Williams RA, Hindmarsh PC, Brook CG. Transsphenoidal surgery for pituitary tumours. *Arch Dis Child.* 1997;76:398–404.
436. Magiakou MA, Mastorakos G, Chrousos GP. Final stature in patients with endogenous Cushing's syndrome. *J Clin Endocrinol Metab.* 1994;79:1082–1085.
437. Lebrethon MC, Grossman AB, Afshar F, Plowman PN, Besser GM, Savage MO. Linear growth and final height after treatment for Cushing's disease in childhood. *J Clin Endocrinol Metab.* 2000;85:3262–3265.
438. Davies JH, Storr HL, Davies K, Monson JP, Besser GM, Afshar F, et al. Final adult height and body mass index after cure of paediatric Cushing's disease. *Clin Endocrinol (Oxf).* 2005;62:466–472.
439. Storr HL, Plowman PN, Carroll PV, Francois I, Krassas GE, Afshar F, et al. Clinical and endocrine responses to pituitary radiotherapy in pediatric Cushing's disease: an effective second-line treatment. *J Clin Endocrinol Metab.* 2003;88:34–37.
440. Nieman LK. Medical therapy of Cushing's disease. *Pituitary.* 2002;5:77–82.
441. Schulte HM, Benker G, Reinwein D, Sippell WG, Allolio B. Infusion of low dose etomidate: correction of hypercortisolemia in patients with Cushing's syndrome and dose–response relationship in normal subjects. *J Clin Endocrinol Metab.* 1990;70:1426–1430.
442. Greening JE, Brain CE, Perry LA, et al. Efficient short-term control of hypercortisolaemia by low-dose etomidate in severe paediatric Cushing's disease. *Horm Res.* 2005;64:140–143.
443. Banerjee RR, Marina N, Katznelson L, Feldman BJ. Mifepristone Treatment of Cushing's Syndrome in a Pediatric Patient. *Pediatrics.* 2015;136. e1377–1381.
444. Wannachalee T, Turcu AF, Auchus RJ. Mifepristone in the treatment of the ectopic adrenocorticotropic hormone syndrome. *Clin Endocrinol (Oxf).* 2018;89:570–576.
445. Else T, Auchus RJ, Miller WL. Adrenocortical carcinoma in a 17th-century girl. *J Steroid Biochem Mol Biol.* 2017;165:109–113.
446. Perry RR, Nieman LK, Cutler Jr GB, Chrousos GP, Loriaux DL, Doppman JL, et al. Primary adrenal causes of Cushing's syndrome. Diagnosis and surgical management. *Ann Surg.* 1989;210:59–68.
447. Michalkiewicz E, Sandrini R, Figueiredo B, Miranda EC, Caran E, Oliveira-Filho AG, et al. Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. *J Clin Oncol.* 2004;22:838–845.
448. Ribeiro RC, Sandrini F, Figueiredo B, Zambetti GP, Michalkiewicz E, Lafferty AR, et al. An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A.* 2001;98:9330–9335.
449. Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, et al. Adrenocortical carcinoma. *Endocr Rev.* 2014;35:282–326.
450. Mohan DR, Lerario AM, Hammer GD. Therapeutic targets for adrenocortical carcinoma in the genomics era. *J Endocr Soc.* 2018;2:1259–1274.
451. Beuschlein F, Fassnacht M, Assie G, Calebiro D, Stratakis CA, Osswald A, et al. Constitutive activation of PKA catalytic subunit in adrenal Cushing's syndrome. *N Engl J Med.* 2014;370:1019–1028.
452. Wolthers OD, Cameron FJ, Scheimberg I, Honour JW, Hindmarsh PC, et al. Androgen secreting adrenocortical tumours. *Arch Dis Child.* 1999;80:46–50.
453. Wieneke JA, Thompson LD, Heffess CS. Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. *Am J Surg Pathol.* 2003;27:867–881.
454. Ribeiro RC, Pinto EM, Zambetti GP, Rodriguez-Galindo C. The International Pediatric Adrenocortical Tumor Registry initiative: contributions to clinical, biological, and treatment advances in pediatric adrenocortical tumors. *Mol Cell Endocrinol.* 2012;351:37–43.
455. Lacroix A. Heredity and cortisol regulation in bilateral macronodular adrenal hyperplasia. *N Engl J Med.* 2013;369:2147–2149.
456. Lacroix A, Ndiaye N, Tremblay J, Hamet P. Ectopic and abnormal hormone receptors in adrenal Cushing's syndrome. *Endocr Rev.* 2001;22:75–110.
457. Louiset E, Duparc C, Young J, Renouf S, Tetsi Nomigni M, Boutelet I, et al. Intraadrenal corticotropin in bilateral macronodular adrenal hyperplasia. *N Engl J Med.* 2013;369:2115–2125.
458. Assie G, Libe R, Espiard S, Rizk-Rabin M, Guimier A, Luscip W, et al. ARMC5 mutations in macronodular adrenal hyperplasia with Cushing's syndrome. *N Engl J Med.* 2013;369:2105–2114.
459. Gatta-Cherifi B, Chabre O, Murat A, Niccoli P, Cardot-Bauters C, Rohmer V, et al. Adrenal involvement in MEN1. Analysis of 715 cases from the Groupe d'étude des Tumeurs Endocrines database. *Eur J Endocrinol.* 2012;166:269–279.
460. Hsiao HP, Kirschner LS, Bourdeau I, Keil MF, Boikos SA, Verma S, et al. Clinical and genetic heterogeneity, overlap with other tumor syndromes, and atypical glucocorticoid hormone secretion in adrenocorticotropic-independent macronodular adrenal hyperplasia compared with other adrenocortical tumors. *J Clin Endocrinol Metab.* 2009;94:2930–2937.
461. Matyakhina L, Freedman RJ, Bourdeau I, Wei MH, Stergiopoulos SG, Chidakel A, et al. Hereditary leiomyomatosis associated with bilateral, massive, macronodular adrenocortical disease and atypical cushing syndrome: a clinical and molecular genetic investigation. *J Clin Endocrinol Metab.* 2005;90:3773–3779.
462. Young Jr WF, Carney JA, Musa BU, Wulffraat NM, Lens JW, Drexhage HA. Familial Cushing's syndrome due to primary pigmented nodular adrenocortical disease. Reinvestigation 50 years later. *N Engl J Med.* 1989;321:1659–1664.
463. Stratakis CA. Cushing syndrome caused by adrenocortical tumors and hyperplasias (corticotropin-independent Cushing syndrome). *Endocr Dev.* 2008;13:117–132.
464. Rothenbuhler A, Stratakis CA. Clinical and molecular genetics of Carney complex. *Best Pract Res Clin Endocrinol Metab.* 2010;24:389–399.
465. Storr HL, Mitchell H, Swords FM, Main KM, Hindmarsh PC, Betts PR, et al. Clinical features, diagnosis, treatment and molecular studies in paediatric Cushing's syndrome due to primary nodular adrenocortical hyperplasia. *Clin Endocrinol (Oxf).* 2004;61:553–559.
466. Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, et al. Mutations of the gene encoding the protein kinase A type I- α regulatory subunit in patients with the Carney complex. *Nat Genet.* 2000;26:89–92.
467. Kirschner LS, Sandrini F, Monbo J, Lin JP, Carney JA, Stratakis CA. Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. *Hum Mol Genet.* 2000;9:3037–3046.
468. Bossis I, Stratakis CA. PRKAR1A: normal and abnormal functions. *Endocrinology.* 2004;145:5452–5458.
469. Bertherat J, Horvath A, Groussin L, Grabar S, Boikos S, Cazabat L, et al. Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *J Clin Endocrinol Metab.* 2009;94:2085–2091.

470. Almeida MQ, Stratakis CA. Carney complex and other conditions associated with micronodular adrenal hyperplasias. *Best Pract Res Clin Endocrinol Metab.* 2010;24:907–914.
471. Bourdeau I, Matyakhina L, Stergiopoulos SG, Sandrini F, Boikos S, Stratakis CA. 17q22-24 chromosomal losses and alterations of protein kinase a subunit expression and activity in adrenocorticotropin-independent macronodular adrenal hyperplasia. *J Clin Endocrinol Metab.* 2006;91:3626–3632.
472. Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, et al. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. *Nat Genet.* 2006;38:794–800.
473. Kirk JM, Brain CE, Carson DJ, Hyde JC, Grant DB. Cushing's syndrome caused by nodular adrenal hyperplasia in children with McCune-Albright syndrome. *J Pediatr.* 1999;134:789–792.
474. Neary NM, Lopez-Chavez A, Abel BS, Boyce AM, Schaub N, Kwong K, et al. Neuroendocrine ACTH-producing tumor of the thymus—experience with 12 patients over 25 years. *J Clin Endocrinol Metab.* 2012;97:2223–2230.
475. Salunke P, Bhansali A, Dutta P, Bansal A, Gupta K, Vasishta RK, Singh P, Mukherjee KK. Congenital immature teratoma mimicking Cushing's disease. *Pediatr Neurosurg.* 2010;46:46–50.
476. Koo BK, An JH, Jeon KH, Choi SH, Cho YM, Jang HC, et al. Two cases of ectopic adrenocorticotropin hormone syndrome with olfactory neuroblastoma and literature review. *Endocr J.* 2008;55:469–475.
477. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004;116:281–297.
478. Hill DA, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D, et al. DICER1 mutations in familial pleuropulmonary blastoma. *Science.* 2009;325:965. 2009.
479. Sahakitrungruang T, Srichomthong C, Pornkunwilai S, Amornfa J, Shuangshoti S, Kulawonganchai S, et al. Germline and somatic DICER1 mutations in a pituitary blastoma causing infantile-onset Cushing's disease. *J Clin Endocrinol Metab.* 2014;99: E1487–1492.
480. Choong CS, Priest JR, Foulkes WD. Exploring the endocrine manifestations of DICER1 mutations. *Trends Mol Med.* 2012;18:503–505.
481. de Kock L, Sabbaghian N, Plourde F, Srivastava A, Weber E, Bouron-Dal Soglio D, Hamel N, et al. Pituitary blastoma: a pathognomonic feature of germ-line DICER1 mutations. *Acta Neuropathol.* 2014;128:111–122. 2014.
482. Scheithauer BW, Horvath E, Abel TW, Robital Y, Park SH, Osamura RY, et al. Pituitary blastoma: a unique embryonal tumor. *Pituitary.* 2012;15:365–373.
483. Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev.* 1998;19:647–672.
484. Raff H, Raff JL, Findling JW. Late-night salivary cortisol as a screening test for Cushing's syndrome. *J Clin Endocrinol Metab.* 1998;83:2681–2686.
485. Stratakis CA, Kirschner LS, Carney JA. Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metab.* 2001;86:4041–4046.
486. Stratakis CA, Sarlis N, Kirschner LS, Carney JA, Doppman JL, Nieman LK, et al. Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. *Ann Intern Med.* 1999;131:585–591.
487. Lienhardt A, Grossman AB, Dacie JE, Evanson J, Huebner A, Afshar F, et al. Relative contributions of inferior petrosal sinus sampling and pituitary imaging in the investigation of children and adolescents with ACTH-dependent Cushing's syndrome. *J Clin Endocrinol Metab.* 2001;86:5711–5714.
488. Ilias I, Chang R, Pacak K, et al. Jugular venous sampling: an alternative to petrosal sinus sampling for the diagnostic evaluation of adrenocorticotropin hormone-dependent Cushing's syndrome. *J Clin Endocrinol Metab.* 2004;89:3795–3800.
489. Zennaro MC, Boulkroun S, Fernandes-Rosa F. An update on novel mechanisms of primary aldosteronism. *J Endocrinol.* 2015;224: R63–77.
490. Vaidya A, Mulatero P, Baudrand R, Adler GK. The expanding spectrum of primary aldosteronism: implications for diagnosis, pathogenesis, and treatment. *Endocr Rev.* 2018;39:1057–1088.
491. Charmandari E, Sertedaki A, Kino T, Merakou C, Hoffman DA, Hatch MM, et al. A novel point mutation in the KCNJ5 gene causing primary hyperaldosteronism and early-onset autosomal dominant hypertension. *J Clin Endocrinol Metab.* 2012;97: E1532–1539.
492. Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, et al. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. *Science.* 2011;331:768–772.
493. Mulatero P, Tauber P, Zennaro MC, Monticone S, Lang K, Beuschlein F, Fischer E, et al. KCNJ5 mutations in European families with nonglucocorticoid remediable familial hyperaldosteronism. *Hypertension.* 2012;59:235–240.
494. Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, et al. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. *Proc Natl Acad Sci U S A.* 2012;109:2533–2538.
495. Fernandes-Rosa FL, Williams TA, Riestter A, Steichen O, Beuschlein F, Boulkroun S, et al. Genetic spectrum and clinical correlates of somatic mutations in aldosterone-producing adenoma. *Hypertension.* 2014;64:354–361.
496. Boulkroun S, Beuschlein F, Rossi GP, Golib-Dzib JF, Fischer E, Amar L, et al. Prevalence, clinical, and molecular correlates of KCNJ5 mutations in primary aldosteronism. *Hypertension.* 2012;59:592–598.
497. Akerstrom T, Maharjan R, Sven Willenberg H, Cupisti K, Ip J, Moser A, et al. Activating mutations in CTNNB1 in aldosterone producing adenomas. *Sci Rep.* 2016;6:19546.
498. Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, et al. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. *Nat Genet.* 2013;45:1055–1060.
499. Beuschlein F, Boulkroun S, Osswald A, Wieland T, Nielsen HN, Lichtenauer UD, et al. Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension. *Nat Genet.* 2013;45:440–444. 444e441–442.
500. Zennaro MC, Boulkroun S, Fernandes-Rosa F. Genetic causes of functional adrenocortical adenomas. *Endocr Rev.* 2017;38:516–537.
501. Scholl UI, Stolting G, Schewe J, Thiel A, Tan H, Nelson-Williams C, et al. CLCN2 chloride channel mutations in familial hyperaldosteronism type II. *Nat Genet.* 2018;50:349–354.
502. Perez-Rivas LG, Williams TA, Reincke M. Inherited forms of primary hyperaldosteronism: new genes, new phenotypes and proposition of a new classification. *Exp Clin Endocrinol Diabetes.* 2019;127(2–03):93–99.
503. Nishimoto K, Tomlins SA, Kuick R, Cani AK, Giordano TJ, Hovelson DH, et al. Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands. *Proc Natl Acad Sci U S A.* 2015;112: E4591–4599.
504. Omata K, Anand SK, Hovelson DH, Liu CJ, Yamazaki Y, Nakamura Y, et al. Aldosterone-producing cell clusters frequently harbor somatic mutations and accumulate with age in normal adrenals. *J Endocr Soc.* 2017;1:787–799.
505. Nanba K, Vaidya A, Williams GH, Zheng I, Else T, Rainey WE. Age-related autonomous aldosteronism. *Circulation.* 2017;136:347–355.
506. Charmandari E, Kino T, Chrousos GP. Familial/sporadic glucocorticoid resistance: clinical phenotype and molecular mechanisms. *Ann N Y Acad Sci.* 2004;1024:168–181.
507. van Rossum EF, van den Akker EL. Glucocorticoid resistance. *Endocr Dev.* 2011;20:127–136.
508. Hurley DM, Accili D, Stratakis CA, Karl M, Vamvakopoulos N, Rorer E, et al. Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J Clin Invest.* 1991;87:680–686.
509. Karl M, Lamberts SW, Detera-Wadleigh SD, Encio IJ, Stratakis CA, Hurley DM, et al. Familial glucocorticoid resistance caused by a splice site deletion in the human glucocorticoid receptor gene. *J Clin Endocrinol Metab.* 1993;76:683–689.
510. Kino T, Stauber RH, Resau JH, Pavlakis GN, Chrousos GP. Pathologic human GR mutant has a transdominant negative effect on the wild-type GR by inhibiting its translocation into the nucleus: importance of the ligand-binding domain for intracellular GR trafficking. *J Clin Endocrinol Metab.* 2001;86:5600–5608.

511. Zennaro MC, Hubert EL, Fernandes-Rosa FL. Aldosterone resistance: structural and functional considerations and new perspectives. *Mol Cell Endocrinol*. 2012;350:206–215.
512. Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, et al. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypaldosteronism type 1. *Nat Genet*. 1996;12:248–253.
513. Kerem E, Bistrizter T, Hanukoglu A, Hofmann T, Zhou Z, Bennett W, et al. Pulmonary epithelial sodium-channel dysfunction and excess airway liquid in pseudohypaldosteronism. *N Engl J Med*. 1999;341:156–162.
514. Geller DS, Rodriguez-Soriano J, Vallo Boado A, et al. Mutations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypaldosteronism type I. *Nat Genet*. 1998;19:279–281.
515. Sartorato P, Lapeyraque AL, Armanini D, Kuhnle U, Khaldi Y, Salomon R, et al. Different inactivating mutations of the mineralocorticoid receptor in fourteen families affected by type I pseudohypaldosteronism. *J Clin Endocrinol Metab*. 2003;88:2508–2517.
516. Geller DS, Farhi A, Pinkerton N, Fradley M, Moritz M, Spitzer A, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science*. 2000;289:119–123.
517. Chandar J, Abitbol C, Zilleruelo G, Gosalbez R, Montane B, Strauss J. Renal tubular abnormalities in infants with hydronephrosis. *J Urol*. 1996;155:660–663.
518. Min J, Perera L, Krahn JM, Jewell CM, Moon AF, Cidlowski JA, Pedersen LC. Probing dominant negative behavior of glucocorticoid receptor beta through a hybrid structural and biochemical approach. *Mol Cell Biol*. 2018;38(8). pii, e00453–17.
519. Taniguchi Y, Iwasaki Y, Tsugita M, Nishiyama M, Taguchi T, Okazaki M, et al. Glucocorticoid receptor- β and receptor- γ exert dominant negative effect on gene repression but not on gene induction. *Endocrinology*. 2010;151:3204–3213.
520. Weikum ER, Knuesel MT, Ortlund EA, Yamamoto KR. Glucocorticoid receptor control of transcription: precision and plasticity via allostery. *Nat Rev Mol Cell Biol*. 2017;18:159–174.
521. Harcken C, Riether D, Kuzmich D, Liu P, Betageri R, Ralph M, et al. Identification of highly efficacious glucocorticoid receptor agonists with a potential for reduced clinical bone side effects. *J Med Chem*. 2014;57:1583–1598.
522. Wang JC, Shah N, Pantoja C, et al. Novel arylpyrazole compounds selectively modulate glucocorticoid receptor regulatory activity. *Genes Dev*. 2006;20:689–699.
523. McDonnell DP. The molecular pharmacology of estrogen receptor modulators: implications for the treatment of breast cancer. *Clin Cancer Res*. 2005;11:871s–877s.
524. Charmandari E. Primary generalized glucocorticoid resistance and hypersensitivity. *Horm Res Paediatr*. 2011;76:145–155.
525. Santen RJ, Jewell CM, Yue W, Heitjan DF, Raff H, Katen KS, Cidlowski JA. Glucocorticoid receptor mutations and hypersensitivity to endogenous and exogenous glucocorticoids. *J Clin Endocrinol Metab*. 2018;103:3630–3639.
526. Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF. Clinical features associated with glucocorticoid receptor polymorphisms. An overview. *Ann N Y Acad Sci*. 2009;1179:179–198.
527. Oakley RH, Cidlowski JA. Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. *J Biol Chem*. 2011;286:3177–3184.
528. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, et al. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature*. 1985;318:635–641.
529. Aggarwal A, Costa MJ, Rivero-Gutierrez B, Ji L, Morgan SL, Feldman BJ. The circadian clock regulates adipogenesis by a Per3 crosstalk pathway to Klf15. *Cell Rep*. 2017;21:2367–2375.
530. Gibbs J, Ince L, Matthews L, Mei J, Bell T, Yang N, et al. An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. *Nat Med*. 2014;20:919–926.
531. Kino T. Circadian rhythms of glucocorticoid hormone actions in target tissues: potential clinical implications. *Sci Signal*. 2012;5:244:pt4.
532. So AY, Bernal TU, Pillsbury ML, Yamamoto KR, Feldman BJ. Glucocorticoid regulation of the circadian clock modulates glucose homeostasis. *Proc Natl Acad Sci U S A*. 2009;106:17582–17587.
533. German A, Suraiya S, Tenenbaum-Rakover Y, Koren I, Pillar G, Hochberg Z. Control of childhood congenital adrenal hyperplasia and sleep activity and quality with morning or evening glucocorticoid therapy. *J Clin Endocrinol Metab*. 2008;93:4707–4710.
534. Grossmann C, Scholz T, Rochel M, Bumke-Vogt C, Oelkers W, Pfeiffer AF, et al. Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties. *Eur J Endocrinol*. 2004;151:397–406.
535. Derendorf H, Mollmann H, Barth J, Mollmann C, Tunn S, Krieg M. Pharmacokinetics and oral bioavailability of hydrocortisone. *J Clin Pharmacol*. 1991;31:473–476.
536. Hindmarsh PC, Charmandari E. Variation in absorption and half-life of hydrocortisone influence plasma cortisol concentrations. *Clin Endocrinol (Oxf)*. 2015;82:557–561.
537. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab*. 2002;13:35–43.
538. Streck WF, Lockwood DH. Pituitary adrenal recovery following short-term suppression with corticosteroids. *Am J Med*. 1979;66:910–914.

15

Pheochromocytoma/Paraganglioma, Medullary Thyroid Carcinoma, and Hereditary Endocrine Neoplasia Syndromes

Steven G. Waguespack, Lauren Fishbein

CHAPTER OUTLINE

INTRODUCTION	491		
GENETIC COUNSELING AND TESTING	491		
PHEOCHROMOCYTOMA AND PARAGANGLIOMA	493		
Biosynthesis and Actions of Catecholamines	495		
Clinical Presentation	502		
Evaluation	496		
Genetic Issues	498		
Management	499		
Prognosis and Follow-Up	500		
MEDULLARY THYROID CARCINOMA	500		
Clinical Presentation	502		
Evaluation and Management	502		
Prognosis	503		
HEREDITARY ENDOCRINE NEOPLASIA SYNDROMES	503		
Carney Complex	503		
Familial Isolated Pituitary Adenomas	504		
Hereditary Paraganglioma-Pheochromocytoma Syndromes	504		
Hyperparathyroidism-Jaw Tumor Syndrome	505		
Multiple Endocrine Neoplasia Type 1	505		
Multiple Endocrine Neoplasia Type 2	510		
Multiple Endocrine Neoplasia Type 4	513		
X-Linked Acrogigantism	513		
OTHER TUMOR SYNDROMES ASSOCIATED WITH ENDOCRINE NEOPLASIA	514		
Adenomatous Polyposis Coli-Associated Polyposis	514		
Beckwith-Wiedemann Syndrome	514		
Carney Triad	514		
DICER1 Syndrome	514		
Li-Fraumeni Syndrome	515		
Lynch Syndrome (Hereditary Nonpolyposis Colon Cancer)	515		
Neurofibromatosis Type 1	515		
Peutz-Jeghers Syndrome	515		
PTEN Hamartoma Tumor Syndrome	516		
Tuberous Sclerosis Complex	516		
Von Hippel-Lindau Disease	516		
SUMMARY AND FUTURE DEVELOPMENTS	517		
References	517		

INTRODUCTION

Endocrine neoplasms comprise a variety of benign and malignant tumors that arise from the endocrine glands or other neuroendocrine tissues, such as the paraganglia. Most childhood endocrine tumors, typified by papillary thyroid carcinoma, are sporadic and not attributable to an identifiable germline mutation whereas others, epitomized by catecholamine-producing tumors (Table 15.1) and medullary thyroid carcinoma (MTC), are familial and occur within the context of a broader hereditary tumor syndrome. In familial endocrine neoplasms, the mode of inheritance is autosomal dominant and mutations are primarily inactivating mutations causing loss of function in a tumor suppressor gene. Advances in genetic testing and research have led to the ongoing discovery of novel tumor-susceptibility genes, in addition to a better understanding of the underlying pathophysiology of these unique disorders. Knowledge regarding genotype-phenotype relationships continues to evolve, as has clinical practice regarding the age of performing predictive genetic testing, screening for endocrine tumors in an asymptomatic carrier, and the timing of therapeutic intervention. Given the rapidly changing field, it is beneficial for patients with an endocrine tumor to be evaluated in programs with known multidisciplinary expertise. In addition, the results of genetic testing and formal genetic counseling should be fully incorporated

into treatment planning and long-term follow-up. This chapter reviews the pathophysiology, diagnosis, and management of pediatric endocrine tumors and the most common genetic syndromes associated with their diagnosis.

GENETIC COUNSELING AND TESTING

A diagnosis of an endocrine tumor in a child, especially one that is neuroendocrine in origin, should raise concern for the possibility of an underlying genetic predisposition. Genetic counseling is a process of communication that promotes understanding, decision-making, and coping related to the impact of genetic disease.¹ It should be incorporated into all stages of care, both at diagnosis and during long-term follow-up, because patients' needs change over time and also because genetic testing and management recommendations are likely to evolve.

Genetic testing, nicely exemplified in multiple endocrine neoplasia (MEN) type 2,² is a multistep process that begins with a patient who already has clinical manifestations of disease. In most cases, such as in MEN1 and MEN2A, this is likely to be a parent or another blood relative, but in other heritable disorders with low disease penetrance, such as the familial paraganglioma (PGL) syndromes, genetic testing of the family may actually begin with the child who is the index case. Genetic

TABLE 15.1 Major Disorders and Genes Associated With Pheochromocytoma and Paraganglioma

Hereditary Syndrome	Gene (Chromosome)	Tumor Type	Clinical Phenotype ^a	Earliest Age ^b	Screening Guidelines ^c
Multiple endocrine neoplasia type 2A (MEN2A)	<i>RET</i> (10q11.21)	PHEO/rare PGL & composite PHEO ^d	<ul style="list-style-type: none"> • Adrenergic • Malignancy rare • Background of adrenal medullary hyperplasia 	8 ⁴⁵⁵	Start by age 11 yrs. in codon 634 mutations; by age 16 yrs. in other codons
Multiple endocrine neoplasia type 2B (MEN2B)	<i>RET</i> (10q11.21)	PHEO/rare PGL	<ul style="list-style-type: none"> • Adrenergic • Malignancy rare • Background of adrenal medullary hyperplasia 	10 ³⁵⁵	Start by age 11 yrs. in codon 918 and 883 mutations
Neurofibromatosis type 1 (NF1)	<i>NF1</i> (17q11.2)	PHEO/rare PGL & composite PHEO ^d	<ul style="list-style-type: none"> • Adrenergic • Malignancy uncommon 	7 ⁴⁵⁶	Screen any NF1 patient with HTN, signs/symptoms of catecholamine excess, or an incidental adrenal or paraaortic mass
Familial paraganglioma syndrome 1 (PGL1)	<i>SDHD</i> (11q23.1)	PGL (primarily head & neck > thorax/abdominal/pelvic)/PHEO	<ul style="list-style-type: none"> • Nonfunctional or noradrenergic • Malignancy rare 	5 ^{11,14}	Start by age 10 yrs. in patients with paternal inheritance of disease ^e
Familial paraganglioma syndrome 3 (PGL3)	<i>SDHC</i> (1q23.3)	PGL (primarily head & neck/ thorax)	<ul style="list-style-type: none"> • Nonfunctional or noradrenergic • Malignancy rare 	13 ⁴⁵⁷	Start by age 10 yrs ^f
Familial paraganglioma syndrome 4 (PGL4)	<i>SDHB</i> (1p36.13)	PGL (Abdominal/ pelvic >head & neck)/PHEO	<ul style="list-style-type: none"> • Noradrenergic or nonfunctional • Malignancy ~25% 	3 ⁴⁵⁸	Start by age 5 yrs ^g
Familial paraganglioma syndrome 5 (PGL5)	<i>SDHA</i> (5p15.33)	PGL/PHEO	<ul style="list-style-type: none"> • Nonfunctional or noradrenergic • Malignancy uncommon 	8 ²²⁸	Start by age 10 yrs ^h
von Hippel-Lindau disease (VHL)	<i>VHL</i> (3p25.3)	PHEO/PGL	<ul style="list-style-type: none"> • Noradrenergic • Malignancy rare 	2 ⁴⁴⁴	Start by age 5 yrs

HTN, Hypertension; PGL, paraganglioma; PHEO, pheochromocytoma.

^aNoradrenergic tumors exclusively secrete norepinephrine/normetanephrine, whereas adrenergic tumors secrete both epinephrine and norepinephrine, in addition to their metabolites (metanephrine and normetanephrine). Nonfunctional tumors are parasympathetic tumors that arise in the head and neck.

^bEarliest age of diagnosis of PHEO/PGL and reference.

^cData from consensus guidelines and other relevant publications.^{55,63,65–67,235,236} The age to start annual screening (blood pressure; fractionated plasma or 24-hour urine metanephrines) and the frequency/type of radiologic screening (for *SDHx* carriers) is not an absolute and should be tailored to patient symptoms, physical examination (blood pressure), biochemical test results, and family preference. Consider testing in young women of childbearing age who plan to become pregnant and in any patient before an elective surgical procedure, assuming they have not been recently screened.

^dA composite PHEO is a mixed tumor comprised of PHEO and a developmentally related neurogenic tumor, such as neuroblastoma, ganglioneuroma, or ganglioneuroblastoma.⁶

^eThere are parent-of-origin effects in PGL1, such that children develop clinical disease only if a mutation is inherited from their father. In children with maternal transmission of an *SDHD* mutation, routine screening is not needed, although comprehensive testing should be considered at least once, as there are reports of maternally inherited *SDHD* mutations causing disease.

^fThe authors also consider head and neck ultrasonography for screening of cervical PGL, in addition to biennial (or less frequent) whole-body magnetic resonance imaging (MRI) as recommended by others.

^gGiven the higher rate of malignancy, earlier screening is recommended for *SDHB* mutation carriers. In addition to biochemical screening, the authors consider ultrasound of the abdomen/pelvis starting at age 5 years and biennial whole-body MRI when the patient is old enough to tolerate an MRI without sedation.

^hDisease penetrance in young *SDHA* mutation carriers is extremely low^{228,459} and thus intensive screening during childhood may not be warranted.

testing serves multiple purposes, including confirmation of the genetic cause of an endocrine tumor, identification of asymptomatic patients who are at risk for clinical disease, and guidance regarding clinical management and family planning.³ When ordering predictive genetic testing, it is important to understand how the results will be used in patient management. In some cases (e.g., MEN2), knowledge of the genotype will lead to a disease-preventing intervention (early thyroidectomy), whereas in other hereditary syndromes, such as MEN1 and von Hippel-Lindau (VHL) disease, it will only lead to an earlier diagnosis.

The process of genetic testing in children is engrained with various ethical, legal, and psychosocial implications.^{4,5} Despite the

clear medical benefits afforded from an early diagnosis, genetic testing in children has the potential for harm to the patient and family: alteration of the child's self-image and of the parents' perception of the child, modification of the patient's outlook on life, worry about the potential for genetic discrimination, changes in family relationships, early "medicalization" of an otherwise healthy child, and concerns regarding future reproductive issues. Timely medical benefit to the child should be the primary justification for genetic testing. In all cases where the risks versus benefits of genetic testing are unclear, the provider should respect the decision of the family. There are several online resources regarding genetic counseling and testing, including the National Society of Genetic Counselors (www.nsgc.org), the National Cancer

Institute's "The Genetics of Cancer" website (www.cancer.gov/cancertopics/genetics), and the internet site *GeneReviews*[®] (<https://www.ncbi.nlm.nih.gov/books/NBK1116/>).

PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Pheochromocytomas (PHEOs) and PGLs are neuroendocrine tumors (NETs) that arise from neural crest-derived paraganglion cells. PHEO (Fig. 15.1) is the term used for a

catecholamine-producing tumor that evolves from chromaffin cells in the adrenal medulla, whereas PGLs (Fig. 15.2) are extra-adrenal tumors that arise from both sympathetic and parasympathetic paraganglia located in the autonomic nervous system outside the cerebrospinal axis.^{6–8} Historically, the term *PHEO* was used interchangeably with PGL, but it is best to maintain a distinction between these two neoplasms because of underlying differences in genetics, clinical presentation, and malignant potential (see Table 15.1).

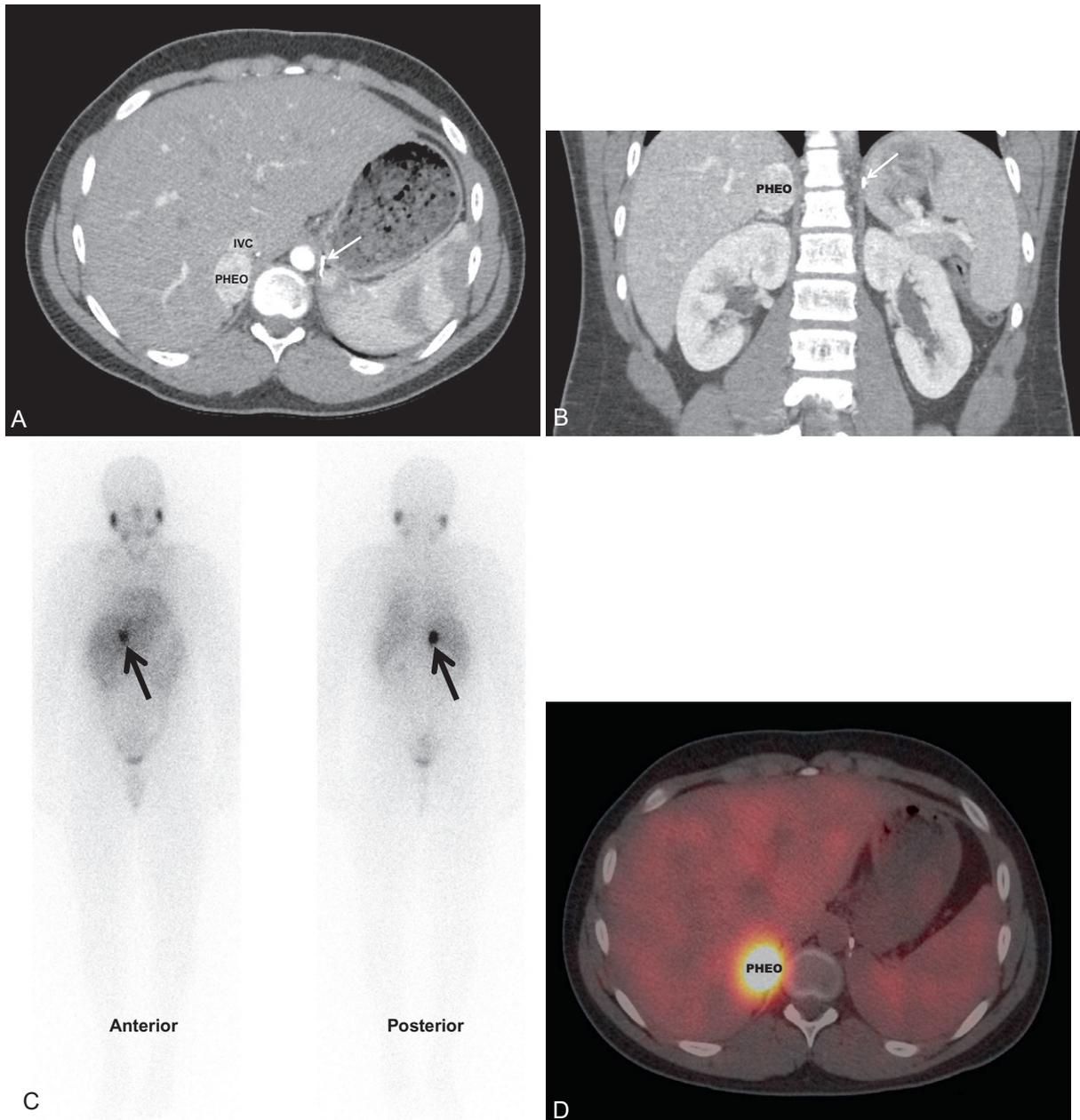


Fig. 15.1 A normotensive 14-year-old male with von Hippel-Lindau disease and a history of a left pheochromocytoma and abdominal paraganglioma diagnosed at the age of 7 years was found to have elevated normetanephrine levels after screening. Axial computed tomography (CT) postcontrast (A) with coronal reconstruction (B) identified a vascular neoplasm arising from the superior right adrenal gland (arrow, surgical clips from previous adrenalectomy). Metaiodobenzylguanidine (MIBG) scan confirmed the functional nature of the tumor and ruled out other synchronous tumors. Planar (C) and fused single photon emission CT/CT axial images (D) in the same patient, 24 hours after the administration of ¹²³I MIBG. IVC, Inferior vena cava; PHEO, pheochromocytoma.

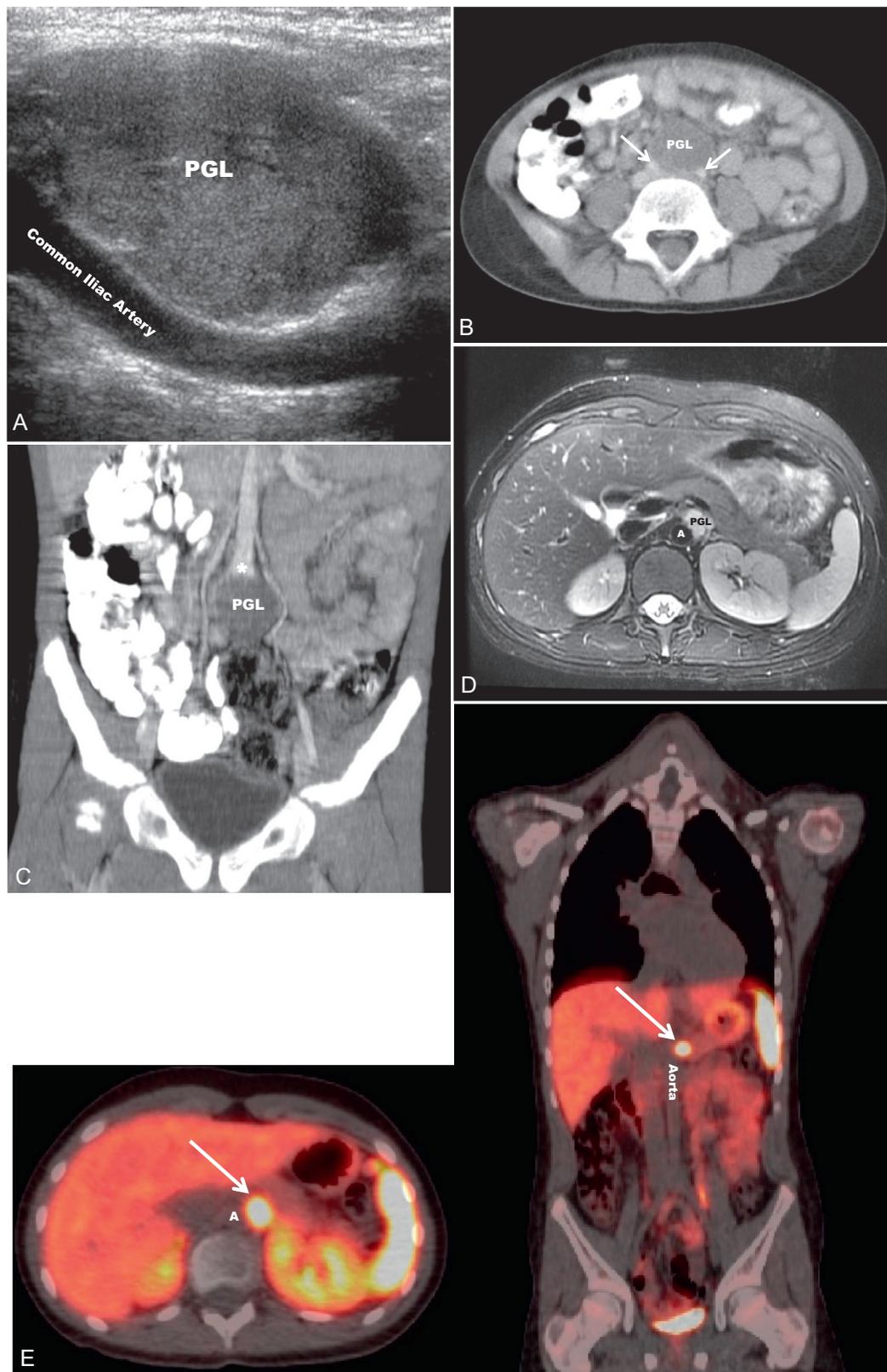


Fig. 15.2 A 6-year-old female with a paraganglioma (PGL) and an *SDHB* mutation presented with severe hypertension during a well-child examination. A, Abdominal ultrasound (sagittal view) revealed a homogeneous, hypervascular 3.3-cm mass next to the common iliac arteries. Axial computed tomography (CT) postcontrast (B) (arrows, common iliac arteries) with coronal reconstruction (C) confirmed a tumor at the aortic bifurcation (asterisk). A 13-year-old boy with an *SDHB* mutation developed elevated blood pressures and abnormal norepinephrine, normetanephrine, and chromogranin A levels during prospective screening. T2-weighted axial images from abdominal magnetic resonance imaging (D) revealed a 2-cm paraaortic mass (PGL) between the origins of the celiac axis and superior mesenteric artery that was T2 hyperintense. This was subsequently confirmed via functional imaging with ⁶⁸Ga-DOTATATE positron emission tomography/CT (E) to be a paraganglioma (arrows). A, Aorta.

PHEO/PGL are rare tumors with an annual incidence in the United States of 500 to 1600 cases per year.⁹ Around 10% of PHEO/PGL are diagnosed during childhood at an average age of 13 years; there is a slight predominance in boys, particularly when diagnosed under the age of 10 years.^{10–22} Less than 2% of children diagnosed with hypertension will harbor a catecholamine-producing neoplasm.^{23,24}

PGL occur in all locations where paraganglia are found (from the skull base to the pelvis) and are either functioning (sympathetic) or nonfunctioning (parasympathetic) neoplasms, depending on the site of origin and underlying pathophysiology^{6,11,25–27} (see Table 15.1). PGL arising in the head and neck region, which are rarer in children,^{11,28} are almost exclusively nonfunctioning, parasympathetic tumors, whereas most intraabdominal PGL (commonly occurring within the organ of Zuckerkandl [see Fig. 15.2] in a periaxillary location, or around the renal hilum⁶) are sympathetic and thus secretory tumors. The majority of PHEO/PGL diagnosed during childhood are functional and synthesize and secrete catecholamines (dopamine, norepinephrine, and/or epinephrine) and their metabolites (including 3-methoxytyramine, normetanephrine, and metanephrine, respectively)^{25,29,30} (Fig. 15.3). All functioning PHEO/PGL contain chromaffin tissue, which refers to the brown-black color resulting from the oxidation of catecholamines after staining with chromium salts. Multicentric tumors are common in childhood presentations of PHEO/PGL.^{10,11,13,15,20–22,31}

Biosynthesis and Actions of Catecholamines

Dopamine, norepinephrine, and epinephrine (collectively known as *catecholamines*) are chemical neurotransmitters and hormones that play important roles in the regulation of numerous physiologic processes and the development of neurologic, psychiatric, endocrine, and cardiovascular diseases.^{32–35} The catecholamines are comprised of a catechol (1,2-dihydroxybenzene) moiety and a side-chain amine group. They are synthesized from the amino acid tyrosine, which is converted to 3,4-dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis (see Fig. 15.3). Subsequent enzymatic decarboxylation and hydroxylation of dopa yields dopamine and norepinephrine, respectively, and norepinephrine is subsequently converted to epinephrine via the cytosolic enzyme phenylethanolamine N-methyltransferase (PNMT).

The catecholamines are synthesized and stored in granules within the adrenal medulla, where they are released via exocytosis into the systemic circulation in response to stressful stimuli. Dopamine and norepinephrine are also produced by postganglionic neurons in the sympathetic nervous system. Epinephrine is made only in the adrenal medulla, where it represents the predominant catecholamine (~80%) because PNMT expression is dependent upon and regulated by high local concentrations of glucocorticoids (as occurs only in the adrenal medulla, surrounded by the cortisol-synthesizing adrenal cortex, with a distinct concentration gradient towards the adrenal medulla).^{32,36,37} The effects of catecholamines are terminated via rapid reuptake into nerve terminals by the norepinephrine transporter and via metabolism by two major enzymes: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT)^{25,32,35} (see Fig. 15.3).

The complex actions of norepinephrine and epinephrine are mediated by the G-protein-coupled α - and β -adrenergic receptors, whereas dopamine binds to a different class of G-protein-coupled dopamine receptors (five distinct receptors [D1–D5]) that are divided into two families: D1-like and

D2-like)^{32–34} (Table 15.2). The initial classification of adrenergic receptors was based upon epinephrine's ability to both excite (α -receptor) and inhibit (β -receptor) smooth muscle. Specific agonists and antagonists characterize the adrenergic receptor subtype (α_1 , α_2 , β_1 , β_2 , and β_3) and can be used as therapeutic agents. The D2 receptor is the primary dopamine receptor that is targeted for drug therapy.

Clinical Presentation

The clinical presentation of pediatric PHEO/PGL is highly variable. Children with these tumors can come to attention because of symptomatic catecholamine hypersecretion, symptoms caused by tumor mass effect (e.g., pain), an incidental radiographic finding, or because of prospective screening in one of the associated hereditary tumor syndromes^{6,8,12,38–41} (see Table 15.1). PHEO/PGL may also arise in the setting of cyanotic congenital heart disease.^{42,43} Given their neuroendocrine origin, PHEO/PGL can very rarely cosecrete other hormones that result in a clinical syndrome of ectopic hormone excess, primarily Cushing syndrome, because of overproduction of adrenocorticotropic hormone (ACTH).^{25,44}

The clinical presentation of a functioning PHEO/PGL depends on inherent differences in catecholamine production, as well as individual patient sensitivities to catecholamines.⁴⁵ Signs and symptoms of catecholamine excess include: hypertension, which is typically sustained in the majority of pediatric cases; severe headaches; paroxysmal episodes with the classic triad of headaches, palpitations, and diaphoresis (less common in children); orthostatic hypotension and syncope; pallor; tremor; and/or anxiety.^{7,13,17,18,23,25,26,38,44,46} PHEO/PGL in children can also cause nonspecific signs and symptoms, such as blurred vision; abdominal pain, constipation, diarrhea and other gastrointestinal symptoms; weight loss; hyperglycemia; polyuria and polydipsia; low-grade fever; and behavioral problems, attention deficit hyperactivity disorder, and/or a decline in school performance.^{13,17,18,23,25,26,46–49} Bladder PGL can present with hematuria and paroxysmal symptoms during micturition.^{44,50}

Complications of catecholamine excess can include hypertensive crisis, cardiomyopathy (takotsubo cardiomyopathy), arrhythmias, pancreatitis, severe constipation and intestinal pseudoobstruction, stroke, seizures, and even multisystem crisis and death.^{26,44,46,48,51–53} Symptoms of parasympathetic PGL (usually located in the head and neck region) include hearing loss, pulsatile tinnitus, neck mass, and other symptoms of mass effect, such as voice hoarseness, pharyngeal fullness, and dysphagia.⁵⁴

Up to 80% of the pediatric population with PHEO/PGL have a hereditary predisposition syndrome (see Table 15.1 and later section),^{6,10,11,14,20–22,31,55} and a subset of these will be diagnosed only after a tumor is identified during a screening program. Compared with sporadic disease, hereditary PHEO/PGL identified during the course of prospective presymptomatic screening are smaller and less symptomatic (or even asymptomatic) tumors.^{56–58} In all cases of pediatric PHEO/PGL, genetic counseling and testing should be pursued^{39,55,59–62}; identifying a germline mutation early after diagnosis appears to have a positive impact on the management and clinical outcome of patients with heritable disease.⁶¹ The American Association of Cancer Researchers developed guidelines for screening the pediatric population with hereditary PHEO/PGL syndromes,⁶³ and others have also published screening recommendations in pediatric populations with MEN2^{64,65} and VHL disease.^{66,67}

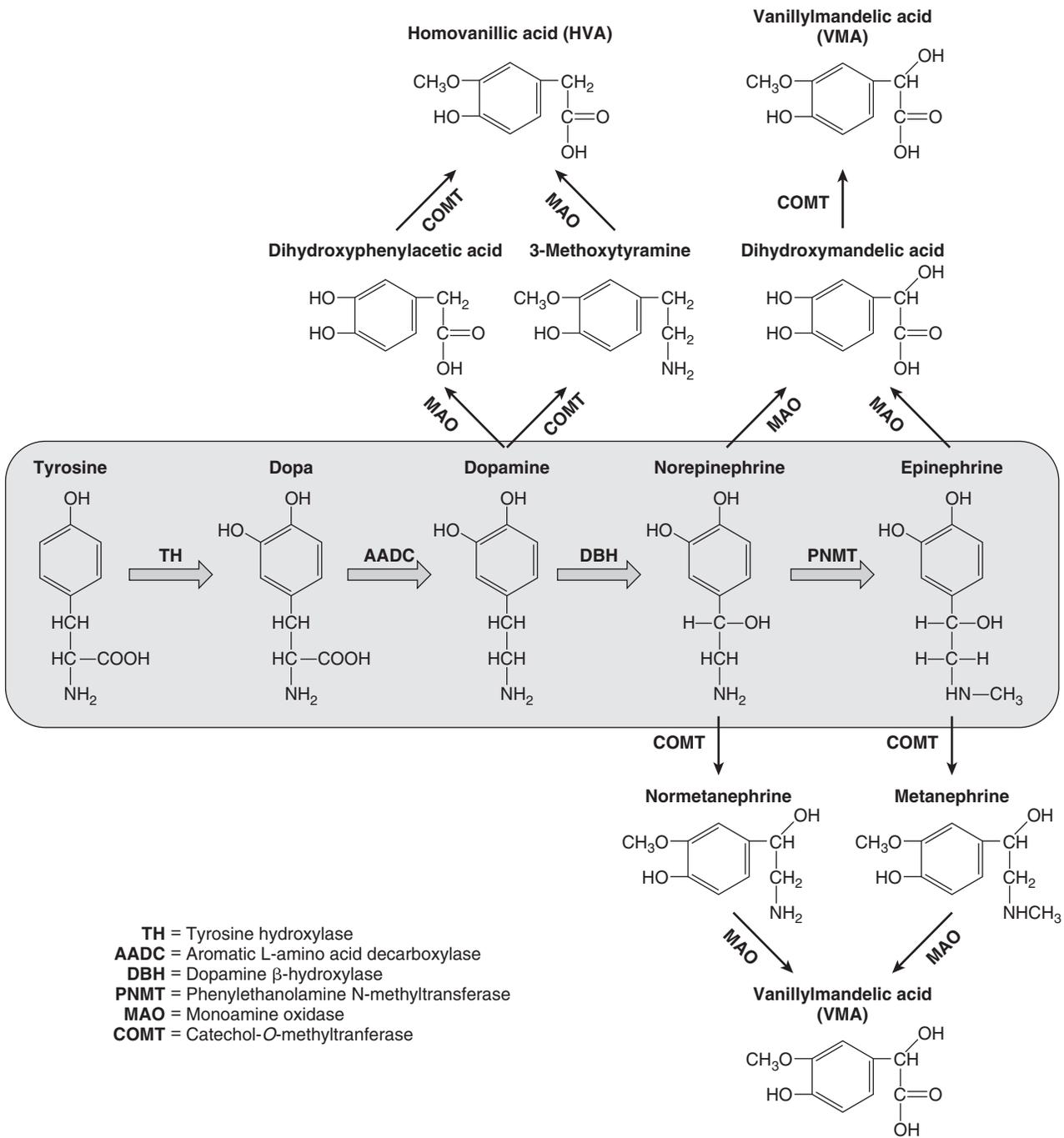


Fig. 15.3 The catecholamines are synthesized from the amino acid tyrosine, which is converted to 3,4-dihydroxyphenylalanine (dopa) by the enzyme tyrosine hydroxylase (TH), the rate-limiting step in catecholamine biosynthesis. Subsequent enzymatic decarboxylation (aromatic L-amino acid decarboxylase; AADC) and hydroxylation (dopamine β-hydroxylase; DBH) yields dopamine and norepinephrine, respectively, and norepinephrine is subsequently converted to epinephrine via the cytosolic enzyme phenylethanolamine N-methyltransferase (PNMT). The catecholamines are metabolized by two major enzymes: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

Evaluation

Biochemical Diagnosis

The diagnosis of PHEO/PGL has been simplified by advances in the assays used to detect and quantify levels of catecholamines and their metabolites in blood and urine. The measurement of fractionated plasma and/or urine metanephrines

(metanephrine and normetanephrine) is the most sensitive test (approaching 100% sensitivity) for the diagnosis of PHEO/PGL and should be the primary diagnostic test^{7,25,59,68-72} (Fig. 15.4).

The high sensitivity of metanephrine testing is based upon the fact that there is continuous intratumoral metabolism of catecholamines, a process that occurs independently of catecholamine release, which can occur intermittently or at

TABLE 15.2 Catecholamine Receptor Classification, Function, and Pharmacology

Receptor Type	Primary Pharmacologic Agonist(s)	Primary Pharmacologic Antagonist(s)	Major Biologic Effects
α_1	Phenylephrine, midodrine	Doxazosin, prazosin, terazosin, etc.	Vasoconstriction; promotes cardiac growth and structure
α_2	Clonidine, methyl dopa, tizanidine	Yohimbine	Inhibition of norepinephrine release; vasoconstriction; inhibits adrenal catecholamine release and modulates CNS dopamine neurotransmission
β_1	Dobutamine	Atenolol, bisoprolol, esmolol, metoprolol, etc.	Increases heart rate and contractility
β_2	Albuterol, levalbuterol, salmeterol, terbutaline, etc.	Propranolol (prototypic β_2 antagonist that is also a β_1 antagonist) and others	Smooth muscle relaxation (arteriolar and venous dilation; relaxation of tracheobronchial muscles)
β_3	Mirabegron	Investigational	Metabolic effects in adipose tissue and skeletal muscle
D1-like (D_1 and D_5)	Levodopa	No primary agents	Vasodilation; increased renin secretion; promotes norepinephrine and epinephrine release; various CNS functions
D2-like (D_2 , D_3 , and D_4)	Bromocriptine, cabergoline, pramipexole, ropinirole	Aripiprazole, chlorpromazine, haloperidol, metoclopramide, prochlorperazine, etc.	Decreases prolactin secretion; decreases renin secretion; inhibits norepinephrine and epinephrine release; various CNS functions

CNS, central nervous system.

(From Westfall, T.C., Westfall, D.P. (2011). Neurotransmission: the autonomic and somatic motor nervous systems. In: Brunton, L.L., ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill, 171–218; Westfall, T.C., Westfall, D.P. (2011). Catecholamines and sympathomimetic drugs. In: Brunton, L.L., ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill, 277–333; Sanders-Bush, E., Hazelwood, L. (2011). 5-Hydroxytryptamine (Serotonin) and Dopamine. In: Brunton, L.L., ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill, 335–361.)

low rates.⁶⁹ An elevation of metanephrines greater than 4-fold above the reference range is associated with almost 100% probability of the presence of a catecholamine-secreting tumor⁷³; however, a PHEO/PGL can still be present with metanephrine levels more than two times the upper limit of normal, especially in the setting of hereditary disease.⁷⁴ Any drugs known to interfere with these assays (e.g., acetaminophen, tricyclic antidepressants, phenoxybenzamine, and decongestants, among others⁵⁹) should be discontinued before testing. Dietary restrictions need not be routinely used but should be considered if the assay used measures only deconjugated normetanephrines or if a dopamine-secreting tumor is suspected.⁷⁵ The measurement of the catecholamine metabolites vanillylmandelic acid (VMA) and homovanillic acid (HVA) is no longer recommended for the evaluation of PHEO/PGL. However, testing for HVA and VMA in spot urine samples remains a critical component of the evaluation of neuroblastoma, where these analytes have a high sensitivity and specificity for tumor detection.⁷⁶

In patients with mildly elevated metanephrine levels, in whom a false positive test result is suspected, consideration should be given to measuring these plasma analytes drawn from patients in the supine position, 30 minutes after an indwelling needle or catheter is inserted into the vein.^{7,59,71} Clonidine suppression and glucagon stimulation tests^{59,77,78} have been a component of the diagnostic algorithm in adults but are rarely required and have not been validated in the diagnosis of childhood PHEO/PGL.

Catecholamine-producing tumors can be subclassified as being either noradrenergic or adrenergic based upon their pattern of catecholamine release.^{79,80} Noradrenergic PHEO/PGL secrete norepinephrine and normetanephrine, as seen in VHL disease and in tumors associated with the hereditary PGL/PHEO syndromes.^{19,22,59,79,81,82} Adrenergic tumors secrete both epinephrine and norepinephrine and their metabolites, and these tumors are more commonly PHEO that arise

sporadically or within the clinical context of MEN2 or neurofibromatosis type 1 (NF1).^{59,69,79,81,82} This differential secretion of catecholamines is caused by decreased expression of PNMT in noradrenergic tumors secondary to PNMT promoter hypermethylation.^{81,83}

Dopamine-secreting tumors are rare and are typically extraadrenal succinate dehydrogenase (*SDH*)x-associated PGLs, as well as head and neck PGLs.^{84–86} The measurement of methoxytyramine, a metabolite of dopamine, (see Fig. 15.3) may help to identify more aggressive tumors, particularly in the context of an *SDHx* mutation,⁸⁷ but this test is not widely available. A dopamine-secreting tumor should be considered in normotensive patients identified to have a mass that appears consistent with a PHEO/PGL, in which case dopamine and its metabolites, HVA and methoxytyramine (if available), should be measured.^{85,87–89} For prospective screening in patients with a known *SDHx* mutation, total catecholamines should be checked in addition to total metanephrines because of this possibility.

Chromogranin A is a major secretory protein present in the soluble matrix of chromaffin granules that serves as a tumor marker that may correlate with PHEO/PGL size and malignant potential.^{19,68,90} Chromogranin A also appears to be a useful marker in the biochemically silent, *SDHB*-related PGL,^{27,86} making it a potentially useful test in the screening of asymptomatic *SDHx* mutation carriers. However, chromogranin A can be falsely elevated in many circumstances, including concomitant use of proton-pump inhibitors and serotonin reuptake inhibitors, renal failure, and pancreatic disorders,⁹¹ so caution is needed in the interpretation.

Radiographic Studies

Once a diagnosis of catecholamine excess is established from biochemical testing, radiographic studies should be performed to identify the location of the tumor(s)^{26,71} (see Figs. 15.1, 15.2, and 15.4).

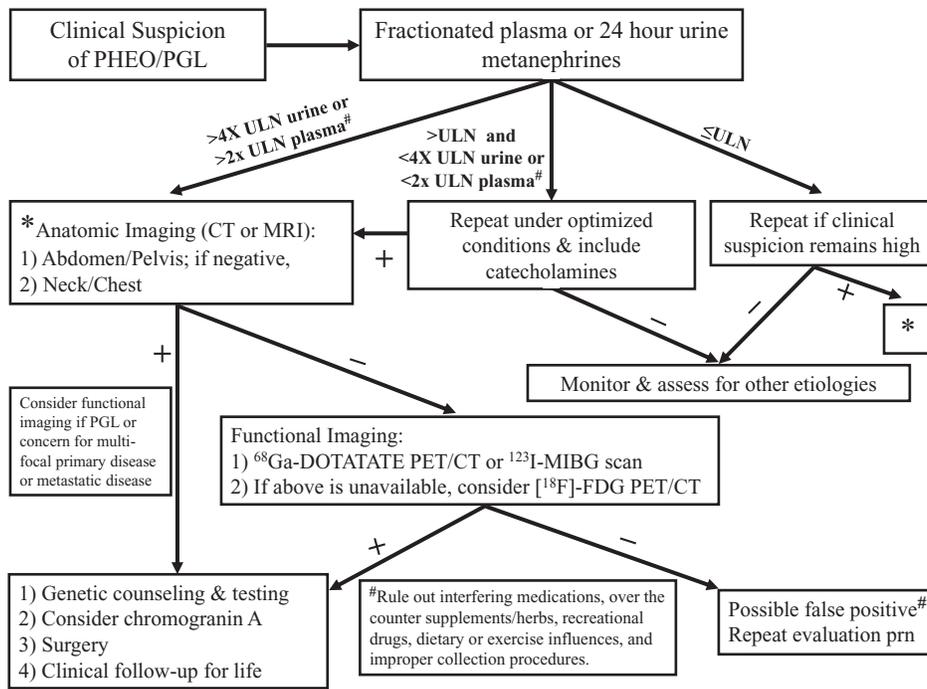


Fig. 15.4 Diagnosis of pediatric pheochromocytoma (PHEO) /paraganglioma (PGL). ULN, Upper limit of normal. (Modified from Waguespack, S.G., Rich, T., Grubbs, E., et al. (2010). A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. *J Clin Endocrinol Metabol*, 95(5), 2023–2037.)

Initial imaging studies should include anatomic cross-sectional imaging of the abdomen and pelvis (computed tomography [CT] or magnetic resonance imaging [MRI]), followed by imaging of the neck and chest if the initial studies are unrevealing^{25,69} (see Fig. 15.4). CT and MRI have similar diagnostic sensitivities, and so the imaging test of choice is best determined by local practices and patient preference.^{71,92} Abdominal ultrasound (US) may also be considered in young children, if local expertise permits (see Fig. 15.2). PHEO/PGL are highly vascular (and hence enhancing) neoplasms that commonly contain necrotic, cystic, and/or hemorrhagic areas. On CT, the noncontrast Hounsfield units are almost exclusively greater than 10, and on MRI, PHEO/PGL exhibit a classic hyperintense appearance on T2-weighted images (called the *lightbulb sign*) and no loss of signal intensity on in and out of phase imaging^{25,93} (see Fig. 15.2).

Nuclear medicine, or functional imaging can be useful in certain circumstances to diagnose PHEO/PGL (especially to confirm the diagnosis in an extraadrenal tumor) or to evaluate the patient for multifocal or metastatic disease, particularly in those patients with risk factors for malignancy and a noradrenergic phenotype.^{21,94–97} There are several nuclear medicine scans that can be helpful.⁹⁸ Metaiodobenzylguanidine (MIBG) is a synthetic compound that bears structural similarity to norepinephrine (except it also has a guanidine side chain that resists metabolism), and it accumulates preferentially in adrenergic tissues, namely because of reuptake via the norepinephrine transporter system.^{99,100} MIBG used for diagnostic purposes is radiolabeled with either ¹²³I or ¹³¹I, although ¹²³I MIBG is the agent of choice because of its superior imaging properties and substantially lower radiation dose because it is not a β emitter.^{98,103} ¹²³I MIBG scanning (see Fig. 15.1) can confirm the

catecholamine-producing nature of a tumor, localize tumors not seen with cross-sectional imaging, and potentially identify other sites of disease, although its sensitivity is not that high and therefore its use is limited for this reason, unless considering treatment with ¹³¹I-MIBG for metastatic disease.^{59,69,70,94,104} Before ¹²³I-MIBG scanning, care should be taken to ensure that the patient is not taking medications (e.g., decongestants, calcium-channel blockers, or labetalol) that are known to decrease MIBG uptake, and potassium iodide should be administered to block thyroid uptake of radioactive iodine.^{100,105} Because of the limitations of MIBG testing, the use of other nuclear imaging modalities that incorporate positron-emission tomography (PET) with CT imaging have become more commonplace, especially [18F] fluorodeoxyglucose (FDG) PET/CT and ⁶⁸Ga-DOTATATE PET/CT^{69,92,98,106–109} (see Fig. 15.2). ⁶⁸Ga-DOTATATE PET/CT has become the functional imaging modality of choice, given its specificity for somatostatin receptors commonly found on NETs, such as PHEO/PGL and its superior sensitivity over FDG-PET/CT and ¹²³I-MIBG imaging.^{97,106,108,109}

Genetic Issues

Knowledge regarding the hereditary causes of catecholamine-producing tumors is rapidly expanding.^{8,41,110} The syndromes most commonly associated with pediatric PHEO/PGL are VHL disease, followed by the hereditary PGL/PHEO syndromes, particularly those caused by mutations in *SDHB* (PGL4) and *SDHD* (PGL1), then *NF1* and *MEN2*^{10,11,20–22,31} (see Table 15.1 and separate sections later in the chapter). Additional genes associated with the development of PHEO/

PGL, but with a much lower prevalence, include fumarate hydratase (*FH*)¹¹¹; hypoxia-inducible factor 2 alpha (*HIF2A*, also known as [aka] *EPAS1*)¹¹²; kinesin family member 1B (*KIF1B*)¹¹³; *MEN1*¹¹⁴; MYC associated factor X (*MAX*)¹¹⁵; prolyl hydroxylase domain 1 (*PHD1*, aka *EGLN2*) and 2 (*PHD2*, aka *EGLN1*)^{116,117}; succinate dehydrogenase complex assembly factor 2 (*SDHAF2*)¹¹⁸; succinate dehydrogenase complex, subunit A (*SDHA*)¹¹⁹; succinate dehydrogenase complex, subunit C (*SDHC*)¹²⁰; and transmembrane protein 127 (*TMEM127*)¹²¹. Recently, even more genes have been described as associated with PHEO/PGL and further research is needed to determine causation. These include BRCA1-associated protein-1 (*BAP1*)¹²²; malate dehydrogenase 2 (*MDH2*)¹²³; MER protooncogene, tyrosine kinase (*MERTK*)¹²⁴; MET protooncogene (*MET*)¹²⁴; solute carrier family 25 member 11 (*SLC25A11*)¹⁰¹; and others. Family history, clinical presentation of the patient, and differences in the biochemical phenotype (noradrenergic versus adrenergic) help to prioritize genetic testing,^{39,83} but the availability of next-generation sequencing has led to the development of validated targeted gene panels that are now more commonly used,¹¹⁰ especially when testing an index case.⁸³

Based on genomic analysis, the PHEO/PGL susceptibility genes are now divided into two major clusters: cluster 1 (a pseudohypoxia subtype, subdivided into tricarboxylic acid [TCA] cycle-dependent and *VHL/EPAS1*-dependent) and cluster 2 (a kinase signaling subtype).^{41,111,125} Most pediatric PHEO/PGL will fall into cluster 1 (*SDHx*, *VHL*) instead of cluster 2 (*NF1*, *RET*).^{22,31} PHEO/PGL that arise in the context of *VHL* or the hereditary PGL syndromes (cluster 1) occur at younger ages and are typically noradrenergic tumors, producing almost exclusively norepinephrine and normetanephrine.^{19,22,82,83} Adrenergic tumors (which secrete epinephrine and metanephrine, in addition to norepinephrine and normetanephrine) are seen in cluster 2 (*MEN2*, *NF1*) and sporadic cases.

Management

Surgical Therapy

Surgical resection is the mainstay in the treatment of PHEO/PGL. A preoperative biopsy is not indicated and potentially dangerous.¹²⁶ The procedure of choice for most PHEO is laparoscopic adrenalectomy, either using transperitoneal or retroperitoneal approaches.^{59,127,128} Laparotomy should be contemplated in patients with large PHEO and/or a concern for underlying malignancy based upon the clinical presentation, genetic background, or radiographic appearance of the tumor.^{128,129} In the setting of bilateral PHEO or known hereditary PHEO, a cortical-sparing procedure should be performed to minimize the risk of the life long glucocorticoid and mineralocorticoid replacement and the attendant risks of primary adrenal insufficiency.^{102,129–134} Because it is extremely difficult to preserve a vascularized portion of adrenal cortex sufficient to prevent corticosteroid dependence without also leaving some residual adrenal medulla, there is a risk for recurrent PHEO in the remnant. Recent data suggest that recurrence rates after cortical-sparing surgery are 10% or less.^{102,131,132,135} The surgical approach for removal of a PGL depends upon the location of the tumor, but in selected cases of abdominal disease can also be performed laparoscopically. Head and neck PGL can be expectantly monitored, if small, or treated with surgery or radiation.^{129,136}

It is important that the anesthesiologist have experience with the intraoperative management of PHEO/PGL because

dysrhythmias can occur and blood pressures can be quite labile.^{137,138} Both intravenous antihypertensive medications (e.g., nitroprusside, phentolamine, esmolol, labetalol, etc.) and vasopressors (e.g., phenylephrine and norepinephrine) should be readily available for intraoperative use. The greatest risk for hypertension occurs during anesthesia induction and manipulation of the tumor, whereas hypotension is most likely to occur after ligation of the adrenal vein, when the abrupt decline in catecholamine concentrations leads to vasodilation.¹²⁹ Postoperatively, the patient should be monitored for the two major complications of hypotension and hypoglycemia.^{7,129,137,138} In patients who have had a cortical-sparing adrenalectomy in the context of bilateral PHEO resection, stress glucocorticoids should be provided perioperatively and a high-dose cosyntropin stimulation test obtained to determine the need for ongoing adrenal steroid replacement.

Medical Preparation for Surgery

Once the diagnosis of a PHEO/functioning PGL has been confirmed, medical therapy to normalize the blood pressure and mitigate the signs and symptoms of catecholamine excess should be initiated (Table 15.3). If surgery is planned, medical treatment should be taken for at least 10 to 14 days before surgery to minimize the potential complications that may arise from acute catecholamine surges.^{16,26,59,137} No universal algorithm exists for the medical management of a PHEO/PGL before surgery. For adults, the Endocrine Society guidelines recommend first-line therapy with an α -adrenergic receptor blocker and second-line therapy with a calcium channel blocker.⁵⁹ Limited data exist for children, but small studies suggest these regimens are safe.¹³⁹

Effective α -receptor blockade improves symptoms, lowers blood pressure, and expands the vascular bed and blood volume. Tachycardia without hypotension is a sign of good alpha blockade and should be treated with addition of a β -adrenergic receptor blocker rather than a dose reduction of the alpha blocker. Beta blockers should not be started without good alpha blockade because of the theoretical risk of reflex hypertension from uncontrolled α -adrenergic receptor activity. Phenoxybenzamine is a noncompetitive α -receptor antagonist that has a long half-life; therefore it may also increase the risk of postoperative hypotension.^{7,16,70,140} Selective α 1-receptor blockers, such as prazosin and doxazosin, can also be used.^{16,59,140,141} Although labetalol and carvedilol, drugs with both α - and β -receptor antagonist activity, sound like attractive options for preoperative blockade, they are not recommended for primary medical treatment because of their lower α -adrenergic receptor blockade relative to their β -antagonist activity.^{59,141} Symptomatic postural hypotension may be seen at the beginning of medical therapy, particularly with large biochemically active tumors, so it is imperative to start at low doses and increase the dose and/or frequency every few days, until the blood pressure is normal for age and height and the patient is minimally orthostatic. Phenoxybenzamine is only supplied as a single dose (10-mg capsule) and so it will need to be compounded by the pharmacy to allow for the administration of the lower doses needed in younger children. Phenoxybenzamine is also an expensive drug, which makes the selective α 1-receptor blockers a more attractive option in many cases.

Metyrosine is a competitive inhibitor of tyrosine hydroxylase, the rate-limiting step of catecholamine biosynthesis (see Fig. 15.3) and it can also be used as part of the preoperative preoperative regimen, although data in children are limited.^{141,142} However, not all centers use metyrosine routinely because of its

TABLE 15.3 Preoperative Medical Management of Pheochromocytoma/Sympathetic Paraganglioma

Drug Class	Drug	Mechanism of Action	Initial Pediatric Dose
α-adrenergic receptor blockers	Doxazosin	α ₁ -antagonist	0.5–1 mg daily
	Phenoxybenzamine	α ₁ - and α ₂ -antagonist	0.2–0.25 mg/kg/d divided BID (max 10 mg BID)
	Prazosin	α ₁ -antagonist	0.05–0.1 mg/kg/d divided TID (max 1 mg TID)
β-adrenergic receptor blockers	Atenolol	β ₁ - antagonist	0.5–1 mg/kg/d daily or BID (max 50 mg daily)
	Metoprolol	β ₁ - antagonist	1–2 mg/kg/d divided BID (max 50 mg BID)
Calcium channel blockers	Propranolol	β ₁ - and β ₂ -antagonist	1–2 mg/kg/d divided BID or TID (max 80 mg daily)
	Amlodipine	Calcium channel blocker	0.1 mg/kg/d (<6 years); 2.5–5 mg daily (age ≥6 years)
	Nifedipine (sustained release)	Calcium channel blocker	0.25–0.5 mg/kg/d daily or BID (max 60 mg total daily dose)
Inhibitors of catecholamine synthesis	Metyrosine	Tyrosine hydroxylase inhibitor	125–250 mg QID

BID, Twice per day; *QID*, four times a day; *TID*, three times per day.

cost and potential significant side effects (sedation, diarrhea, and extrapyramidal manifestations) and unclear benefit in most cases.^{39,129} A few days before surgery, oral salt loading (either via increased dietary intake or with sodium chloride tablets) is recommended to expand the blood volume to mitigate postoperative hypotension. Some centers also routinely admit patients for intravenous fluids before PHEO/PGL resection,¹⁴¹ and this can be considered for very symptomatic children with large tumors.

Prognosis and Follow-Up

The long-term prognosis of most children diagnosed with PHEO/PGL is excellent. In all cases, however, there is risk of the development of synchronous and/or metachronous tumors in both adrenal glands, as well as extraadrenal sites. In fact, up to 50% of patients diagnosed with a childhood PHEO/PGL will develop an additional primary tumor in their lifetime (25% at 9 years and 50% at 31 years postdiagnosis),¹¹ underscoring the need for life-long follow-up and appropriate genetic counseling and testing.⁶⁰

Malignancy in PHEO/PGL is defined as the presence of metastases in a site where chromaffin cells and paraganglia are not normally located (primarily bones but also lymph nodes, liver, and/or lungs).^{19,22,30} In fact, the most recent World Health Organization (WHO) guidelines removed the term malignant PHEO/PGL in favor of metastatic PHEO/PGL.⁶ There is no single histologic feature or immunohistochemical profile that is independently able to predict metastatic potential in a resected PHEO/PGL, but histopathologic scoring systems (Pheochromocytoma and the Adrenal Gland Scaled score¹⁴³ and grading system for adrenal PHEO and PGL^{144,145}) have tried to create predictions for features more frequently noted in metastatic tumors. Unfortunately, these scoring systems are limited by a lot of inter- and intraobserver variability.¹⁴⁶ The most reliable features of an increased risk of metastatic potential include extraadrenal location, size greater than 5 cm, and a germline *SDHB* mutation.^{11,55,147,148} The highest risk for malignancy and death in the pediatric population is in *SDHB*-related sympathetic PGL, which represents a majority of metastatic tumors.^{11,19–22,148,149}

Based upon data from a British tumor registry, the incidence of malignant disease in children is estimated to be 0.02 per million per year.¹² Approximately 9% to 16% of pediatric PHEO/PGL become metastatic,^{10,11,13,21} although some referral centers report a much higher malignancy rate (up to 65%) in pediatric PHEO/PGL,^{19,38} likely reflecting a referral bias. On the other hand, the latency period between diagnosis and confirmation of metastatic disease is 9 years on average¹⁹; thus the true rate of malignancy may be higher than previously recognized because it can only be identified during long-term systematic follow-up. Children with metastatic disease typically demonstrate a more indolent clinical course with average overall survival of more than 6 years after the diagnosis of metastatic disease.^{11,19}

Because PHEO/PGL can have unpredictable clinical behavior and because children are at risk for the development of metachronous primary tumors, delayed metastatic disease, and local recurrence, long-term follow-up with biochemical screening and intermittent imaging studies is required for all children with PHEO/PGL.^{11,12,39,60,63} For asymptomatic children with an identified genetic mutation predisposing them to the development of a PHEO/PGL, annual biochemical screening is advised, with the age of initial screening determined by the specific gene mutation (see Table 15.1). Furthermore, occasional cross-sectional imaging, typically MRI because of the lack of radiation exposure, is recommended periodically for follow-up of patients at high risk of recurrence or malignant disease (e.g., abdominal PGLs) or at risk for developing a PHEO/PGL that may not be identified on biochemical testing alone (e.g., the hereditary PGL-PHEO syndromes).

MEDULLARY THYROID CARCINOMA

MTC is a malignant NET that arises from the calcitonin-producing, parafollicular C-cells of the thyroid gland.^{6,150,151} In childhood, MTC comprises 5% or less of thyroid malignancies and has an annual incidence of 0.3 cases/million/year¹⁵²; it is the most common thyroid cancer in patients younger than 5 years of age. In the pediatric population, MTC has a fairly equal female:male ratio,¹⁵³ unlike the differentiated thyroid carcinomas, which are much more frequent in girls than

boys.¹⁵² Pediatric MTC almost always results from an inherited or de novo activating mutation in the *rearranged during transfection* (*RET*) protooncogene^{154–157} and is diagnosed in the context of either MEN2A or MEN2B, depending on the specific mutation.^{2,65,158–161} Because of the close association of MTC with a germline *RET* deoxyribonucleic acid (DNA) variant, genetic testing is recommended in all cases.⁶² Although sporadic nonhereditary tumors account for 70% to 75% of adult MTC cases,^{6,159} such tumors are rare in children. Sporadic MTC is primarily associated with somatic (not germline) mutations in *RET* (specifically the p.M918T mutation) and *RAS*.^{65,159,161}

RET is a member of the cadherin superfamily and encodes a receptor tyrosine kinase that has an extracellular binding domain and an intracellular tyrosine kinase domain (Fig. 15.5). The endogenous ligands (which activate *RET* via a high-affinity ligand-binding coreceptor, GFR α) are members of the glial cell derived neurotrophic factor (GDNF) family, which are involved in the regulation of neural tissue development.¹⁶² The *RET* protein, therefore plays a crucial role in the development of neural crest-derived cells, the urogenital system, and the central and peripheral nervous systems, notably the enteric nervous

system.^{163,164} In MEN2, ligand-independent constitutive activation of the *RET* receptor tyrosine kinase, either as a dimer (because of mutations in the extracellular cysteine-rich domain; MEN2A) or monomer (because of mutations in the intracellular tyrosine kinase domain; MEN2B), stimulates multiple downstream pathways that promote cell growth, proliferation, survival, and differentiation.¹⁶¹

Hereditary MTC is typically multifocal, bilateral, and located in the middle to upper regions of the thyroid lobes (Fig. 15.6), an area where C-cells are the most highly concentrated.^{6,151,165} Microscopic examination and calcitonin staining of the thyroid will often identify C-cell hyperplasia, the initial stage in an oncologic cascade that leads to the development of microscopic noninvasive MTC and ultimately lymph node and distantly metastatic disease because of frankly invasive carcinoma^{2,151,166–168} (see Fig. 15.6). Patients with familial MTC have an age-related progression of malignant disease, with lymph node and distant metastases typically occurring years after the onset of C-cell hyperplasia.^{166,168} The cervical and mediastinal lymph node basins are the most common sites of metastatic disease, whereas distant sites for MTC spread include the lungs, liver, and bone/bone marrow.

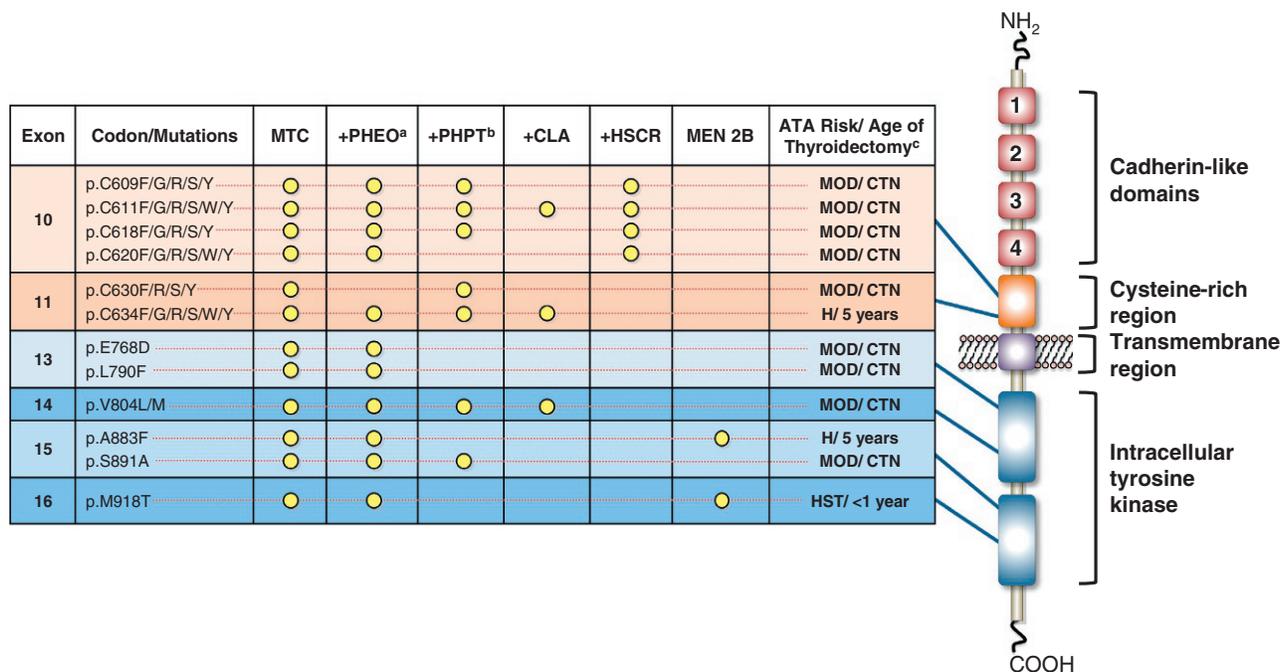


Fig. 15.5 The *RET* receptor tyrosine kinase, commonly mutated *RET* codons, associated MEN2 phenotypes, and the 2015 ATA risk stratification and recommendations regarding the timing of thyroidectomy and prospective screening for other MEN2 manifestations.⁶⁵ ATA, American Thyroid Association; CTN, basal serum calcitonin level; CLA, cutaneous lichen amyloidosis; H, ATA high risk; HSCR, Hirschsprung disease; HST, ATA highest risk; MEN2, multiple endocrine neoplasia Type 2; MOD, ATA moderate risk; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism. ^a Annual screening for PHEO is recommended at age 11 years for mutations in *RET* codons 634, 883, and 918 and at age 16 years for *RET* codons 609, 611, 618, 620, 768, 790, 804, and 891. Patients with mutations not yet known to be associated with PHEO can be screened periodically. ^b Annual screening for PHPT is recommended at age 11 years for mutations in *RET* codon 634 and at age 16 years for *RET* codons 609, 611, 618, 630, 804, and 891. Patients with mutations not yet known to be associated with PHPT can be screened periodically. ^c Age of thyroidectomy is the age by which surgery should be considered for presymptomatic *RET* carriers. The age of testing for the presence of a *RET* mutation should be pursued at an earlier age after appropriate genetic counseling and based upon the family's wishes. CTN denotes that timing of thyroidectomy can be determined by the basal serum calcitonin level, in addition to the preference of the parent/guardian after multidisciplinary input. For a more comprehensive list of *RET* mutations, the reader is referred elsewhere.^{65,327} (Modified from Waguespack, S.G., Rich, T.A., Perrier, N.D., Jimenez, C., Cote, G.J. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol*, 7(10), 596–607.)

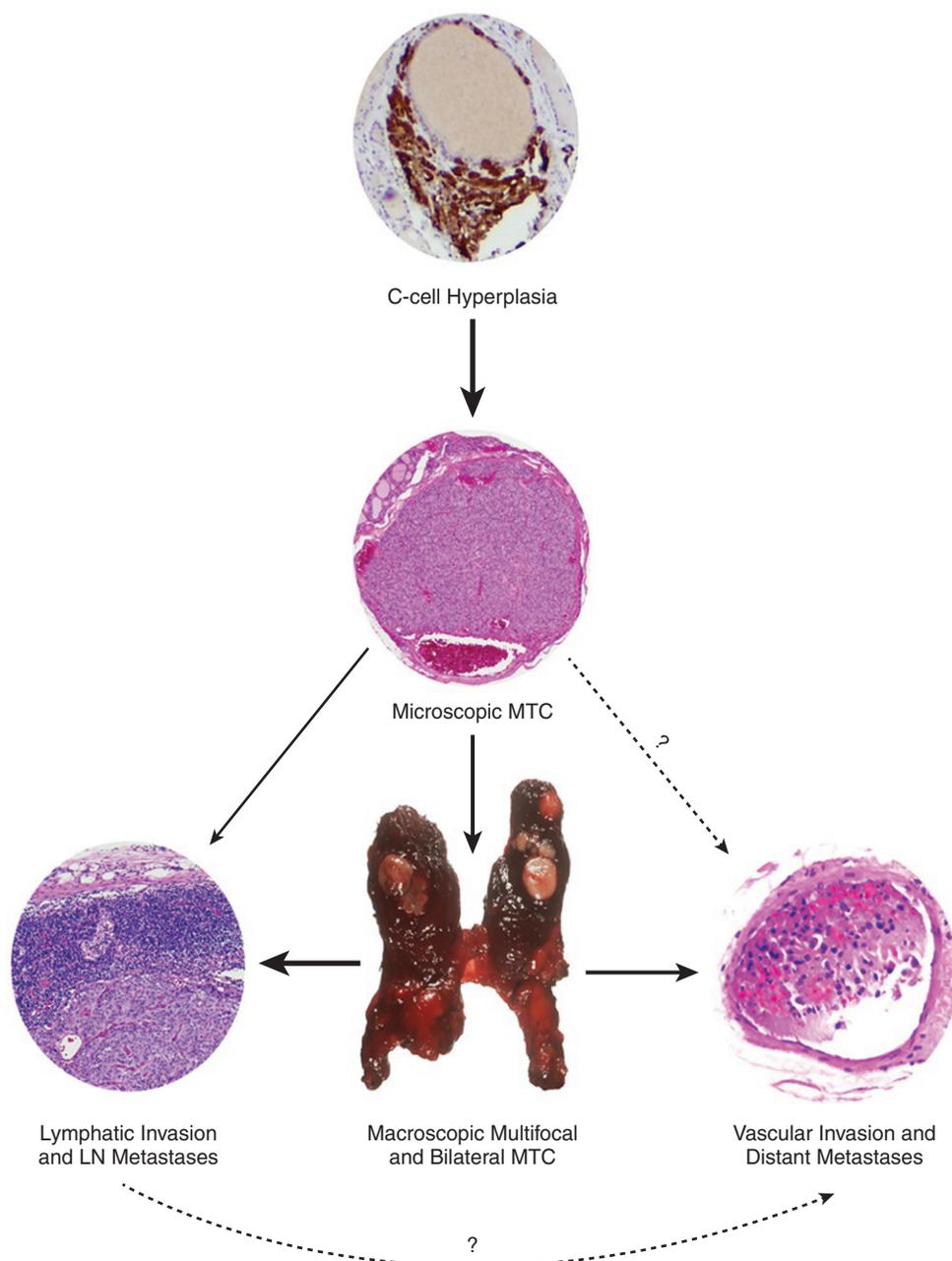


Fig. 15.6 A germline *RET* mutation causes C-cell hyperplasia, the initial stage in an oncologic cascade that ultimately leads to the development of microscopic noninvasive MTC and possibly lymph node and distant metastatic disease caused by frankly invasive carcinoma. The weight of the arrows denotes the hypothetical probability of the event occurring in the typical pediatric patient with a *RET* codon 634 mutation. *LN*, Lymph node; *MTC*, medullary thyroid carcinoma. (From Waguespack, S.G., Rich, T.A., Perrier, N.D., Jimenez, C., Cote, G.J. (2011). Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol*, 7(10), 596–607.)

Clinical Presentation

Children with sporadic MTC present similar to other thyroid malignancies, typically with a palpable thyroid nodule and/or cervical lymphadenopathy; diarrhea also can rarely be the initial clinical presentation (primarily attributed to extremely high calcitonin levels). On the other hand, children with hereditary disease because of a germline *RET* mutation less frequently present with overt clinical disease, except for MTC associated with MEN2B, which is a diagnosis that remains uniformly delayed.^{169,170} Therefore in the current era, the

predominant presentation of MTC during childhood is one of the presymptomatic identification of a *RET* mutation and the ultimate identification of microscopic MTC after early thyroidectomy.

Evaluation and Management

There are several guidelines and review papers available to assist the clinician in the specific evaluation and management of the child with suspected or proven MTC.^{2,64,65,159,160,171–173} Similar to other pediatric thyroid malignancies, surgery is the

cornerstone of therapy and the only chance for MTC cure. Given the higher surgical complication rates in children¹⁷⁴ and the unique aspects of the medical and surgical care of patients with MTC, treatment should be pursued at tertiary care centers with multidisciplinary, high-volume expertise.^{65,173,175} Apart from the decision of when to intervene, the thyroid surgeon must incorporate knowledge regarding the patient's *RET* genotype and clinical data into the decision-making process to determine the optimal surgical approach.² Meticulous and safe removal of all thyroid tissue, including the posterior capsule, is the goal of a prophylactic/early thyroidectomy for the MEN2 patient without clinical MTC.¹⁷¹ Routine central compartment (level VI) neck dissection is not performed in the setting of a purely prophylactic procedure because lymph node metastases are exceedingly rare in that setting.^{65,166,171,176–179} However, if the operation is for a structurally evident hereditary tumor (especially if the calcitonin level exceeds 40 pg/mL) or for sporadic MTC, total thyroidectomy and a concomitant central neck dissection should be performed.⁶⁵ Dissection of the lateral cervical lymph node compartments (levels IIA–V) is generally performed, only in cases where there is clinical evidence of lateral neck involvement, although some centers base this decision on the preoperative calcitonin level.

Fortunately, life-threatening MTC rarely occurs during childhood and primarily only in the clinical context of MEN2B. In advanced MTC, response rates to traditional cytotoxic chemotherapy (dacarbazine, vincristine, and cyclophosphamide) are low. More recently, two targeted molecular therapies (vandetanib and cabozantinib) that inhibit *RET* and other receptor tyrosine kinases have been approved in adults for the treatment of metastatic MTC.^{159,180} Studies using these drugs in children with MEN2B have suggested comparable efficacy and safety.^{181–183} Novel therapeutics that selectively inhibit the *RET* kinase in tumors with a *RET* mutation or fusion are showing promise in clinical trials that commenced in 2017.¹⁸⁰

Prognosis

Overall, the prognosis of childhood MTC is good, with 5- and 15-year survival rates of 97% and 89%, respectively.¹⁸⁴ In general, positive lymph node status and a higher stage at diagnosis predict lower disease-free survival and higher mortality in MTC.^{153,176,179,185,186} As the tumor stage increases, the risk of both locoregional and distant metastases rises,^{153,187} although lymph node metastases may still occur when the tumor is less than 1 cm in size.^{188,189} Patients with MEN2B have an especially guarded prognosis, with a fifth of patients dying at a median age of 25 years (range <1–59 years), albeit not always caused by MTC.¹³¹

Many children who present with clinical MTC already have metastatic disease at diagnosis. Consequently, the majority of cases of childhood MTC not diagnosed before lymph node metastases occur represent incurable yet indolent cancers. The aggressiveness of the clinical course can be predicted by the specific *RET* mutation (see MEN2 section later), the child's clinical presentation, and by the use of the serologic tumor markers, calcitonin and carcinoembryonic antigen (CEA). The loss of calcitonin expression, a CEA level out of proportion to calcitonin, and a rapid CEA and/or calcitonin doubling time are all harbingers of an aggressive disease course.^{65,173,185,190,191}

HEREDITARY ENDOCRINE NEOPLASIA SYNDROMES

Carney Complex

First described in 1985,¹⁹² the Carney complex (CNC) is a genetically and clinically heterogeneous familial lentiginosis syndrome with autosomal dominant inheritance. It is characterized by blue nevi and/or spotty skin pigmentation affecting the lips, conjunctiva, and other mucosal surfaces (Fig. 15.7); myxomas of the breast, heart, and skin; endocrine tumors and/or



Fig. 15.7 Carney complex and primary pigmented nodular adrenocortical disease (PPNAD). A, A 10-year-old female with adrenocorticotropic hormone (ACTH)-independent Cushing syndrome and the Carney complex demonstrates facial rounding, plethora, and classic facial lentiginosis. B, Gross pathologic specimen of the right adrenal gland in another patient with PPNAD and the Carney complex.

overactivity, classically primary pigmented nodular adrenocortical disease (PPNAD)¹⁹³ and pituitary growth hormone/prolactin hypersecretion; and psammomatous melanotic schwannoma, among other clinical manifestations.^{192,194–196} Two or more distinct genetic loci are associated with CNC: the gene *PRKARIA*^{197,198} on chromosome 17q24.2 and an unknown gene on chromosome 2p16.¹⁹⁹ *PRKARIA* encodes for the RI- α subunit of protein kinase A, the major mediator of intracellular cyclic adenosine monophosphate (cAMP) signaling. Most *PRKARIA* mutations are inactivating point mutations, and about 30% of CNC patients have de novo disease.²⁰⁰ In patients with CNC, the overall *PRKARIA* mutation detection rate is 62%.²⁰⁰

The major endocrine phenotype of the CNC is PPNAD, occurring either alone or with other CNC manifestations, in 60% of cases²⁰⁰; adrenocortical carcinoma also has been rarely reported.²⁰¹ PPNAD is a rare form of ACTH-independent hypercortisolism pathologically associated with multiple small (<1 cm) black or brown nodules (containing lipofuscin) in an otherwise atrophic cortex.^{193,201} (see Fig. 15.7). The median age of onset is 34 years (with about 20% of cases occurring during childhood), and there is a female predominance after puberty.²⁰⁰ The hypercortisolism is typically insidious in its onset, and a pathognomonic finding in PPNAD-related Cushing syndrome is the paradoxical increase in glucocorticoid excretion on the second day of high-dose dexamethasone administration during the Liddle test.²⁰² Adrenal imaging is of limited value for the confirmation of PPNAD because the lesions are small, but it can suggest diffuse micronodular disease on contrast-enhanced CT.^{203,204} The treatment of symptomatic PPNAD is usually bilateral adrenalectomy, although this may not be necessary in all patients.²⁰¹

Growth-hormone hypersecretion (causing gigantism or acromegaly, depending on the age of onset) is the primary pituitary phenotype of the CNC, occurring in 10% to 12% of CNC patients.^{192,200} Pathologically, this may be associated with an adenoma or hyperplasia.^{205–207} Concomitant hyperprolactinemia is prevalent and is similarly caused by underlying hyperplasia or frank adenomas in rare cases.^{205,208} Subtle abnormalities in growth hormone and prolactin secretion may be identified in up to 75% of patients with CNC.²⁰⁶ Corticotroph adenomas have also been described in rare cases.^{207,209} Thyroid neoplasia occurs in 25% of CNC patients with most being benign nodules, although differentiated thyroid carcinoma (both papillary and follicular carcinomas) have been identified in 2.5% of cases.²⁰⁰ Involvement of the thyroid occurs around a median age of 33 years (range 12–57 years) and can be associated with thyrotoxicosis.²¹⁰ Males with CNC are at risk for testicular tumors, primarily bilateral large-cell calcifying Sertoli cell tumors (LCCSCT), clinically identified in 33% to 41% of patients.^{200,206} Similar to the Peutz-Jeghers syndrome (see later), gynecomastia and/or testicular enlargement may be a clinical presentation of LCCSCT,²¹¹ which can be malignant in very rare cases.¹⁹⁵

Once CNC is diagnosed clinically or an asymptomatic child is found to harbor a germline *PRKARIA* mutation, prospective monitoring for the development of the tumors and endocrine disorders characteristic of the syndrome can be undertaken using published recommendations for screening.^{195,196,206}

Familial Isolated Pituitary Adenomas

Hereditary pituitary adenomas can occur as a distinct entity within families who do not have MEN1, and the term *familial isolated pituitary adenomas* (FIPA) was first proposed in 2006.²¹²

FIPA is defined by the occurrence of pituitary adenomas in two or more members of the same family with no other clinical manifestations suggestive of MEN1 or CNC.²¹³ With all hormonal subtypes represented, pituitary tumors occurring within FIPA families present similarly to sporadic pituitary adenomas, and there can be significant intrafamilial clinical heterogeneity.²⁰⁷

AIP-Associated Familial Isolated Pituitary Adenomas

In a subset of FIPA kindreds, the disorder was ultimately identified to be caused by germline mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene (chromosome 11q13.2).^{214,215} Mutations in *AIP* represent only 15% to 30% of FIPA cases and disease penetrance is incomplete, estimated to be less than 30%.^{207,213,216} FIPA individuals with *AIP* mutations have tumors that are larger (overwhelmingly macroadenomas) and diagnosed at a much younger age compared with *AIP*-negative patients.^{215,217,218} Median age of diagnosis is 16 to 23 years and 50% to 60% of cases present during childhood or adolescence.^{217,218} The vast majority of *AIP*-mutated adenomas are somatotroph or mammosomatotroph adenomas, which typically present with gigantism in up to 30% of cases (especially males) and are more recalcitrant to the typical therapies prescribed for gigantism/acromegaly.^{207,213,215,217} In patients younger than age 18 years presenting with an apparently sporadic pituitary macroadenoma, especially a somatotroph adenoma, 20% may harbor a germline *AIP* mutation.²¹⁹ In a large series of pediatric patients with macroprolactinomas, 9% of tested cases were found to have an *AIP* mutation, and these children responded similarly well to a dopamine agonist as sporadic cases.²²⁰

Given the relatively recent characterization of *AIP*-related FIPA, there are no evidence-based consensus guidelines as to the timing of genetic testing for *AIP* mutations in sporadic pituitary adenoma cases or for at-risk members of an affected kindred with an *AIP* mutation. Recommendations for prospective clinical screening in asymptomatic *AIP*-mutation carriers are also not well established, but some experts have proposed an approach to care that includes educating patients about symptoms of pituitary disease and performing an annual hormonal and auxologic evaluation.^{213,216,221} Because of the low clinical penetrance of *AIP* mutations and the high likelihood of functioning pituitary tumors, which most likely would be identified via biochemical testing and review of the growth chart in children, it is unclear if routine screening MRI is indicated.

Hereditary Paraganglioma-Pheochromocytoma Syndromes

Hereditary paraganglioma-pheochromocytoma syndromes (HPS) are characterized primarily by the development of parasympathetic and sympathetic PGL and PHEO.⁴⁰ Five autosomal dominantly inherited HPS syndromes (PGL1-5) have been described (see Table 15.1) and are caused by germline mutations in discrete genes encoding any of the subunits of the succinate dehydrogenase enzyme gene (*SDHA*, *SDHB*, *SDHC*, and *SDHD*) or a protein necessary for the flavination of *SDHA* (*SDHAF2*): PGL1 (*SDHD*),²²² PGL2 (*SDHAF2*),¹¹⁸ PGL3 (*SDHC*),¹²⁰ PGL4 (*SDHB*),²²³ and PGL5 (*SDHA*).¹¹⁹

The *SDHx* genes comprise the subunits for complex II of the mitochondrial respiratory chain that, when mutated, lead to stabilization of hypoxia inducible factor 1 with a subsequent state of pseudohypoxia.²²⁴ The clinical phenotype somewhat

differs among the five syndromes and remains poorly characterized in some cases, such as PGL5 (*SDHA*). As an example, mutations in *SDHD* primarily cause benign parasympathetic head and neck PGL (also referred to as *glomus tumors* or *chemodectomas*), whereas mutations in *SDHB* are associated more with abdominal PGL and a higher risk of malignancy (see Table 15.1 and Fig. 15.2). In all cases, penetrance of the clinical phenotype is less than 100% and increases with age.^{28,40,55,225–228} Mutations in *SDHD* (and also *SDHAF2*) demonstrate parent-of-origin effects, with disease occurring only when the mutation is inherited from the father,^{40,229} although recent reports of PHEO/PGL development in individuals with maternal inheritance of an *SDHD* mutation have been published.²³⁰ Besides PGL and PHEO, *SDHx*-related tumors include gastrointestinal stromal tumors (GIST), a finding that has also been denoted the Carney-Stratakis syndrome or dyad.²³¹ Other nonparanglial neoplasms attributed to an *SDHx* mutation include clear cell renal cell carcinoma, pituitary adenomas, and other NETs.^{28,40,207,225,232,233} The association of PHEO/PGL and pituitary adenoma occurring in the context of a germline *SDHx* mutation has been named the “3PAs.”²³⁴ The optimal screening program for children with HPS (see Table 15.1) remains poorly defined but generally includes annual physical examination/blood pressure monitoring, annual plasma or urine metanephrines, and biennial whole body MRI.^{55,63,235} Similar to presymptomatic screening in other tumor-predisposition syndromes, MRI is the preferred imaging modality in HPS.²³⁶

Hyperparathyroidism-Jaw Tumor Syndrome

The hyperparathyroidism-jaw tumor (HPT-JT) syndrome is a heritable disorder with incomplete penetrance characterized by parathyroid neoplasia/primary hyperparathyroidism (PHPT) associated with ossifying fibromas of the maxilla and/or mandible. First reported in 2002, *CDC73* (chromosome 1q31.2; formerly known as *HRPT2*) is the only known gene in which mutations cause HPT-JT syndrome, and *CDC73* mutations are identified in about 60% of HPT-JT syndrome kindreds.²³⁷ PHPT caused by a single benign parathyroid adenoma is the most common clinical manifestation, but parathyroid carcinomas are prevalent, representing 10% to 15% of cases.²³⁸ The youngest age of diagnosis of PHPT has been age 7 years.²³⁹ Ossifying fibromas, also known as *cementoossifying fibromas*, can be locally aggressive and occur in 30% to 40% of individuals with HPT-JT syndrome²³⁸; they are chiefly treated with surgical extirpation. Other clinical manifestations of the HPT-JT syndrome include renal lesions (cysts, hamartomas, and nephroblastoma [Wilms tumor]) and uterine tumors.^{240,241} Prospective clinical monitoring of asymptomatic individuals with a *CDC73* mutation is recommended (starting at age 5–10 years) and includes annual biochemistries to assess for PHPT and imaging (panoramic dental x-ray and renal US) every 5 years; female adolescents of reproductive age should get a pelvic US if any abnormal uterine bleeding occurs.^{64,238}

Multiple Endocrine Neoplasia Type 1

MEN1 is an inherited tumor syndrome characterized by glandular hyperplasia and benign or malignant neoplasms occurring in two or more endocrine glands, classically the parathyroids, pituitary, and neuroendocrine pancreas^{242–247} (Table 15.4). MEN1 tumors can be hormonally active or nonfunctioning and multifocality is common, except for pituitary tumors. Patients are also at risk for developing adrenocortical tumors (ACTs), PHEO, extraabdominal NETs (thymus

and bronchopulmonary), benign tumors of the skin (angiofibromas, collagenomas, lipomas), central nervous system (CNS) tumors (meningiomas and ependymomas), and/or uterine leiomyomas.^{114,242,246–250} Breast cancer risk in women with MEN1 may be heightened.²⁵¹

The hereditary nature of multiple endocrine adenomas and the link to a monogenic disorder was first proposed by Wermer in 1954.^{247,252} The precise genetic cause of the syndrome was identified and confirmed in 1997.^{253–256} The *MEN1* gene is a tumor suppressor gene that encodes a ubiquitous nuclear protein (menin), which plays a role in transcriptional regulation, genome stability, cell division, and proliferation.^{245,257,258} Most patients with MEN1 inherit the mutation from an affected parent, but about 10% of individuals with MEN1 represent de novo disease;^{255,257,259} about one-quarter of patients younger than age 21 years will represent index cases.^{249,260}

MEN1 represents a prime example of the “two-hit” mechanism of disease (Fig. 15.8), which was first described by Knudson in hereditary retinoblastoma²⁶¹ and is applicable to any disease caused by inactivating mutations of a tumor suppressor gene. An inherited heterozygous germline *MEN1* mutation is insufficient to induce tumor formation. Consequently, a somatic mutation (the second hit) in the wild-type *MEN1* allele is required to cause disease. This second hit usually deletes the only normally functioning *MEN1* gene, leading to a loss of heterozygosity at the *MEN1* locus in tumor DNA and attenuating the ordinary constraints by menin on cell growth. More than 90% of tumors from MEN1 patients have loss of heterozygosity caused by a subchromosomal rearrangement or deletion of the entire chromosome; other mechanisms for the second hit include point mutations and small insertions/deletions within the wild-type *MEN1* gene.^{244,245,257} To date, there have been several hundred unique germline mutations of the *MEN1* gene characterized, and these occur via multiple mechanisms and are distributed throughout the *MEN1* gene without hot spots.^{258,262} Testing by direct DNA sequencing identifies most *MEN1* mutations, but approximately 5% to 10% of people with MEN1 do not have a mutation in the coding region or splicing sites of *MEN1*.²⁵⁷ Such patients may represent a phenocopy (see section on MEN4) or they may harbor a mutation in untranslated regions or introns or a large gene deletion, the latter of which requires other technologies, such as multiplex ligation-dependent probe amplification (MLPA) to detect.²⁵⁸

Clinical Presentation and Management

Most children will be diagnosed with MEN1 after the identification of a germline *MEN1* mutation, as a result of cascade genetic testing in a known MEN1 family.²⁶³ The clinical presentation of MEN1-related disease is highly variable, even among members of the same kindred, and will depend on the location and functionality of the underlying tumor(s). Not surprisingly, functional tumors typically present 5 to 10 years earlier than nonsecretory neoplasms.²⁶⁴ Despite the variable expressivity, there is almost complete penetrance of the phenotype, such that MEN1 clinical and biochemical manifestations will generally develop in 80% and more than 98% of patients, respectively, by the fifth decade of life.^{243,248,259,264} Penetrance of the clinical phenotype in children with MEN1 has been previously estimated to be less than 1% before age 5 years, 7% by age 10 years, 28% by age 15 years, and 52% by age 20 years²⁵⁹ (Fig. 15.9). Most of the more recent studies found similar high penetrance in childhood. In two studies, 73% to 78% of patients younger than age 21 years had at least one MEN1

TABLE 15.4 Earliest Clinical Manifestations and Suggested Biochemical/Imaging Screening Protocol for Neuroendocrine and Adrenocortical Tumors in Children with Multiple Endocrine Neoplasia 1^a

Tumor	Earliest Age of Diagnosis (Years) With Reference	Age to Begin Screening (Years)	Clinical Signs/Symptoms of Disease	Biochemical ^b and Imaging Tests Used for Screening Asymptomatic Patients	
Pituitary	5 ^{c,460}	5–10	No symptoms, headaches, visual loss, abnormal linear growth, delayed puberty, galactorrhea, menstrual irregularity	Prolactin, IGF-1	Pituitary MRI (start at 15 years and then every 3 years)
Parathyroid	4 ^{d,249} 8 ^{e,249}	5–10	No symptoms, nephrolithiasis, polyuria, difficulty concentrating, fatigue, poor appetite, abdominal pain, constipation	Total calcium, intact PTH	No routine imaging indicated
Thymic	16 ²⁷⁷	15	No symptoms, chest/shoulder pain, cough, dyspnea, wheezing, hemoptysis	No routine biochemical testing (unless lesion or clinical syndrome identified)	Chest MRI (start at 15 years and then every 3 years)
Bronchial	15 ²⁶⁵				
Gastric	NA	NA	No symptoms	No routine biochemical testing	No routine imaging
Duodenal/pancreatic					
-Insulinoma	5 ²⁴⁹	5	Confusion, loss of consciousness, neuroglycopenic symptoms, weakness, seizures	Glucose, insulin	No routine imaging (only once diagnosis made)
-Gastrinoma	7 ²⁴⁹	15	Peptic ulcer disease, dyspepsia, abdominal pain, diarrhea	Gastrin ^f	No routine imaging (will typically not identify duodenal tumors)
-Nonfunctional	12 ⁴⁶¹	15	No symptoms	Chromogranin A, pancreatic polypeptide ^g	Abdominal MRI (start at age 15 years and then every 3 years) or endoscopic ultrasound
-Other functional	NA	NA	Diabetes, skin rash, weight loss, stomatitis, anemia (glucagonoma) Watery diarrhea, hypokalemia, achlorhydria (VIPoma)	No routine biochemical testing	No routine imaging
Adrenocortical	3 ²⁴⁹	15	Virilization (primary sign), Cushing syndrome, hypertension, abdominal/back pain	No routine biochemical testing (unless lesion or clinical syndrome identified)	Abdominal MRI (done in conjunction with screening for pancreatic NETs)

IGF-1, Insulin-like growth factor 1; MEN, multiple endocrine neoplasia; MRI, magnetic resonance imaging; NETs, neuroendocrine tumors; PTH, parathyroid hormone; PHPT, primary hyperparathyroidism.

^aData from consensus guidelines and other relevant publications.^{64,246,249,262,265,289,293,312} The age to start and the frequency of radiologic screening is not an absolute and should be tailored to patient symptoms and whether or not they already have clinical manifestations of MEN1. In current practice, the authors typically start clinical and biochemical screening at age 5 years and imaging in asymptomatic patients around age 15 years.

^bScreening laboratories should always be done after an overnight fast.

^cThis patient had a mammosomatotroph tumor, cosecreting prolactin and growth hormone.

^dBiochemical PHPT only.

^eSymptomatic PHPT.

^fGastrin levels will be elevated in patients taking a proton-pump inhibitor, which should be stopped at least 7 days before testing.

^gThese tests have a low diagnostic accuracy but, if significantly elevated, should prompt imaging.

clinical manifestation.^{249,260} In another series, 54.5% of children monitored using a specific screening program developed at least one clinical manifestation of MEN1 by a median age of 22.5 years (range 12–31 years), 83% of whom were clinically asymptomatic.²⁶³ However, another study documented a much lower penetrance (12%) of the MEN1 clinical phenotype before the age of 19 years.²⁶⁵ In contrast with MEN2, as discussed subsequently, there are no clear genotype-phenotype correlations in MEN1, so a provider cannot rely on the specific mutation or family history to predict the age of onset, severity, or type of clinical manifestation.^{245,259,264}

In the absence of treatment, MEN1-associated endocrine tumors are associated with higher mortality (50% probability of death by the age of 50 years.), and the cause of death in 50% to 70% of patients with MEN1 is usually a malignant tumor process or sequela of the disease.^{246,266,267} Currently, the greatest risk of mortality in MEN1 is chiefly from malignant duodenopancreatic and thymic NETs.^{246,249,264,266}

The specific management of each MEN1-associated endocrine neoplasm is generally similar to that for the respective sporadic tumors occurring in patients without MEN1, with notable exceptions for the parathyroid and pancreas (see later).

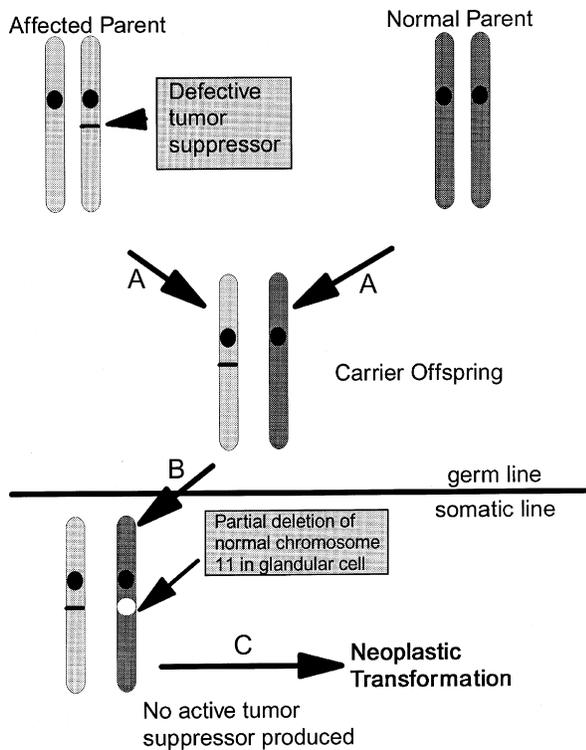


Fig. 15.8 “Two-hit” theory of tumorigenesis as exemplified by multiple endocrine neoplasia (MEN)1. A, An affected parent passes a mutated *MEN1* gene to an offspring, who inherits a normal (wild-type) gene from the other parent. B, Loss of heterozygosity in a somatic cell (a “second hit” that typically occurs via a subchromosomal rearrangement or deletion of the entire chromosome) disrupts the function of the remaining normal *MEN1* allele. C, The absence of menin tumor suppressor activity in a cell then leads to tumor formation.

This section will therefore focus more on the unique aspects of these disorders as they relate to MEN1 in children. As with most hereditary tumor syndromes, patients with MEN1 and their families should be managed by a multidisciplinary team consisting of relevant specialists with experience in the management of endocrine tumors.^{246,247}

Primary Hyperparathyroidism

PHPT is the most prevalent and earliest endocrine manifestation of MEN1.^{248,249,264} It is diagnosed in 75% to 91% of cases of MEN1 patients with clinical manifestations before age 21 years, with or without other MEN1 manifestations; most diagnoses are made after 10 years of age.^{248,249,263,265} MEN1 patients have multigland parathyroid hyperplasia rather than single gland adenomas. The majority of children with MEN1-related PHPT are asymptomatic, and nephrolithiasis is the major presenting sign of symptomatic disease.^{249,263}

The diagnosis of PHPT is based on an elevated serum calcium level in the setting of an inappropriately normal or frankly elevated PTH level; at times, the PTH can be elevated with a normal calcium level.²⁶³ Given the multifocal parathyroid hyperplasia in MEN1 patients, preoperative imaging is generally not beneficial.^{251,268} However, in the case of reoperation, localization studies, such as technetium (^{99m}Tc) sestamibi scan (typically with concomitant single-photon emission CT [SPECT] imaging), US, contrast-enhanced CT, four-dimensional (4D)-CT, or less commonly MRI can be helpful for surgical planning.^{269–271}

Surgical removal of the hyperfunctioning parathyroid glands is the definitive treatment of PHPT in MEN1 patients,

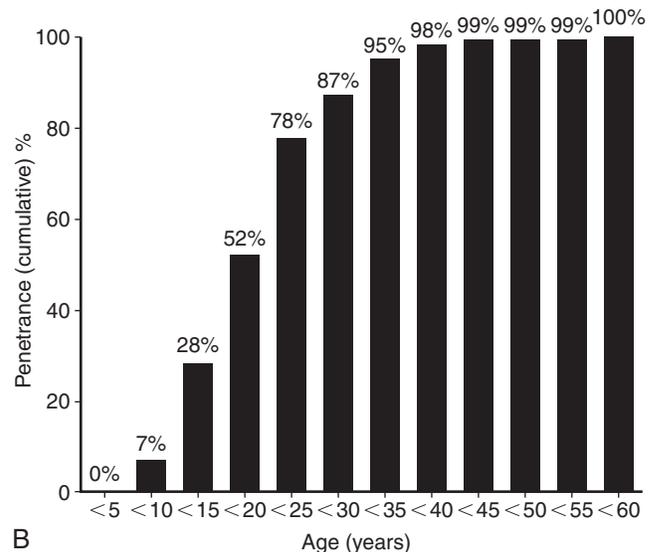
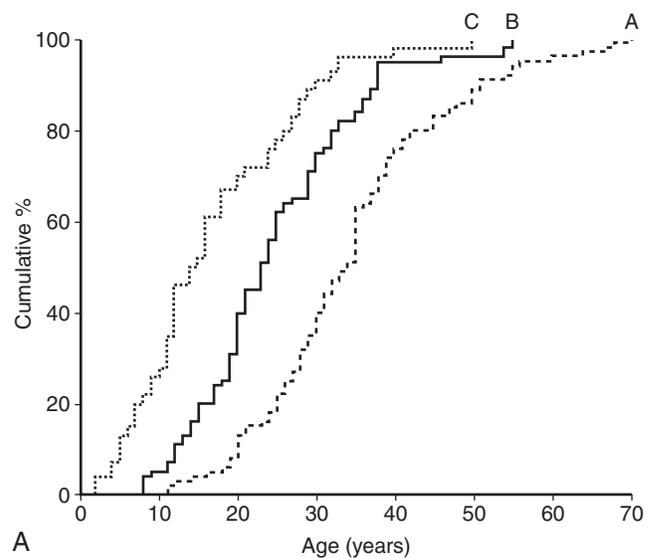


Fig. 15.9 Penetrance of the multiple endocrine neoplasia (MEN)1 phenotype. Age distributions (A) and age-related penetrance (B) of MEN1 clinical manifestations in 201 carriers of a mutated *MEN1* gene. Group A patients presented with clinical symptoms at the age depicted. Group B patients were asymptomatic but had positive biochemical screening at the age depicted. Group C patients were asymptomatic with negative biochemical screening with the age of their last biochemical testing shown. Patients in Groups B and C were significantly younger than Group A ($P < .001$). The age-related penetrance (B) is the proportion of mutation carriers who have developed a clinical manifestation by a given age. (From Bassett, J.H., Forbes, S.A., Pannett, A.A., et al. (1998). Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet*, 62(2), 232–244).

but the surgical approach remains controversial.^{242,272} Subtotal (3.5 gland) parathyroidectomy with transcervical thymectomy or total (four gland) parathyroidectomy with transcervical thymectomy and parathyroid autotransplantation to the nondominant forearm are the primary options for surgical therapy. Resection of less than three glands results in the highest risk of persistent and recurrent disease (odds ratio [OR], 3.11; 95% confidence interval [CI] = 2.00–4.84).^{273,274} Although the risk for persistent or recurrent HPT is not significantly different between a subtotal versus total procedure,

the risk for permanent hypoparathyroidism is significantly lower with subtotal parathyroidectomy,²⁷³ which makes this the preferred approach.^{246,272} However, some studies have shown equivalent outcomes between the two procedures in young patients.²⁷⁵ In patients operated for PHPT before age 21 years, 67% remain normocalcemic at last follow-up, whereas 22% have persistent hypercalcemia and 11% have hypoparathyroidism.²⁴⁹ At the initial surgery, concomitant transcervical thymectomy is recommended to remove any potential ectopic parathyroid glands and to prophylactically remove the bulk of the thymus, which is at risk for the development of a thymic NET.^{246,273,274,276,277}

Surgical indications for asymptomatic PHPT in children with MEN1 remain poorly developed, and the risk of permanent hypoparathyroidism must be balanced against the risks of PHPT. In addition to the calcium level, factors, such as surgical experience, patient or parent preference, and availability of long-term monitoring of calcium levels, should be considered in the timing of surgery.²⁴⁶ In select MEN1 patients with PHPT for whom surgery is not feasible, medical treatment with the calcimimetic agent, cinacalcet, can be considered.^{251,263,278}

Pituitary Adenomas

Pituitary tumors are the second most common clinical manifestation in children, occurring in 29% to 55% of MEN1 patients with penetrance of the MEN1 phenotype younger than 21 years of age, a little over half of whom will be symptomatic.^{207,248,249,263,265} Diagnosis before age 10 years is unlikely. Similar to adults, prolactinomas are the most prevalent, although all subtypes of pituitary adenomas have been reported.^{248,249,263,265,279–281}

In MEN1, there does seem to be a higher prevalence of pituitary adenomas in females, a higher rate of invasiveness and macroadenomas (especially in males), and more pluripotent tumors.^{249,279,281–285} Hypersecreting MEN1-related pituitary tumors may be more resistant to standard medical therapies,^{220,279,281,282} although other data suggest that medical treatment is equally effective compared with the general population.²⁵¹ There is no increased risk of death from having an MEN1-associated pituitary tumor,^{266,282} although one case of pituitary carcinoma occurring in the context of MEN1 has been published.²⁸⁰

Duodenopancreatic Neuroendocrine Tumors

NETs that arise from the neuroendocrine cells of the duodenum and pancreas represent the third most common clinical manifestation of MEN1 in children, reported to be present in 9% to 42% of all patients with MEN1 manifestations occurring before age 21 years.^{248,249,260,263,265} These gastrointestinal (GI) NETs can often be multifocal in the setting of MEN1. Gastrinomas primarily occur in the duodenum (within the so-called *gastrinoma triangle*), whereas other hormonally active tumors arise from the pancreatic islet cells at various locations throughout the pancreas.^{6,128,246,284} Furthermore, pancreatic NETs can ectopically secrete growth hormone–releasing hormone and ACTH.^{285,286} Nonfunctioning pancreatic NETs by definition are not associated with clinical symptoms from hormonal hypersecretion and thus are diagnosed via routine imaging.²⁴⁹ Such tumors can overproduce clinically silent hormones, such as pancreatic polypeptide and chromogranin A,^{6,287,288} although these serologic markers have a low diagnostic accuracy.^{289,290}

Because NETs can be small and grow slowly, localization can be challenging and often multiple techniques may need to be used.^{289,291,292} Guidelines recommend multiphasic CT,

MRI, and/or endoscopic ultrasound (EUS) for the evaluation of pancreatic NETs.^{128,246,287,293} EUS affords the highest diagnostic sensitivity, especially with smaller tumors,^{251,260,289} but this improved sensitivity may not change management because small tumors less than 2 cm in size are typically monitored in the absence of symptomatic hormonal hypersecretion.²⁹³ NETs generally express somatostatin receptors. Thus somatostatin receptor scintigraphy, especially ⁶⁸Ga-DOTATATE PET/CT, also plays an important role in the management of pancreatic NETs, especially in the identification of metastatic disease.^{128,287,289,291,294} FDG-PET is not routinely recommended because of the low proliferation rate of these neoplasms, but poorly differentiated tumors can be localized with FDG-PET.^{128,287} A diagnosis of a malignant NET would be extraordinarily rare in childhood. Guidelines regarding the diagnosis and management of NETs are available.^{128,250,287}

Insulinoma. Insulinomas are the most frequent functioning pancreatic NET in children with MEN1, having been reported to account for 54% to 78% of pancreatic NETs in large published series and diagnosed in 12% to 25% of all patients with MEN1 manifestations that occur at younger than age 21 years.^{248,249,265} Patients present with hypoglycemic symptoms that improve with glucose intake, thus fulfilling Whipple's triad. Two main symptoms attributed to hypoglycemia include loss of consciousness or coma (55%) and weakness (45%), associated with seizures in some cases.²⁴⁹ The gold standard diagnostic test is a 72-hour fast. The diagnosis is established if the serum glucose is less than 55 mg/dL with a concomitant insulin of 3 μ U/mL or higher by immunochromiluminescent assay (ICMA), c-peptide of 0.2 nmol/L or higher, and no detectable oral hypoglycemic in the blood.²⁹⁵ One-third of insulinoma patients will develop hypoglycemia at 12 hours, 80% at 24 hours, 90% at 48 hours, and 100% by 72 hours.²⁹⁶ Because medical treatment with frequent carbohydrate meals, diazoxide, and/or somatostatin analogs is not always successful, surgery is considered the standard of care.^{128,296} In contrast with other functioning NETs, insulinomas have a lower malignant potential.²⁹⁷

Gastrinoma. Gastrinomas occur primarily in the duodenum and are usually multiple, small (<1 cm) tumors that result in elevated gastric acid production and recurrent peptic ulcer disease, referred to as the *Zollinger-Ellison syndrome* (ZES).^{246,293} Other symptoms can include diarrhea and steatorrhea. They are the most common functional tumor in adults with MEN1.²⁴⁸ Exceedingly rare during childhood and occurring in 0% to 7% of patients (0% to 22% of pancreatic NETs) in series that include children,^{248,249,260,263,265} gastrinomas are usually diagnosed during the second decade of life in pediatric patients.

The diagnosis is established by an increased serum fasting gastrin concentration (often \geq 1000 pg/mL) and elevated basal gastric acid secretion (gastric pH < 2).^{6,287,298,299} Patients with borderline hypergastrinemia should be considered for the secretin stimulation test; after a 2-U/kg intravenous bolus,³⁰⁰ a rise in gastrin of 120 pg/mL or more from baseline affords the highest diagnostic sensitivity and specificity.³⁰¹ False positive elevations of gastrin can occur because of use of proton-pump inhibitors or antacids, conditions of autoimmune chronic atrophic gastritis with achlorhydria, and/or chronic renal insufficiency.^{302,303} Patients should therefore be off proton-pump inhibitors for at least 1 week before testing, but this may not be possible in highly symptomatic patients.^{287,299} Hypercalcemia can also stimulate gastrin secretion.²⁹⁹

Medical therapy with a potent oral proton-pump inhibitor is the primary treatment of gastrinoma in MEN1 patients.^{246,293}

Some patients may also require the addition of an H₂ receptor antagonist. The role of surgery in treating MEN1 patients with gastrinoma is controversial. Tumors are generally multiple and in the duodenum, so cure is less likely, unless extensive surgery is undertaken. If surgery is performed, both duodenal inspection and lymph node dissection are required.²⁸⁴ For pancreatic gastrinomas or lesions larger than 2 cm, surgery should be considered to reduce the risk of lymph node or hepatic metastasis.²⁹³

Other Functional Pancreatic Neuroendocrine Tumors.

Glucagonomas secrete glucagon and have not been previously described in children with MEN1, outside of a single case of an apparently silent glucagonoma.²⁶³ The classic glucagonoma syndrome is characterized by a skin rash in the groin and extremities (called *necrolytic migratory erythema*) in 70% of patients, mild diabetes mellitus in 87%, weight loss in almost all patients, stomatitis, and anemia.^{288,293}

VIPomas, which also have not been reported during childhood, are very rare tumors that secrete vasoactive intestinal peptide (VIP) and are associated with the Verner-Morrison syndrome of watery diarrhea, hypokalemia, and achlorhydria (i.e., pancreatic cholera).^{288,293}

Nonfunctioning Pancreatic Neuroendocrine Tumors. In children with MEN1, nonfunctioning pancreatic NETs represent the second most common NET arising in the duodenum/pancreas (40% of pancreatic NETs; 9% of all cases) and are identified via imaging alone.²⁴⁹ With improved screening methods, they have been identified in 42% of adolescents subjected to testing and represented 75% of pancreatic NETs,²⁶⁰ and so their true penetrance may be underestimated in older studies that primarily include functional, and thus symptomatic, tumors. Epidemiologic studies have shown that 27% of patients with nonfunctioning pancreatic NETs measuring 2.1 to 3.0 cm have metastasis compared with 11% of those 2 cm or less.³⁰⁴ More importantly, nonfunctioning tumors are now understood to confer an increased risk of death in MEN1 patients, comparable with thymic NETs and functioning pancreatic NETs, except insulinomas.^{266,267} Although controversies remain as to the indications for surgical therapy, general consensus is that tumors 2 cm (or larger) or tumors that demonstrate a relatively rapid rate of growth should be considered for resection.^{128,246,247,293,305}

Other Clinical Manifestations

Thymic, Bronchial and Gastric Neuroendocrine Tumors. In MEN1, NETs also occur outside of the duodenum and pancreas and can be found in the thymus, bronchopulmonary system, and stomach. Historically referred to as *carcinoid tumors*, the preferred term is *NET*.^{6,128} The diagnosis of such tumors almost universally occurs in adulthood, but one case of a malignant thymic NET has been reported in a 16-year-old MEN1 patient,^{249,277} and a bronchial NET has been found in a 15-year-old girl.²⁶⁵

Thymic NETs are more prevalent in smokers and males (male/female ratio 20:1), whereas bronchial NETs are more prevalent in women (male/female ratio 1:4).^{306,307} Bronchial NETs behave more indolently compared with thymic tumors, which impart a significant increased risk for death in MEN1 patients.²⁶⁶ Thymic NETs can still develop despite previous prophylactic transcervical thymectomy,^{251,277} presumably because the entire thymus is not removed via a cervical approach, underscoring the importance of continuing prospective screening even if thymectomy has already been performed. Thymic and

bronchial NETs can ectopically secrete growth hormone–releasing hormone and ACTH.^{277,285,286}

Gastric NETs are well-differentiated tumors with malignant potential that arise from the enterochromaffin-like cells of the stomach mucosa.³⁰⁸ Type II gastric NETs are those that occur in MEN1, developing secondary to chronic hypergastrinemia in the setting of gastrinoma/ZES.³⁰⁹ Given the rarity of gastrinoma in childhood, type II gastric NETs have not been reported in a pediatric patient.

Adrenocortical Tumors. Unilateral and bilateral ACTs are reported in 10% to 26% of MEN1 patients, with a mean age at diagnosis of an adrenal lesion of around 45 years of age^{310,311}; in children with clinical MEN1 younger than age 21 years, the prevalence ranges from 0% to 4%.^{248,249,263,265} Adrenocortical hyperplasia, adenomas, and adrenocortical carcinoma are the types of adrenal lesions seen; PHEO has also been reported in adults with MEN1. Most ACTs are nonfunctioning, and functioning tumors primarily present with hypersecretion of aldosterone or cortisol, although hyperandrogenism/virilization is seen in children.^{249,310} In the large MEN1 series published by Goudet et al. and in the study by Gatta-Cherifi and colleagues, all cases of ACT arising in childhood before age 20 years presented with hyperandrogenism and were classified as malignant.^{249,310}

Cutaneous Manifestations. Often underappreciated as a component of the MEN1 phenotype, skin manifestations (Fig. 15.10) include angiofibromas, collagenomas, and lipomas.¹⁹⁴ More commonly identified in older MEN1 patients, the dermatologic manifestations can also be found in children, even as an initial manifestation of disease.²⁶³ Angiofibromas are benign tumors of blood vessels and connective tissue, similar to those seen in tuberous sclerosis. They present as pink to tan, dome-shaped papules that occur on the face, especially the nose. Collagenomas are well-defined, skin-colored cutaneous papules that have a predilection for the neck and trunk. The prevalence rates of angiofibromas, collagenomas, and lipomas in MEN1 patients are 64% to 88%, 63% to 72%, and 3% to 34%, respectively.¹⁹⁴ In general, MEN1-related skin lesions do not require treatment, although lipomas can grow quite large and may need to be resected because of local symptoms.

Genetic Testing and Presymptomatic Screening

An integrated program of both genetic counseling/testing of at-risk individuals and clinical screening of MEN1 mutation carriers is undertaken with the assumption that an earlier diagnosis and treatment may help to reduce morbidity and mortality. Guidelines for the care of MEN1 patients based on systematic reviews of the medical literature and developed by experts in the field were introduced in 2001 and updated in 2012.^{243,246} Subsequent publications have proposed alterations to the 2012 screening guidelines, especially as relate to nonfunctioning pancreatic NETs.^{64,249,265,289,293,312}

At the present time, there is no specific therapy that will prevent a MEN1-associated disease nor are any prophylactic therapies warranted. In addition, an earlier identification of asymptomatic disease may potentially lead to premature and unnecessary treatment that may in fact have a negative long-term impact. Thus the goal of screening in MEN1 is primarily to detect a clinical manifestation at an earlier stage, specifically so that treatment can be rendered before malignancy develops in high-risk organs or serious ramifications from hormone hypersecretion occur. Because of the long latency for disease manifestations, many unaffected individuals will potentially

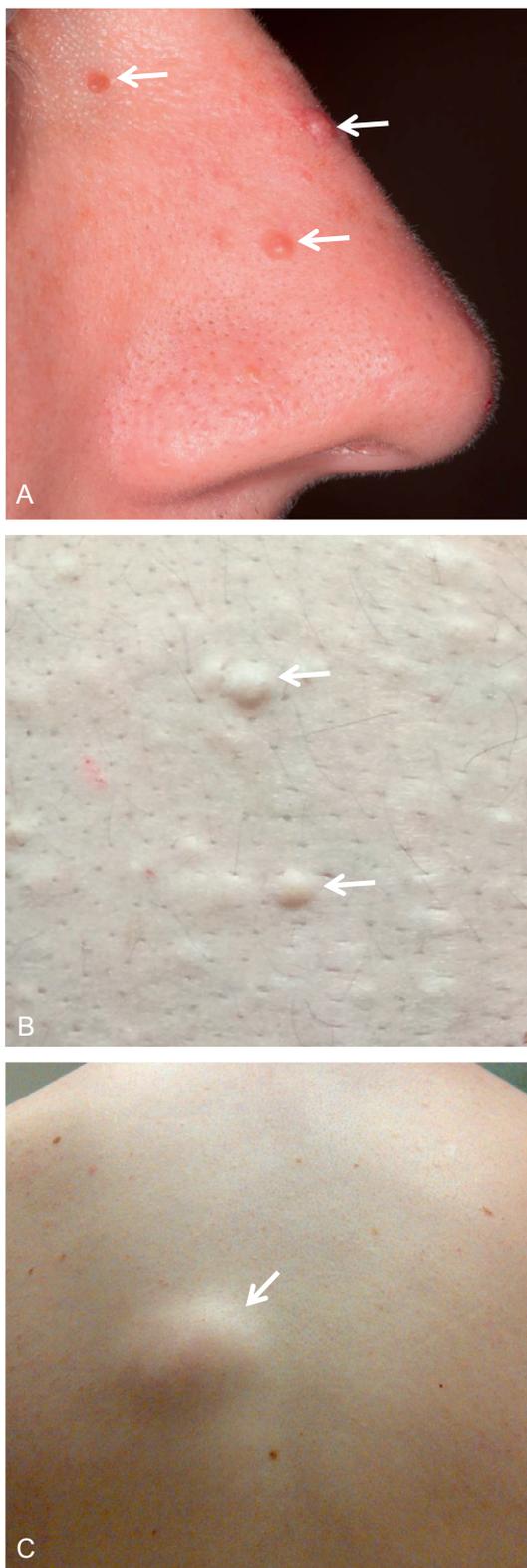


Fig. 15.10 Dermatologic manifestations of multiple endocrine neoplasia (MEN)1. Angiofibromas (A), collagenomas (B), and lipomas (C) are more commonly identified in adult MEN1 patients, but they can also develop in children, even as an initial manifestation of disease. Angiofibromas are telangiectatic papules characterized by fibrous tissue and vascular proliferation and distributed primarily on the nose and adjacent cheeks. Collagenomas are cutaneous stromal lesions that represent areas of excess collagen and tend to occur on the upper torso, neck, and shoulders. Lipomas are benign neoplasms that contain mature fat cells enclosed by thin fibrous capsules.

undergo years of episodic testing, which may have psychosocial and financial consequences,⁵ and this must be kept in mind as a prospective screening program is planned for any given MEN1 patient. In all cases, patient/parent preference, risks of screening, local resources, and clinical judgment should be considered when prospectively monitoring a patient with MEN1.

MEN1 germline mutation testing should be offered to all index patients and first-degree relatives of those individuals with a known MEN1 mutation, regardless of symptomatology.²⁴⁶ Because relevant MEN1 disease has been diagnosed in children as young as 5 years of age (see Table 15.4), current practice is to offer testing at the earliest opportunity, but only after comprehensive genetic counseling so that patients and families with MEN1 receive the medical and genetic information they require.⁵

Subsequent to genetic diagnosis, a specific screening program should be devised to detect the development of tumors in presymptomatic patients, understanding that the optimal ages to begin screening for disease have not been clearly established. Table 15.4 summarizes a suggested MEN1 screening program based upon consensus guidelines and adapted from recent publications on the subject.^{64,246,249,262,265,289,293,312}

Debate remains as to the optimal screening procedures, which could be less intensive than what is currently prescribed.^{247,262,265,312} Although annual laboratory evaluation is used by most clinical centers, not all centers order radiographic screening for presymptomatic children and young adults, as the 2012 guidelines proposed, and the frequency and type of imaging (CT, MRI, EUS) is an important area that warrants further research. Important to note are the real risks of using CT (which exposes MEN1 patients to a significant ionizing radiation dose over the course of their lives³¹³) in a hereditary tumor disorder, especially one that requires a somatic second hit for tumorigenesis to occur. Therefore given the lengthy follow-up period required in MEN1 patients, MRI is generally preferred over CT to reduce the risks from ionizing radiation,^{249,262,293} although CT still plays an important role, especially in surgical planning.

Multiple Endocrine Neoplasia Type 2

The original description of MEN2A is attributed to Sipple when he reported the case of a 33-year-old man who died of intracranial hemorrhage and was found on autopsy to have bilateral PHEO, bilateral MTC, and probable parathyroid hyperplasia.³¹⁴ In 1962 Cushman proposed an association between these endocrine tumors,³¹⁵ and MEN2 was subsequently named as a distinct clinical syndrome by Steiner et al.³¹⁶ Linkage analysis studies in large MEN2 kindreds led to the localization of the putative gene to chromosome 10.^{317,318} Ultimately, mutations in the *RET* protooncogene (chromosome 10q11.21) were found to cause MEN2A.^{154,155}

The original characterization of the MEN2B phenotype in the English literature was made by Williams and Pollock in 1966,³¹⁹ and MEN2B was further distinguished as a variant of hereditary MTC with a mucosal neuroma phenotype.³²⁰ In 1994 MEN2B was reported to also be secondary to activating mutations in *RET*.^{156,157}

As a result of the initial collaborative efforts of the International *RET* Mutation Consortium, it quickly became apparent that only a limited number of mutations were associated with MEN2 and that strong genotype–phenotype correlations were present¹⁵⁸ (see Fig. 15.5). Because of the very high penetrance of MTC in individuals with a *RET* mutation, there was a rapid incorporation of genetic testing into management algorithms for patients with MTC and their families. Predictive genetic testing thus ushered in the era of performing total prophylactic

thyroidectomies for presymptomatic individuals carrying a high-risk *RET* germline mutation.¹⁶⁵

Multiple Endocrine Neoplasia 2A

MEN2A accounts for 90% to 95% of MEN2 cases and is characterized by the lifetime development of MTC in more than 90% of *RET* mutation carriers. Depending on the specific mutation, PHEO and/or PHPT occur in 0% to 50% and 0% to 20% of individuals with MEN2A, respectively^{65,160}; rare cases of adrenal ganglioneuroma have also been described.³²¹ Furthermore, activating mutations in *RET* are also associated with Hirschsprung disease³²² and cutaneous lichen amyloidosis,^{194,323} a dermatologic disorder of intense pruritus and secondary skin changes that is typically located in the interscapular region of the back. Although historically discussed as a separate syndrome, familial medullary thyroid carcinoma (FMTC), in which MTC is the only clinical manifestation of a germline *RET* mutation, is now considered to be a variant of MEN2A.^{65,160}

In MEN2A, mutations are located mostly in the extracellular cysteine-rich domain of the *RET* protooncogene, usually in exon 10 (codons 609, 611, 618, or 620) or exon 11 (codon 634), but mutations can also be found in the intracellular tyrosine kinase domain^{65,158,177,324,325} (see Fig. 15.5). Codon 634 mutations account for the vast majority of classical MEN2A cases, but more recently, the prevalence of mutations in other codons (especially codon 804) has increased.^{325,326} As molecular testing becomes widespread in patients with MTC, more *RET* mutations and DNA variants are being identified, which is contributing to an everchanging spectrum of the MEN2A genotype and phenotype.^{160,327}

Medullary Thyroid Carcinoma in Multiple Endocrine Neoplasia 2A. MEN2A-related MTC, when identified during childhood, is usually diagnosed after early thyroidectomy directed by genetic testing results and is usually a microcarcinoma <1 cm in size.^{176,328,329} Most children with MEN2A will have a positive family history; *de novo* *RET* mutations represent only 2% to 9% of cases.^{330,331} A strong genotype-phenotype relationship is present, such that the rapidity with which a mutation carrier will develop MTC can be estimated, but the natural history of MTC in MEN2A can be highly variable, even among members of the same kindred.^{2,158,327,332,333} Patients with mutations in *RET* codon 634 have the highest risk of MTC, followed by those with mutations in codons 609, 611, 618, 620, or 630, whereas mutations in other codons impart the lowest risk for early-onset MTC.^{65,160,168,334} However, once MTC is diagnosed, overall survival and the development of distantly metastatic disease are similar among patients with high- and moderate-risk *RET* mutations,³³⁵ further suggesting that the genotype drives the age of onset of MTC more than it does its clinical aggressiveness after diagnosis.¹⁷³

Pheochromocytoma in Multiple Endocrine Neoplasia 2A. MEN2-associated PHEO is an adrenergic tumor that usually arises within a background of adrenal medullary hyperplasia,^{167,336–338} although some groups prefer the term *microPHEO* to indicate medullary proliferations smaller than 1 cm.³³⁹ With a diagnosis most likely during the fourth decade of life, PHEO develops primarily because of codon 634 mutations and to a much lesser degree with mutations in exon 10 (codons 609, 611, 618, and 620) and other codons.^{82,102,158,336,340–344} Typically, a PHEO is diagnosed synchronously with MTC or only after MTC is identified; in more contemporary studies, it is the initial clinical presentation in up to 15% of cases.^{102,343} Guidelines currently recommended prospective metanephrine screening for PHEO in children with MEN2A, starting at age 11 years, in

patients with a codon 634 mutation, and at age 16 years in all others⁶⁵ (see Table 15.1).

Primary Hyperparathyroidism in Multiple Endocrine Neoplasia 2A. Patients with MEN2A are at risk of developing PHPT because of parathyroid adenomas and hyperplasia. PHPT is chiefly associated with codon 634 mutations and less commonly described in other *RET* mutations.^{65,133,344–346} Onset during childhood is extraordinarily rare but has been described in a child as young as 5 years of age.³⁴⁷ Usually PHPT is diagnosed in the fourth decade of life, and therefore it is rarely the first manifestation of MEN2A.^{133,160,344} Screening for PHPT is recommended at age 11 years, in children with a codon 634 mutation, and at age 16 years in all others⁶⁵ (see Fig. 15.5).

Multiple Endocrine Neoplasia 2B

MEN2B is a completely penetrant disease that accounts for 5% to 10% of MEN2 cases.³⁴⁸ MEN2B is almost always (>95%) because of a single mutation in exon 16 (p.M918T) that is located in the intracellular tyrosine kinase domain of the *RET* receptor^{158,324} (see Fig. 15.5). Rare MEN2B cases can be attributed to double *RET* mutations involving codon 804 or a mutation in codon 883 (p.A883F, exon 15).^{65,348} Carriers of the p.A883F mutation have a later onset of disease and a more indolent natural history compared with those who harbor the p.M918T mutation.³⁴⁹ Unlike MEN2A, 84% to 90% of MEN2B cases arise as a result of a *de novo* mutation, with the child having unaffected parents.^{131,169,350,351} Because of this and because of the rarity of the syndrome, the diagnosis of MEN2B is almost always delayed, even in the presence of obvious clinical features that can start as early as infancy.^{169,170}

MEN2B is characterized by the development of MTC (100% of cases), PHEO (up to 50% of cases; rare cases of adrenal ganglioneuroma have also been described³²¹), and a highly penetrant and characteristic clinical phenotype (100% of cases; Fig. 15.11).^{131,169,170,348,350,352,353} Although the clinical phenotype is present in all patients, individual manifestations have a variable presentation and are age dependent.¹⁷⁰ Multiple mucosal neuromas¹⁹⁴ are a major clinical manifestation and occur on the lips, tongue, and/or conjunctiva, in addition to the urinary system and gastrointestinal tract. The symptoms of intestinal ganglioneuromatosis include constipation and feeding problems in infancy and the development of megacolon. A second component of the MEN2B phenotype is musculoskeletal abnormalities, including a marfanoid body habitus, narrow long facies, pes cavus, pectus excavatum, high-arched palate, scoliosis, and/or slipped capital femoral epiphysis. Other clinical features include joint laxity, hypotonia or proximal muscle weakness, thickened lips, ophthalmologic findings (inability to make tears in infancy [alacrima], thickened and everted eyelids [ectropion], mild ptosis, and prominent corneal nerves), and pubertal delay. The oral manifestations of MEN2B are highly penetrant and often lead to the clinical diagnosis. PHPT is not a feature of MEN2B.³⁴⁴

Medullary Thyroid Carcinoma in Multiple Endocrine Neoplasia 2B. MTC that occurs in MEN2B is an aggressive malignancy with a very early onset of metastasis (lymph node disease has been documented within the first year of life¹⁸⁹), a high cancer stage (defined by the tumor, node, and metastasis [TNM] classification system) at diagnosis, and an average age of MTC onset (second decade of life) that is about 10 years earlier than that observed in MEN2A.^{131,170,176,243,340,348,354} Earlier MTC onset is observed in those children with MEN2B with severe intestinal manifestations, who also seem to have a poorer prognosis than those without these intestinal manifestations.³⁵² Morbidity and

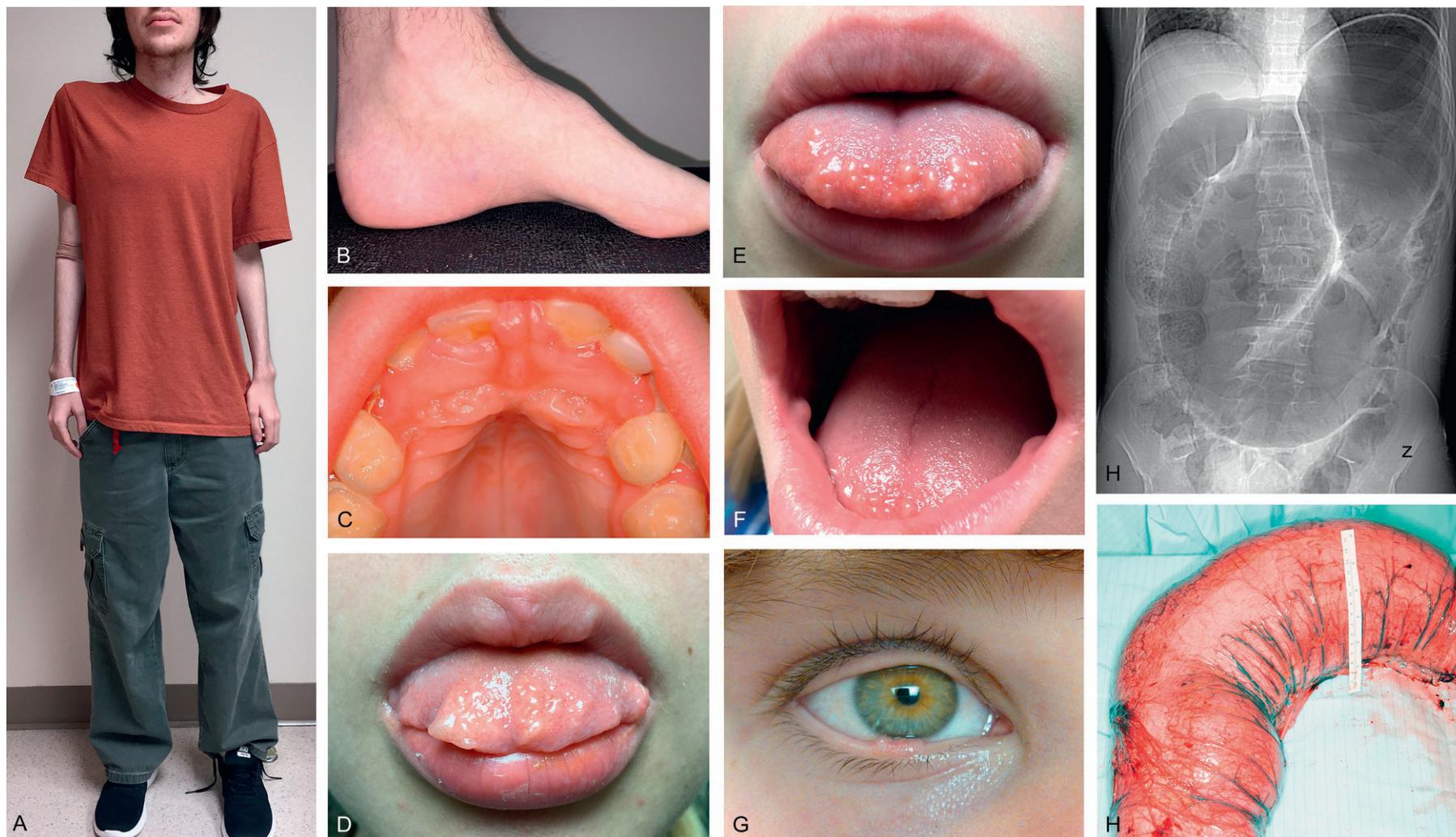


Fig. 15.11 The pathognomonic clinical phenotype of MEN2B: (1) marfanoid body habitus (A) and other skeletal features including scoliosis (A), pes cavus (B), and high-arched palate (C); (2) thickened lips and neuromas affecting the tongue (D) and (E), the oral mucosa (F), the conjunctiva (G), and other mucosal surfaces; (3) ophthalmologic signs including ptosis and everted upper eyelids (ectropion) (G); and (4) gastrointestinal problems primarily related to impaired colonic motility caused by diffuse intestinal ganglioneuromatosis that can lead to megacolon (H). For additional clinical manifestations of MEN2B, please refer to the text. (From Castinetti, F., Moley, J., Mulligan, L., Waguespack, S.G. (2018). A comprehensive review on MEN2B. *Endocr Relat Cancer*, 25(2), T29–T39)

mortality rates are much higher in MEN2B, although the higher mortality rate may reflect the earlier onset of disease and more advanced tumor stage at presentation, rather than an intrinsically more aggressive carcinoma.^{173,185,348,353} Clinical outcomes are improving in the current era because of improved diagnosis and treatment at a younger age and thus a lower stage of MTC.³⁵¹

Pheochromocytoma in Multiple Endocrine Neoplasia 2B.

Compared with the more heterogeneous MEN2A population, patients with MEN2B have a higher lifetime risk (~50%) of developing PHEO.^{131,342} The clinical phenotype of the disease is no different than that seen in MEN2A, although the median age of diagnosis is around 25 years, about a decade earlier than MEN2A.^{102,131,343,344,351} Two other notable differences between MEN2A and MEN2B are that PHEO is even less likely to be diagnosed before MTC and that historical mortality from PHEO is lower compared with death from MTC.^{131,336,342,355} Despite an aggressive MTC phenotype and a constitutively activated RET tyrosine kinase, the risk of malignant PHEO in patients with MEN2B is exceedingly rare (0%–3%).^{131,147,343} To date, the youngest documented case of MEN2B-associated PHEO was in a 10-year-old child.³⁵⁵ Contemporary guidelines recommend initiation of screening at age 11 years.⁶⁵

Timing of Thyroidectomy in Multiple Endocrine Neoplasia 2

When MTC metastasizes beyond the thyroid, it usually becomes an incurable disease that may ultimately cause shortened survival. Given that MEN2 can be easily diagnosed through predictive genetic testing, hereditary MTC has become one of the few malignancies that can be prevented (via prophylactic thyroidectomy) or cured (via early thyroidectomy) before it becomes clinically apparent. In experienced surgeons' hands, children who have a total thyroidectomy performed before the onset of metastatic disease have an excellent chance of remaining disease free; cure is also possible even for children with high-risk MEN2, despite surgery not being performed at the earliest prescribed ages.^{131,176,179,329,350,351,353,356,357} In reality, many thyroidectomy specimens from children with MEN2A harbor microscopic, nonmetastatic MTCs that are often cured after early thyroidectomy^{328,329,357,358}; in those not cured after initial surgery, calcitonin may remain abnormal but without structural evidence of disease.^{328,357} For most children with MEN2B, cure is unobtainable because of the fact that most surgeries in this population are therapeutic, not prophylactic, given the oft-delayed clinical diagnosis; however, in children diagnosed and treated before age 1 year, biochemical cure is achieved in 83% of cases.¹³⁰

There is widespread agreement that the therapeutic goal in a child with MEN2 is to remove the at-risk thyroid before incurable MTC metastasis occurs, while minimizing potential medical and surgical morbidity. Recommendations regarding the timing of surgical intervention have evolved over the last 25 years. Previously based on genotype alone, contemporary practice is to offer an early thyroidectomy in children at an age dictated by genotype, and in the case of mutations less likely to cause MTC in early childhood, basal calcitonin levels^{65,173} (see Fig. 15.5). Although thyroid US is frequently incorporated into clinical decision making, it is an insensitive test for the diagnosis of MTC and should therefore not be used independently to determine the age of surgery.³⁵⁸

In the American Thyroid Association (ATA) guidelines published in 2015, the disease-causing *RET* mutations were stratified into three major risk categories: highest risk (codon 918), high risk (codons 634 and 883), and moderate risk (all others)⁶⁵ (see Fig. 15.5). Recent studies have suggested that the ATA moderate risk mutations are a heterogeneous group and that further subdivisions in this category may be needed to better inform the risk for MTC development.¹⁶⁸ Total thyroidectomy is recommended within the first year of life in carriers of the highest risk mutation and at or before age 5 years for those with high-risk mutations. With all other (moderate risk) *RET* mutations, the detection of an elevated calcitonin level can be used to help decide surgical timing, noting that some ATA moderate risk mutations may be more virulent than others.^{168,334} Calcitonin levels under 30 to 40 pg/mL are associated with an exceedingly low risk of lymph node metastasis,^{173,176,359} and thus operating on a child just as the calcitonin is rising should result in a long-term cure.³⁵⁷ Ultimately, decisions regarding the age of surgery should be made by a multidisciplinary team experienced in MEN2 management and the child's parent(s)/guardian(s). Notably, an elevated calcitonin level does not reliably portend a diagnosis of MTC¹⁷⁶; false positive elevations can be found in nonneoplastic conditions, such as chronic kidney disease, autoimmune thyroiditis, and hyperparathyroidism.^{65,191} Conversely, MTC can be identified pathologically even in the presence of a normal calcitonin level.^{357,358}

Multiple Endocrine Neoplasia Type 4

MEN4 is a syndrome of multiple endocrine tumors that is caused by germline mutations in the *CDKN1B* gene on chromosome 12p13.1, which encodes the cyclin-dependent kinase inhibitor p27^{Kip1} and plays a critical role in regulating cell proliferation.³⁶⁰ Homozygous mutations in rat *Cdkn1b* were originally identified to cause MENX, a multitumor syndrome in the rat that has phenotypic features of both MEN1 and MEN2.^{360,361} Subsequently, studies in humans without a proven *MEN1* mutation confirmed that a small subset will have heterozygous mutations in the human homologue *CDKN1B*, and these patients have an MEN1-like phenotype, subsequently called *MEN4*.³⁶² Individuals with a germline *CDKN1B* mutation are at risk for developing tumors of the parathyroid glands (the main clinical manifestation), pituitary, adrenals, and/or GI neuroendocrine system, among others.^{360–362} Although MEN4 is a very rare cause of multiple endocrine neoplasia, mutational screening of the *CDKN1B* gene should be considered in patients with a clinical diagnosis of MEN1, but negative *MEN1* genetic testing.^{246,362} The optimal surveillance strategy has not been established but at a minimum should include periodic testing for PHPT and other functioning endocrine tumors.⁶⁴

X-Linked Acrogigantism

X-linked acrogigantism (X-LAG) is the most recently characterized hereditary disorder associated with pediatric pituitary tumors. It is a disorder with X-linked inheritance that is characterized by pituitary gigantism with an onset during the first few years of life (<4 years of age).³⁶³ The disease is caused by inherited or de novo (most common) germline microduplications on chromosome Xq26.3 that includes the responsible gene, *GPR101*, which encodes for an orphan G-protein-coupled

receptor.^{364,365} Somatic mosaicism for the Xq26.3 microduplication leading to the X-LAG phenotype has also been reported in male simplex cases.³⁶⁵ A germline and somatic *GPR101* missense variant (p.E308D) has also been identified in some cases of acromegaly,³⁶⁴ although it remains uncertain if this DNA variant plays a causative role in the pathogenesis of somatotroph adenomas.²⁰⁷

Clinically, X-LAG patients present with marked growth hormone excess that is associated with hyperprolactinemia in about 80% to 90% of cases; histologically, mixed somatotroph–lactotroph adenomas and/or pituitary hyperplasia are identified.^{364,365} In distinct contrast with *AIP*-associated FIPA, X-LAG is characterized by a female preponderance, with 24/33 reported cases being female.²⁰⁷ Interestingly, all female probands published to date represent de novo cases and vertical transmission of a *GPR101* duplication from an affected parent to a daughter has not yet been reported.³⁶³

OTHER TUMOR SYNDROMES ASSOCIATED WITH ENDOCRINE NEOPLASIA

Adenomatous Polyposis Coli-Associated Polyposis

The *adenomatous polyposis coli* (*APC*)-associated polyposis conditions result from dominantly inherited germline mutations in the *APC* tumor suppressor gene (chromosome 5q22.2) and include the clinical syndromes of familial adenomatous polyposis (FAP, including the now historical Gardner and Turcot syndromes), attenuated FAP, and gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS).³⁶⁶ Patients with an *APC* mutation are at greatest risk for colon cancer but may also develop endocrine neoplasia. Compared with the general population, ACTs are at least twice as prevalent (16% of patients) in FAP, and similar to sporadic adrenal neoplasia, *APC*-associated ACTs may be hormonally active or nonfunctioning and may rarely be malignant.^{367–369} Patients with an *APC* mutation also have up to a 12% lifetime risk for thyroid malignancy.³⁷⁰ The primary subtype is papillary thyroid carcinoma, classically the cribriform-morular histologic variant,³⁷¹ and it is a diagnosis almost exclusively made in young women during the third decade of life.³⁷⁰ Outside of thyroid palpation, opinions regarding screening for thyroid cancer in an *APC*-associated polyposis condition are varied; routine surveillance for adrenal tumors is not recommended.^{235,366,372}

Beckwith-Wiedemann Syndrome

The Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder caused by dysregulation of gene transcription in an imprinted domain on chromosome 11p15 that includes genes, such as *CDKN1C* and *IGF2*, which are strong regulators of fetal growth.^{373,374} Clinical findings are pleiotropic and include macrosomia and hemihyperplasia, macroglossia, visceromegaly, embryonal tumors (e.g., Wilms tumor, hepatoblastoma, neuroblastoma, pancreatoblastoma, and rhabdomyosarcoma), omphalocele, neonatal hypoglycemia, ear creases/pits, ACTs/adrenal cytomegaly, and renal abnormalities (e.g., medullary dysplasia, nephrocalcinosis, medullary sponge kidney, and nephromegaly). A variety of malignant tumors, typically restricted to childhood onset, have been associated with BWS and occur in 7.4% of all cases.³⁷⁵ ACT, both benign and malignant, are among the more common neoplasms identified in BWS, representing up to 8% of benign and 7% of malignant tumors.³⁷⁶ Fetal adrenocortical cytomegaly (a component of the underlying visceromegaly)

and adrenal cystic lesions also occur.^{376,377} PHEO and thyroid carcinoma are additional endocrine tumors that have been reported.^{375–377} Various protocols have been suggested for tumor surveillance in BWS and algorithms have been developed for the management of children with an identified adrenal mass.³⁷⁷ Children with paternal uniparental disomy appear at highest risk for the development of ACTs and thus screening with abdominal US should be focused more on these children, as well as those with negative molecular tests.^{374,375}

Carney Triad

The Carney triad was originally described in 1977 as the association of gastric epithelioid leiomyosarcoma (later renamed GIST), PGL, and pulmonary chondroma.³⁷⁸ Over time, the phenotype has expanded to include clinically nonfunctioning ACTs and esophageal leiomyomas.^{379,380} The Carney triad affects primarily young women (85%) with a mean age of onset of 20 years (7–48 years).³⁸⁰ Because of incomplete expression of the phenotype, PGL is not present in all patients suspected to have the Carney triad.³⁷⁹ PHEO can occur in a minority of patients, and the aortopulmonary body is a common site for the development of PGL, although PGL occur equally in the head and neck, thorax, and abdomen.³⁸⁰

Although the Carney triad is not a heritable disorder in the traditional sense, about 10% of patients will be identified to have a germline DNA variant in the *SDHA*, *SDHB*, or *SDHC* genes.³⁸¹ Furthermore, hypermethylation of *SDHC* appears to be the main molecular characteristic of the Carney triad.³⁸²

DICER1 Syndrome

Germline mutations in the *DICER1* gene cause the DICER1 syndrome, a rare autosomal dominant disorder with decreased penetrance, characterized by a variety of benign and malignant tumors,^{6,383–385} including pleuropulmonary blastoma (PPB), ovarian sex cord-stromal tumors (primarily Sertoli-Leydig cell tumor but also juvenile granulosa cell tumor and gynandroblastoma), cystic nephroma, and thyroid gland neoplasms (multinodular goiter and differentiated thyroid cancer). Other tumors that are less commonly observed include ciliary body medulloepithelioma, botryoid-type embryonal rhabdomyosarcoma of the cervix or other sites, nasal chondromesenchymal hamartoma, renal sarcoma, pituitary blastoma, and pineoblastoma.

DICER1 (chromosome 14q32.13) is a gene encoding an endoribonuclease that cleaves noncoding small ribonucleic acid (RNA) precursors to generate mature microRNAs that are involved in the posttranscriptional regulation of gene expression. Mutations in *DICER1* were first identified in familial PPB³⁸⁶ and then subsequently found to cause a pleiotropic tumor syndrome, including various other tumor types.³⁸⁷ The *DICER1* syndrome normally results from a germline inactivating mutation, followed by a somatic second hit primarily involving the RNase IIIb catalytic domain of *DICER1*.^{383,385,388} Most of the germline mutations are inherited but 10% to 20% may arise de novo.³⁸³ Somatic mosaic *DICER1* mutations, often accompanied by second somatic truncating mutations or loss of heterozygosity, can also cause the *DICER1* syndrome.³⁸⁹

Thyroid nodules appear to be one of the most common manifestation in patients with germline *DICER1* mutations, and there is a significantly increased risk of multinodular goiter compared with family controls and at least a 16-fold elevated risk of thyroid cancer compared with the general population.³⁹⁰ Familial multinodular goiter may in fact be the only clinical manifestation,^{391,392} and in the child being evaluated

for possible syndromic nodular goiter, macrocephaly may be a clue to the presence of a germline *DICER1* mutation.³⁹³ Similar to nonsyndromic thyroid nodular disease, females are more affected, and by the age of 40 years, the cumulative incidence of multinodular goiter or thyroidectomy is 75% in women and 17% in men.³⁹⁰ Differentiated thyroid cancer occurs in less than 3% of *DICER1* carriers and is a low-risk tumor that does not appear to be more clinically aggressive.^{390,394} Although exposure to chemotherapy or radiation was originally felt to contribute to the risk of thyroid cancer, a germline *DICER1* mutation alone appears sufficient to increase the thyroid cancer risk.^{383,390} Surveillance for thyroid tumors in children with the *DICER1* syndrome include periodic thyroid ultrasonography, starting by age 8 years and then every 3 years or more frequently, if being treated for another tumor,^{383,384} although some centers recommend physical examination alone, starting at age 10 years in children not exposed to alkylating chemotherapy.³⁹⁵

Pituitary blastomas, occurring in less than 1% of patients with a *DICER1* mutation, are extremely rare and aggressive neoplasms that arise from the fetal anterior pituitary and clinically present at an age younger than 24 months with ACTH-dependent Cushing disease and/or ophthalmoplegia because of mass effect.^{6,388} Because this tumor is so rare, it is unclear that routine surveillance is necessary in all cases, although some centers advocate for MRI screening.³⁹⁵ Regardless, assessing for and recognizing the signs and symptoms of a pituitary blastoma (rapid weight gain, ophthalmoplegia) should lead to an early diagnosis.

Li-Fraumeni Syndrome

Li-Fraumeni Syndrome (LFS) is a highly penetrant cancer-predisposition syndrome caused by autosomal dominantly inherited germline mutations in the tumor suppressor gene *TP53* (tumor protein p53; chromosome 17p13.1).³⁹⁶ Soft tissue and bone sarcomas, premenopausal breast cancer, ACTs, and brain tumors represent the core tumors of LFS.^{397–399} The prototypical endocrine tumor associated with LFS is ACT (both benign and malignant), although several cases of thyroid carcinoma have also been reported, especially in Brazilian kindreds.^{400,401} The adrenal cortex is the third most common site of neoplasia development in LFS.³⁹⁷ ACTs have an early age of onset, with the diagnosis most common in very young children, who may in fact represent the proband for an LFS kindred.^{397,402,403} In southern Brazilian families, the *TP53* mutation p.Arg337His is associated with a higher susceptibility to isolated ACT in children (median age of diagnosis 3 years; range 4 months to 13.5 years),⁴⁰⁴ although cancer risk also appears increased for adult carriers of this mutation.⁴⁰² Children with an ACT typically present with virilization,^{404,405} but at all ages, these tumors can present with other signs/symptoms of adrenocortical hyperfunction (such as Cushing syndrome) and/or with symptoms because of tumor mass effect, particularly in the case of nonfunctioning tumors. Cancer screening recommendations, which include monitoring for the development of an ACT, are available.³⁹⁹

Lynch Syndrome (Hereditary Nonpolyposis Colon Cancer)

Lynch syndrome is a disorder with autosomal dominant inheritance that is secondary to mutations in DNA mismatch repair genes and characterized by an increased risk for colorectal cancer and various other malignancies.⁴⁰⁶ A diagnosis of adrenocortical carcinoma has been reported in individuals and families with Lynch syndrome,^{407,408} although to date there

has not been a pediatric presentation of ACT occurring in this context.

Neurofibromatosis Type 1

NF1, also known as Von Recklinghausen disease, is an autosomal dominant disorder caused by mutations in the *NF1* gene, a tumor suppressor gene located on chromosome 17. Café-au-lait macules, neurofibromas (cutaneous and plexiform), axillary and inguinal freckling, optic pathway gliomas, skeletal dysplasia, and Lisch nodules (benign hamartomas) of the iris are the main clinical features.⁴⁰⁹ NF1 patients are at an increased risk of developing PHEO/PGL and other NETs.

The prevalence of a catecholamine-producing tumor in NF1 patients is 2% to 6%.^{134,297,410} PHEO (up to 17% bilateral) represent the majority of NF1-associated cases but intraabdominal and pelvic PGL are diagnosed in up to 6%.^{134,410} Mean age of diagnosis is in the fifth decade of life,^{134,411} and NF1-associated PHEO are typically characterized by an adrenergic phenotype.^{411,412} Malignancy is uncommon but does occur in 5% to 12% of cases.¹³⁴ Rarely, a composite PHEO (a mixed tumor consisting of PHEO and a developmentally related neurogenic tumor, such as neuroblastoma, ganglioneuroma, or ganglioneuroblastoma) may occur in the setting of NF1.⁶

Gastrointestinal NETs occur in less than 10% of NF1 patients and are almost exclusively duodenal somatostatinoma that occur in the periampullary region and present clinically as a result of the tumor itself (pain, jaundice, bleeding) rather than a somatostatinoma syndrome.^{6,297,413} NF1-associated somatostatinomas are typically diagnosed during the fifth decade of life.⁴¹³ Other types of NETs that have been reported in NF1 patients include insulinomas, gastrinomas, and nonfunctioning pancreatic NETs.^{297,414,415} Case reports of PHPT caused by benign and malignant parathyroid tumors and ACTs have also been reported to occur in NF1.^{369,416,417} Finally, a unique endocrinopathy associated with NF1 in children with infiltrating optic pathway gliomas is the clinical syndrome of gigantism caused by unregulated growth hormone secretion.⁴¹⁸

Peutz-Jeghers Syndrome

Peutz-Jeghers syndrome (PJS) is an autosomal dominantly inherited syndrome with incomplete penetrance characterized by hamartomatous polyps in the GI tract and mucocutaneous hyperpigmentation.^{419,420} It is caused primarily by inactivating mutations in the *STK11* (*LKB1*) gene (chromosome 19p13.3). Individuals with PJS are at increased risk for various malignancies over the course of their lives, chiefly luminal GI cancers (colorectal >stomach >small bowel) and breast cancer, followed by pancreatic cancer, gynecologic malignancies, and lung cancer.^{419,421} Several case reports of differentiated thyroid cancer have been published,⁴²² but it remains unclear if this is a random occurrence or a direct association with PJS.

Patients with PJS are at risk for unique gonadal tumors that may clinically present with an endocrinopathy during childhood: Sertoli cell tumors of the testis in males and ovarian sex cord tumors with annular tubules (SCTATs) in females. Testicular Sertoli cell tumors in PJS are benign lesions that typically occur in prepubertal boys (mean age 6.8 years) who present with gynecomastia and bilateral (rarely unilateral) testicular enlargement.²¹¹ The microscopic findings are distinctive and the term *intratubular large cell hyalinizing Sertoli cell neoplasia* has also been proposed.²¹¹ Calcifications are unusual and not extensive in these lesions, although large-cell calcifying Sertoli cell tumors (similar to those identified in the Carney

complex) can also occur within the PJS spectrum.⁴²³ The gynecomastia is caused by estrogen overproduction secondary to high levels of aromatase activity,²¹¹ making the use of an aromatase inhibitor the optimal approach to medically treating such cases.⁴²⁴ SCTAT is a distinctive neoplasm that has morphologic features that lie between those of a Sertoli cell tumor and a granulosa cell tumor.⁴²⁵ When occurring in the PJS, these tumors are typically small, calcified, multifocal, and bilateral lesions, as compared with non-PJS SCTATs. SCTAT is almost always a benign neoplasm with a mean age of diagnosis of 27 years (range 4–57 years).⁴²⁵ Clinical presentation is often caused by estrogen overproduction and manifested by menstrual irregularity in postmenarchal women and sexual precocity in prepubertal girls. Abdominal pain and a palpable adnexal mass can also be presenting features, and SCTAT can also be an incidental finding.⁴²⁵ Annual physical examination (assessing for gynecomastia/testicular enlargement in boys and precocious puberty in girls) is recommended for children with PJS.³⁷²

PTEN Hamartoma Tumor Syndrome

The *PTEN* hamartoma tumor syndrome (PHTS) results from germline mutations in the *PTEN* (phosphatase and tensin homolog) tumor suppressor gene (chromosome 10q23.31) and is inherited in an autosomal dominant manner.⁴²⁶ Penetrance is high (>80%) and the clinical diagnosis may be missed because of a variable phenotypic presentation and associated tumors that have a high background population prevalence.²³⁵ The PHTS comprises the clinical spectrum of Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, *PTEN*-related Proteus syndrome, and Proteus-like syndrome.⁴²⁷ Cowden syndrome (CS), the most common PHTS phenotype and one that has established diagnostic criteria, is a multiple hamartoma syndrome with an increased risk for both benign and malignant tumors arising from the thyroid follicular cell and, in women, the breast and endometrium; pathognomonic skin manifestations include papillomatous papules, facial trichilemmomas, and acral keratoses.^{426,427} Macrocephaly (occipitofrontal circumference >2 standard deviations [SDs]/97.5th percentile) is a nearly universal finding in children with a *PTEN* mutation, and neurodevelopmental disorders are common (autism spectrum disorder in 50%).⁴²⁸ Thyroid neoplasia eventually occurs in the majority of individuals with CS and includes multinodular goiter, follicular adenomas, and differentiated thyroid carcinoma (papillary and follicular thyroid carcinomas).^{429–431} Follicular carcinomas appears to be overrepresented in *PTEN* mutation-positive patients (youngest reported age is 7 years), but papillary carcinomas are also common.^{430–432} The risk of thyroid neoplasia begins during childhood, and one-quarter of children with PHTS have abnormal thyroid imaging.⁴²⁸ Surveillance of children with the PHTS include annual physical examination and periodic thyroid US.^{235,384,427}

Tuberous Sclerosis Complex

The tuberous sclerosis complex (TSC) is an autosomal dominant, multiorgan hamartomatous disorder characterized by abnormalities of the skin (hypomelanotic macules, confetti skin lesions, facial angiofibromas, shagreen patches, fibrous cephalic plaques, unguis fibromas); brain (cortical dysplasias, subependymal nodules and subependymal giant cell astrocytomas, seizures, intellectual disability/developmental delay); kidney (angiomyolipomas, cysts, renal cell carcinomas); heart (rhabdomyomas, arrhythmias); and lungs (lymphangiomyomatosis).⁴³³ The two

known genes that cause the TSC include *TSC1* (chromosome 9q34.13) and *TSC2* (chromosome 16p13.3), which encode for the proteins hamartin and tuberlin, respectively.^{433,434} Various endocrine neoplasms occurring within the context of the TSC have been reported, including functional pituitary adenomas, parathyroid hyperplasia and adenomas, pancreatic NETs, and case reports of PHEO and papillary thyroid cancer.^{435–438} The pancreatic NETs can be benign or malignant and functioning or nonfunctioning, with a large proportion of these cases associated with *TSC2* gene mutations.^{436,438} Insulinomas appear to be overrepresented in TSC patients and so a low threshold should exist for clinical evaluation in those with classic signs or symptoms of hypoglycemia or worsening neurologic symptoms.⁴³⁵

Von Hippel-Lindau Disease

VHL disease is an autosomal dominant, highly penetrant disorder caused by germline mutations in *VHL* (chromosome 3p25.3), a tumor suppressor gene first identified in 1993,⁴³⁹ and characterized by a variable clinical phenotype that includes hemangioblastomas of the retina (also called *retinal angiomas*) and CNS; renal cysts and clear cell renal cell carcinoma; PHEO/PGL; pancreatic cysts, cystadenomas, and pancreatic NETs; endolymphatic sac tumors; and cystadenomas of the epididymis in males and broad ligament of the uterus in females.^{440–442} About 20% of patients with VHL do not have an affected parent and thus either harbor a de novo mutation or inherit a *VHL* mutation from a parent with germline mosaicism; genetic testing is highly reliable, identifying a mutation in almost all clinically affected individuals.⁴⁴⁰ The majority of VHL mutations are small deletions/insertions or point mutations but large deletions comprise 11% of *VHL* pathogenic variants.⁴⁴⁰

Although the median age of PHEO/PGL diagnosis in VHL is age 28 years, up to 20% of pediatric patients (median age 12 years), primarily those individuals with missense mutations of the *VHL* gene, will be diagnosed with a PHEO/PGL (see Fig. 15.1).^{74,443} PHEO is much more common than PGL and malignancy is rare.^{74,441} These tumors can be one of the earliest clinical manifestations of VHL, having been diagnosed as young as age 2 years,⁴⁴⁴ and it can also be the sole clinical manifestation of VHL, as seen in VHL type 2C.^{440,441} Prospective screening is generally recommended to commence at the age of 5 years in children with missense mutations and/or a family history of PHEO/PGL^{39,66,67,74,440}; others have advocated for earlier testing.^{63,445} Although almost all VHL-related PHEO/PGL are functional noradrenergic tumors (see previous PHEO/PGL section), nonfunctional parasympathetic PGL occurring in the context of VHL have also been described.⁴⁴⁶

Pancreatic NETs develop in 15% of VHL patients.⁴⁴⁷ Few pediatric cases have been reported in the literature,⁴⁴⁸ and the youngest case was in a 10-year-old child.⁴⁴⁹ These tumors are clinically nonfunctioning (thus biochemical screening is unnecessary) and they can be malignant, especially with lesions up to 2.8 to 3 cm or with a fast tumor doubling time, and those in patients with germline missense mutations or a mutation in exon 3 of *VHL*.^{449–452} Prospective screening for pancreatic NETs is performed as a component of testing for other VHL intraabdominal manifestations and include intermittent US (typically starting at age 8 years) or MRI (starting at age 16 years),^{66,67,440,442,445} although some groups have recommended MRI starting at age 10 years.⁶³ Similar to MEN1, MRI is preferred over CT to minimize the lifetime exposure to ionizing radiation.⁴⁵³

Finally, testosterone-secreting ovarian steroid cell tumors have been described,⁴⁵⁴ and this diagnosis should be considered in women with VHL who present with an adnexal mass, amenorrhea, and/or hirsutism.

SUMMARY AND FUTURE DEVELOPMENTS

Although rare, childhood endocrine neoplasms, especially NETs, such as PHEO and MTC, are frequently associated with a known tumor predisposition syndrome. Advances in genomic medicine have improved our understanding of the etiology and pathophysiology of these disorders, which in turn has changed the way providers manage patients with the diseases discussed in this chapter. Because it is a rapidly evolving field, the reader should continue to seek the most current information for important clinical decisions regarding individual patient care. Future research will add to our knowledge regarding genotype-phenotype correlations; optimal screening strategies for asymptomatic children known to carry a disease-causing mutation; timing of interventions, such as early thyroidectomy in MEN2 and parathyroidectomy in MEN1; and the treatment of advanced or inoperable disease with newer targeted therapies.

REFERENCES

1. Resta R, Biesecker BB, Bennett RL, et al. A new definition of Genetic Counseling: National Society of Genetic Counselors' Task Force report. *J Genet Couns*. 2006;15(2):77–83.
2. Waguespack SG, Rich TA, Perrier ND, Jimenez C, Cote GJ. Management of medullary thyroid carcinoma and MEN2 syndromes in childhood. *Nat Rev Endocrinol*. 2011;7(10):596–607.
3. De Sousa SM, Hardy TS, Scott HS, Torpy DJ. Genetic testing in endocrinology. *Clin Biochem Rev*. 2018;39(1):17–28.
4. Botkin JR, Belmont JW, Berg JS, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet*. 2015;97(1):6–21.
5. Stromsvik N, Nordin K, Berglund G, Engebretsen LF, Hansson MG, Gjengedal E. Living with multiple endocrine neoplasia type 1: decent care-insufficient medical and genetic information: a qualitative study of MEN 1 patients in a Swedish hospital. *J Genet Couns*. 2007;16(1):105–117.
6. Lloyd RV, Osamura RY, Klöppel G, Rosai J, eds. *WHO Classification of Tumours of Endocrine Organs*. 4th ed Lyon: IARC Press; 2017.
7. Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Pheochromocytoma. *Lancet*. 2005;366(9486):665–675.
8. Neumann HP, Young Jr WF, Krauss T, et al. 65 YEARS OF THE DOUBLE HELIX: Genetics informs precision practice in the diagnosis and management of pheochromocytoma. *Endocr Relat Cancer*. 2018;25(8):T201–T219.
9. Chen H, Sippel RS, O'Dorisio MS, et al. The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. *Pancreas*. 2010;39(6):775–783.
10. Sarathi V. Characteristics of Pediatric Pheochromocytoma/paraganglioma. *Indian J Endocrinol Metabol*. 2017;21(3):470–474.
11. Bausch B, Wellner U, Bausch D, et al. Long-term prognosis of patients with pediatric pheochromocytoma. *Endocr Relat Cancer*. 2014;21(1):17–25.
12. Spoudeas HA, ed. *A Multi-Disciplinary Consensus Statement of Best Practice from a Working Group Convened Under the Auspices of the British Society of Paediatric Endocrinology & Diabetes and the United Kingdom Children's Cancer Study Group*. Paediatric Endocrine Tumours. West Sussex, United Kingdom: Novo Nordisk Ltd.; 2005.
13. Barontini M, Levin G, Sanso G. Characteristics of pheochromocytoma in a 4- to 20-year-old population. *Ann N Y Acad Sci*. 2006;1073:30–37.
14. Neumann HP, Bausch B, McWhinney SR, et al. Germ-line mutations in nonsyndromic pheochromocytoma. *N Engl J Med*. 2002;346(19):1459–1466.
15. Beltsevich DG, Kuznetsov NS, Kazaryan AM, Lysenko MA. Pheochromocytoma surgery: epidemiologic peculiarities in children. *World J Surg*. 2004;28(6):592–596.
16. Ross JH. Pheochromocytoma. Special considerations in children. *Urol Clin North Am*. 2000;27(3):393–402.
17. Stackpole RH, Melicow MM, Uson AC. Pheochromocytoma in children. Report of 9 case and review of the first 100 published cases with follow-up studies. *J Pediatr*. 1963;63:314–330.
18. Perel Y, Schlumberger M, Marguerite G, et al. Pheochromocytoma and paraganglioma in children: a report of 24 cases of the French Society of Pediatric Oncology. *Pediatr Hematol Oncol*. 1997;14(5):413–422.
19. King KS, Prodanov T, Kantorovich V, et al. Metastatic pheochromocytoma/paraganglioma related to primary tumor development in childhood or adolescence: significant link to SDHB mutations. *J Clin Oncol*. 2011;29(31):4137–4142.
20. Cascon A, Inglada-Perez L, Comino-Mendez I, et al. Genetics of pheochromocytoma and paraganglioma in Spanish pediatric patients. *Endocr Relat Cancer*. 2013;20(3):L1–L6.
21. Babic B, Patel D, Aufforth R, et al. Pediatric patients with pheochromocytoma and paraganglioma should have routine preoperative genetic testing for common susceptibility genes in addition to imaging to detect extra-adrenal and metastatic tumors. *Surgery*. 2017;161(1):220–227.
22. Pamporaki C, Hamplova B, Peitzsch M, et al. Characteristics of pediatric vs adult pheochromocytomas and paragangliomas. *J Clin Endocrinol Metabol*. 2017;102(4):1122–1132.
23. Deal JE, Sever PS, Barratt TM, Dillon MJ. Pheochromocytoma—investigation and management of 10 cases. *Arch Dis Child*. 1990;65(3):269–274.
24. Wyszynska T, Cichocka E, Wieteska-Klimczak A, Jobs K, Januszewicz P. A single pediatric center experience with 1025 children with hypertension. *Acta Paediatr*. 1992;81(3):244–246.
25. Young Jr WF. Endocrine hypertension. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, eds. *Williams Textbook of Endocrinology*. 13 ed. Philadelphia, PA: Elsevier; 2016:556–588.
26. Armstrong R, Sridhar M, Greenhalgh KL, et al. Pheochromocytoma in children. *Arch Dis Child*. 2008;93(10):899–904.
27. Timmers HJ, Pacak K, Huynh TT, et al. Biochemically silent abdominal paragangliomas in patients with mutations in the succinate dehydrogenase subunit B gene. *J Clin Endocrinol Metabol*. 2008;93(12):4826–4832.
28. Andrews KA, Ascher DB, Pires DEV, et al. Tumour risks and genotype-phenotype correlations associated with germline variants in succinate dehydrogenase subunit genes SDHB, SDHC and SDHD. *J Med Genet*. 2018;55(6):384–394.
29. Cryer PE. Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. *N Engl J Med*. 1980;303(8):436–444.
30. Tischler AS. Pheochromocytoma and extra-adrenal paraganglioma: updates. *Arch Pathol Lab Med*. 2008;132(8):1272–1284.
31. Khadilkar K, Sarathi V, Kasaliwal R, et al. Genotype-phenotype correlation in paediatric pheochromocytoma and paraganglioma: a single centre experience from India. *J Pediatr Endocrinol Metab*. 2017;30(5):575–581.
32. Westfall TC, Westfall DP. Neurotransmission: the autonomic and somatic motor nervous systems. In: Brunton LL, ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill; 2011:171–218.
33. Westfall TC, Westfall DP. Catecholamines and sympathomimetic drugs. In: Brunton LL, ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill; 2011:277–333.
34. Sanders-Bush E, Hazelwood L. 5-Hydroxytryptamine (Serotonin) and Dopamine. In: Brunton LL, ed. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12 ed. New York, NY: McGraw-Hill; 2011:335–361.
35. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol Rev*. 2004;56(3):331–349.
36. Wurtman RJ, Pohorecky LA. Adrenocortical control of epinephrine synthesis in health and disease. *Adv Metab Disord*. 1971;5:53–76.
37. Axelrod J, Reisine TD. Stress hormones: their interaction and regulation. *Science*. 1984;224(4648):452–459.
38. Pham TH, Moir C, Thompson GB, et al. Pheochromocytoma and paraganglioma in children: a review of medical and surgical

- management at a tertiary care center. *Pediatrics*. 2006;118(3):1109–1117.
39. Waguespack SG, Rich T, Grubbs E, et al. A current review of the etiology, diagnosis, and treatment of pediatric pheochromocytoma and paraganglioma. *J Clin Endocrinol Metabol*. 2010;95(5):2023–2037.
 40. Else T, Greenberg S, Fishbein L. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *Hereditary paraganglioma-pheochromocytoma syndromes*. Seattle, WA: *GeneReviews*((R)); 2008, [updated 2018 Oct 4].
 41. Alrezk R, Suarez A, Tena I, Pacak K. Update of pheochromocytoma syndromes: genetics, biochemical evaluation, and imaging. *Front Endocrinol (Lausanne)*. 2018;9:515.
 42. Opotowsky AR, Moko LE, Ginns J, et al. Pheochromocytoma and paraganglioma in cyanotic congenital heart disease. *J Clin Endocrinol Metabol*. 2015;100(4):1325–1334.
 43. Zhao B, Zhou Y, Zhao Y, et al. Co-occurrence of pheochromocytoma-paraganglioma and cyanotic congenital heart disease: a case report and literature review. *Front Endocrinol (Lausanne)*. 2018;9:165.
 44. Manger WM. The protean manifestations of pheochromocytoma. *Horm Metab Res*. 2009;41(9):658–663.
 45. Karagiannis A, Mikhailidis DP, Athyros VG, Harsoulis F. Pheochromocytoma: an update on genetics and management. *Endocr Relat Cancer*. 2007;14(4):935–956.
 46. Januszewicz P, Wieteska-Klimczak A, Wyszynska T. Pheochromocytoma in children: difficulties in diagnosis and localization. *Clin Exp Hypertens A*. 1990;12(4):571–579.
 47. Ein SH, Pullerits J, Creighton R, Balfe JW. Pediatric pheochromocytoma. A 36-year review. *Pediatr Surg Int*. 1997;12(8):595–598.
 48. Sullivan J, Groshong T, Tobias JD. Presenting signs and symptoms of pheochromocytoma in pediatric-aged patients. *Clin Pediatr (Phila)*. 2005;44(8):715–719.
 49. Batsis M, Dagalakis U, Stratakis CA, et al. Attention deficit hyperactivity disorder in pediatric patients with pheochromocytoma and paraganglioma. *Horm Metab Res*. 2016;48(8):509–513.
 50. Bissada NK, Safwat AS, Seyam RM, et al. Pheochromocytoma in children and adolescents: a clinical spectrum. *J Pediatr Surg*. 2008;43(3):540–543.
 51. Kassim TA, Clarke DD, Mai VQ, Clyde PW, Mohamed Shakir KM. Catecholamine-induced cardiomyopathy. *Endocr Pract*. 2008;14(9):1137–1149.
 52. Zelinka T, Petrak O, Turkova H, et al. High incidence of cardiovascular complications in pheochromocytoma. *Horm Metab Res*. 2012;44(5):379–384.
 53. Prejbisz A, Lenders JW, Eisenhofer G, Januszewicz A. Cardiovascular manifestations of pheochromocytoma. *J Hypertens*. 2011;29(11):2049–2060.
 54. Offergeld C, Brase C, Yaremchuk S, et al. Head and neck paragangliomas: clinical and molecular genetic classification. *Clinics (Sao Paulo)*. 2012;67(Suppl 1):19–28.
 55. Muth A, Crona J, Gimm O, et al. Genetic testing and surveillance guidelines in hereditary pheochromocytoma and paraganglioma. *J Intern Med*. 2019;285(2):187–204.
 56. Pomares FJ, Canas R, Rodriguez JM, Hernandez AM, Parrilla P, Tebar FJ. Differences between sporadic and multiple endocrine neoplasia type 2A pheochromocytoma. *Clin Endocrinol*. 1998;48(2):195–200.
 57. Walther MM, Reiter R, Keiser HR, et al. Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. *J Urol*. 1999;162(3 Pt 1):659–664.
 58. van Duinen N, Steenvoorden D, Bonsing BA, et al. Pheochromocytomas detected by biochemical screening in predisposed subjects are associated with lower prevalence of clinical and biochemical manifestations and smaller tumors than pheochromocytomas detected by signs and symptoms. *Eur J Endocrinol*. 2010;163(1):121–127.
 59. Lenders JW, Duh QY, Eisenhofer G, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. *J Clin Endocrinol Metabol*. 2014;99(6):1915–1942.
 60. Plouin PF, Amar L, Dekkers OM, et al. European Society of Endocrinology Clinical Practice Guideline for long-term follow-up of patients operated on for a pheochromocytoma or a paraganglioma. *Eur J Endocrinol*. 2016;174(5):G1–G10.
 61. Buffet A, Ben Aim L, Lebouilleux S, et al. Positive impact of genetic test on the management and outcome of patients with paraganglioma and/or pheochromocytoma. *J Clin Endocrinol Metabol*. 2019;104(4):1109–1118.
 62. Hampel H, Bennett RL, Buchanan A, et al. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. *Genet Med*. 2015;17(1):70–87.
 63. Rednam SP, Erez A, Druker H, et al. Von Hippel-Lindau and hereditary pheochromocytoma/paraganglioma syndromes: clinical features, genetics, and surveillance recommendations in childhood. *Clin Cancer Res*. 2017;23(12):e68–e75.
 64. Wasserman JD, Tomlinson GE, Druker H, et al. Multiple endocrine neoplasia and hyperparathyroid-jaw tumor syndromes: clinical features, genetics, and surveillance recommendations in childhood. *Clin Cancer Res*. 2017;23(13):e123–e132.
 65. Wells Jr SA, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*. 2015;25(6):567–610.
 66. Alliance V. *The VHL handbook*. 5th edition. Boston, MA: VHL Alliance; 2015.
 67. Binderup ML, Bisgaard ML, Harbud V, et al. Von Hippel-Lindau disease (vHL). National clinical guideline for diagnosis and surveillance in Denmark. 3rd edition, *Danish Med J*. 2013;60(12):B4763.
 68. d'Herbomez M, Forzy G, Bauters C, et al. An analysis of the biochemical diagnosis of 66 pheochromocytomas. *Eur J Endocrinol*. 2007;156(5):569–575.
 69. Pacak K, Eisenhofer G, Ahlman H, et al. Pheochromocytoma: recommendations for clinical practice from the First International Symposium. October 2005. *Nat Clin Pract Endocrinol Metab*. 2007;3(2):92–102.
 70. Havekes B, Romijn JA, Eisenhofer G, Adams K, Pacak K. Update on pediatric pheochromocytoma. *Pediatr Nephrol*. 2009;24(5):943–950.
 71. Grossman A, Pacak K, Sawka A, et al. Biochemical diagnosis and localization of pheochromocytoma: can we reach a consensus? *Ann N Y Acad Sci*. 2006;1073:332–347.
 72. Sawka AM, Jaeschke R, Singh RJ, Young Jr WF. A comparison of biochemical tests for pheochromocytoma: measurement of fractionated plasma metanephrines compared with the combination of 24-hour urinary metanephrines and catecholamines. *J Clin Endocrinol Metabol*. 2003;88(2):553–558.
 73. Eisenhofer G, Goldstein DS, Walther MM, et al. Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. *J Clin Endocrinol Metabol*. 2003;88(6):2656–2666.
 74. Aufforth RD, Ramakant P, Sadowski SM, et al. Pheochromocytoma Screening Initiation and Frequency in von Hippel-Lindau Syndrome. *J Clin Endocrinol Metabol*. 2015;100(12):4498–4504.
 75. de Jong WH, Eisenhofer G, Post WJ, Muskiet FA, de Vries EG, Kema IP. Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors. *J Clin Endocrinol Metabol*. 2009;94(8):2841–2849.
 76. Brodeur GM, Hogarty MD, Mosse YP, Maris JM. Neuroblastoma. In: Pizzo PA, Poplack DG, eds. *Principles and Practice of Pediatric Oncology*. 6 ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins; 2011:886–922.
 77. Lawrence AM. Glucagon provocative test for pheochromocytoma. *Ann Intern Med*. 1967;66(6):1091–1096.
 78. Bravo EL, Tarazi RC, Fouad FM, Vidt DG, Gifford Jr RW. Clonidine-suppression test: a useful aid in the diagnosis of pheochromocytoma. *N Engl J Med*. 1981;305(11):623–626.
 79. Eisenhofer G, Lenders JW, Linehan WM, Walther MM, Goldstein DS, Keiser HR. Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. *N Engl J Med*. 1999;340(24):1872–1879.
 80. Pacak K, Eisenhofer G, Ilias I. Diagnosis of pheochromocytoma with special emphasis on MEN2 syndrome. *Hormones (Athens)*. 2009;8(2):111–116.

81. Eisenhofer G, Walther MM, Huynh TT, et al. Pheochromocytomas in von Hippel-Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. *J Clin Endocrinol Metabol.* 2001;86(5):1999–2008.
82. Eisenhofer G, Timmers HJ, Lenders JW, et al. Age at diagnosis of pheochromocytoma differs according to catecholamine phenotype and tumor location. *J Clin Endocrinol Metabol.* 2011;96(2):375–384.
83. Eisenhofer G, Klink B, Richter S, Lenders JW, Robledo M. Metabologenomics of pheochromocytoma and paraganglioma: an integrated approach for personalized biochemical and genetic testing. *Clin Biochem Rev.* 2017;38(2):69–100.
84. van Duinen N, Steenvoorden D, Kema IP, et al. Increased urinary excretion of 3-methoxytyramine in patients with head and neck paragangliomas. *J Clin Endocrinol Metabol.* 2010;95(1):209–214.
85. Eisenhofer G, Goldstein DS, Sullivan P, et al. Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. *J Clin Endocrinol Metabol.* 2005;90(4):2068–2075.
86. Timmers HJ, Kozupa A, Eisenhofer G, et al. Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. *J Clin Endocrinol Metabol.* 2007;92(3):779–786.
87. Eisenhofer G, Lenders JW, Timmers H, et al. Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. *Clin Chem.* 2011;57(3):411–420.
88. Proye C, Fossati P, Fontaine P, et al. Dopamine-secreting pheochromocytoma: an unrecognized entity? Classification of pheochromocytomas according to their type of secretion. *Surgery.* 1986;100(6):1154–1162.
89. Dubois IA, Gray DK. Dopamine-secreting pheochromocytomas: in search of a syndrome. *World J Surg.* 2005;29(7):909–913.
90. Bilek R, Safarik L, Ciprova V, Vlcek P, Lisa L. Chromogranin A, a member of neuroendocrine secretory proteins as a selective marker for laboratory diagnosis of pheochromocytoma. *Physiol Res.* 2008;57(Suppl 1):S171–S179.
91. Kidd M, Bodei L, Modlin IM. Chromogranin A: any relevance in neuroendocrine tumors? *Curr Opin Endocrinol Diabetes Obes.* 2016;23(1):28–37.
92. Timmers HJ, Taieb D, Pacak K. Current and future anatomical and functional imaging approaches to pheochromocytoma and paraganglioma. *Horm Metab Res.* 2012;44(5):367–372.
93. Blake MA, Cronin CG, Boland GW. Adrenal imaging. *AJR Am J Roentgenol.* 2010;194(6):1450–1460.
94. Greenblatt DY, Shenker Y, Chen H. The utility of metaiodobenzylguanidine (MIBG) scintigraphy in patients with pheochromocytoma. *Ann Surg Oncol.* 2008;15(3):900–905.
95. Brito JP, Asi N, Gionfriddo MR, et al. The incremental benefit of functional imaging in pheochromocytoma/paraganglioma: a systematic review. *Endocrine.* 2015;50(1):176–186.
96. Jimenez C, Waguespack SG. Functional imaging for pheochromocytoma-paraganglioma: a step closer to understanding its place in clinical practice. *Endocrine.* 2015;50(1):6–8.
97. Jha A, Ling A, Millo C, et al. (2018). Superiority of (68)Ga-DOTATATE over (18)F-FDG and anatomic imaging in the detection of succinate dehydrogenase mutation (SDHx)-related pheochromocytoma and paraganglioma in the pediatric population. *Eur J Nucl Med Mol Imaging.* 2018;45(5):787–797.
98. Kroiss AS. Current status of functional imaging in neuroblastoma, pheochromocytoma, and paraganglioma disease. *Wien Med Wochenschr.* 2019;169(1-2):25–32.
99. Sisson JC, Wieland DM. Radiolabeled meta-iodobenzylguanidine: pharmacology and clinical studies. *Am J Physiol Imaging.* 1986;1(2):96–103.
100. Ilias I, Divgi C, Pacak K. Current role of metaiodobenzylguanidine in the diagnosis of pheochromocytoma and medullary thyroid cancer. *Semin Nucl Med.* 2011;41(5):364–368.
101. Buffet A, Morin A, Castro-Vega LJ, et al. Germline mutations in the mitochondrial 2-oxoglutarate/malate carrier SLC25A11 gene confer a predisposition to metastatic paragangliomas. *Cancer Res.* 2018;78(8):1914–1922.
102. Castinetti F, Qi XP, Walz MK, et al. Outcomes of adrenal-sparing surgery or total adrenalectomy in pheochromocytoma associated with multiple endocrine neoplasia type 2: an international retrospective population-based study. *Lancet Oncol.* 2014;15(6):648–655.
103. Jacobson AF, Deng H, Lombard J, Lessig HJ, Black RR. 123I-metaiodobenzylguanidine scintigraphy for the detection of neuroblastoma and pheochromocytoma: results of a meta-analysis. *J Clin Endocrinol Metabol.* 2010;95(6):2596–2606.
104. Fonte JS, Robles JF, Chen CC, et al. False-negative (1)(2)(3)I-MIBG SPECT is most commonly found in SDHB-related pheochromocytoma or paraganglioma with high frequency to develop metastatic disease. *Endocr Relat Cancer.* 2012;19(1):83–93.
105. Shulkin BL, Shapiro B. Current concepts on the diagnostic use of MIBG in children. *J Nucl Med.* 1998;39(4):679–688.
106. Chang CA, Pattison DA, Tothill RW, et al. (68)Ga-DOTATATE and (18)F-FDG PET/CT in paraganglioma and pheochromocytoma: utility, patterns and heterogeneity. *Cancer Imaging.* 2016;16(1):22.
107. Hofman MS, Lau WF, Hicks RJ. Somatostatin receptor imaging with 68Ga DOTATATE PET/CT: clinical utility, normal patterns, pearls, and pitfalls in interpretation. *Radiographics.* 2015;35(2):500–516.
108. Maurice JB, Troke R, Win Z, et al. A comparison of the performance of (6)(8)Ga-DOTATATE PET/CT and (1)(2)(3)I-MIBG SPECT in the diagnosis and follow-up of pheochromocytoma and paraganglioma. *Eur J Nucl Med Mol Imaging.* 2012;39(8):1266–1270.
109. Han S, Suh CH, Woo S, Kim YJ, Lee JJ. Performance of (68)Ga-DOTA-conjugated somatostatin receptor-targeting peptide pet in detection of pheochromocytoma and paraganglioma: a systematic review and metaanalysis. *J Nucl Med.* 2019;60(3):369–376.
110. The NGS in PPGL Study Group, Toledo RA, Burnichon N, et al. Consensus Statement on next-generation-sequencing-based diagnostic testing of hereditary pheochromocytomas and paragangliomas. *Nat Rev Endocrinol.* 2017;13(4):233–247.
111. Castro-Vega LJ, Buffet A, De Cubas AA, et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum Mol Genet.* 2014;23(9):2440–2446.
112. Lorenzo FR, Yang C, Ng Tang Fui M, et al. A novel EPAS1/HIF2A germline mutation in a congenital polycythemia with paraganglioma. *J Mol Med (Berl).* 2012;91(4):507–512.
113. Yeh IT, Lenci RE, Qin Y, et al. A germline mutation of the KIF1B beta gene on 1p36 in a family with neural and nonneural tumors. *Hum Genet.* 2008;124(3):279–285.
114. Dackiw AP, Cote GJ, Fleming JB, et al. Screening for MEN1 mutations in patients with atypical endocrine neoplasia. *Surgery.* 1999;126(6):1097–1103. discussion 1103–1094.
115. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. *Nat Genet.* 2011;43(7):663–667.
116. Ladrone C, Carcenac R, Leporrier M, et al. PHD2 mutation and congenital erythrocytosis with paraganglioma. *N Engl J Med.* 2008;359(25):2685–2692.
117. Yang C, Zhuang Z, Fliedner SM, et al. Germ-line PHD1 and PHD2 mutations detected in patients with pheochromocytoma/paraganglioma-polycythemia. *J Mol Med (Berl).* 2015;93(1):93–104.
118. Hao HX, Khalimonchuk O, Schradlers M, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science.* 2009;325(5944):1139–1142.
119. Burnichon N, Briere JJ, Libe R, et al. SDHA is a tumor suppressor gene causing paraganglioma. *Hum Mol Genet.* 2010;19(15):3011–3020.
120. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet.* 2000;26(3):268–270.
121. Qin Y, Yao L, King EE, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. *Nat Genet.* 2010;42(3):229–233.
122. Wadt K, Choi J, Chung JY, et al. A cryptic BAP1 splice mutation in a family with uveal and cutaneous melanoma, and paraganglioma. *Pigment Cell Melanoma Res.* 2012;25(6):815–818.

123. Cascon A, Comino-Mendez I, Curras-Freixes M, et al. Whole-exome sequencing identifies MDH2 as a new familial paraganglioma gene. *J Natl Cancer Inst.* 2015;107(5).
124. Toledo RA, Qin Y, Cheng ZM, et al. Recurrent mutations of chromatin-remodeling genes and kinase receptors in pheochromocytomas and paragangliomas. *Clin Cancer Res.* 2016;22(9):2301–2310.
125. Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, et al. Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. *Mol Endocrinol.* 2010;24(12):2382–2391.
126. Vanderveen KA, Thompson SM, Callstrom MR, et al. Biopsy of pheochromocytomas and paragangliomas: potential for disaster. *Surgery.* 2009;146(6):1158–1166.
127. Ball MW, Hemal AK, Allaf ME. International Consultation on Urological Diseases and European Association of Urology International Consultation on Minimally Invasive Surgery in Urology: laparoscopic and robotic adrenalectomy. *BJU Int.* 2017;119(1):13–21.
128. Network NCC. *Neuroendocrine and Adrenal Tumors (Version 4.2018)*. Accessed January 26, 2019.
129. Mannelli M, Dralle H, Lenders JW. Perioperative management of pheochromocytoma/paraganglioma: is there a state of the art? *Horm Metab Res.* 2012;44(5):373–378.
130. Yip L, Lee JE, Shapiro SE, et al. Surgical management of hereditary pheochromocytoma. *J Am Coll Surg.* 2004;198(4):525–534. discussion 534–525.
131. Castinetti F, Waguespack SG, Machens A, et al. Natural history, treatment, and long-term follow up of patients with multiple endocrine neoplasia type 2B: an international, multicentre, retrospective study. *Lancet Diabetes Endocrinol.* 2019;7(3):213–220.
132. Nagaraja V, Eslick GD, Edirimanne S. Recurrence and functional outcomes of partial adrenalectomy: a systematic review and meta-analysis. *Int J Surg.* 2015;16(Pt A):7–13.
133. Guerin C, Romanet P, Taieb D, et al. Looking beyond the thyroid: advances in the understanding of pheochromocytoma and hyperparathyroidism phenotypes in MEN2 and of non-MEN2 familial forms. *Endocr Relat Cancer.* 2018;25(2):T15–T28.
134. Gruber LM, Erickson D, Babovic-Vuksanovic D, Thompson GB, Young Jr WF, Bancos I. Pheochromocytoma and paraganglioma in patients with neurofibromatosis type 1. *Clin Endocrinol.* 2017;86(1):141–149.
135. Grubbs EG, Rich TA, Ng C, et al. Long-term outcomes of surgical treatment for hereditary pheochromocytoma. *J Am Coll Surg.* 2013;216(2):280–289.
136. Smith JD, Harvey RN, Darr OA, et al. Head and neck paragangliomas: a two-decade institutional experience and algorithm for management. *Laryngoscope Invest Otolaryngol.* 2017;2(6):380–389.
137. Hack HA. The perioperative management of children with pheochromocytoma. *Paediatr Anaesth.* 2000;10(5):463–476.
138. Van Braeckel P, Carlier S, Steelaent PJ, Weyne L, Vanfleteren L. Perioperative management of pheochromocytoma. *Acta Anaesthesiol Belg.* 2009;60(1):55–66.
139. Romero M, Kapur G, Baracco R, Valentini RP, Mattoo TK, Jain A. Treatment of hypertension in children with catecholamine-secreting tumors: a systematic approach. *J Clin Hypertens (Greenwich).* 2015;17(9):720–725.
140. Weingarten TN, Cata JP, O'Hara JF, et al. Comparison of two preoperative medical management strategies for laparoscopic resection of pheochromocytoma. *Urology.* 2010;76(2):508 e506–511.
141. Pacak K. Preoperative management of the pheochromocytoma patient. *J Clin Endocrinol Metabol.* 2007;92(11):4069–4079.
142. Wachtel H, Kennedy EH, Zaheer S, et al. Preoperative metyrosine improves cardiovascular outcomes for patients undergoing surgery for pheochromocytoma and paraganglioma. *Ann Surg Oncol.* 2015;22(Suppl 3):S646–S654.
143. Thompson LD. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. *Am J Surg Pathol.* 2002;26(5):551–566.
144. Kimura N, Takayanagi R, Takizawa N, et al. Pathological grading for predicting metastasis in pheochromocytoma and paraganglioma. *Endocr Relat Cancer.* 2014;21(3):405–414.
145. Kimura N, Watanabe T, Noshiro T, Shizawa S, Miura Y. Histological grading of adrenal and extra-adrenal pheochromocytomas and relationship to prognosis: a clinicopathological analysis of 116 adrenal pheochromocytomas and 30 extra-adrenal sympathetic paragangliomas including 38 malignant tumors. *Endocr Pathol.* 2005;16(1):23–32.
146. Wu D, Tischler AS, Lloyd RV, et al. Observer variation in the application of the Pheochromocytoma of the Adrenal Gland Scaled Score. *Am J Surg Pathol.* 2009;33(4):599–608.
147. Ayala-Ramirez M, Feng L, Johnson MM, et al. Clinical risk factors for malignancy and overall survival in patients with pheochromocytomas and sympathetic paragangliomas: primary tumor size and primary tumor location as prognostic indicators. *J Clin Endocrinol Metabol.* 2011;96(3):717–725.
148. Hamidi O, Young Jr WF, Gruber L, et al. Outcomes of patients with metastatic pheochromocytoma and paraganglioma: a systematic review and meta-analysis. *Clin Endocrinol.* 2017;87(5):440–450.
149. Fishbein L, Ben-Maimon S, Keefe S, et al. SDHB mutation carriers with malignant pheochromocytoma respond better to CVD. *Endocr Relat Cancer.* 2017;24(8):L51–L55.
150. Hazard JB, Hawk WA, Crile Jr G. Medullary (solid) carcinoma of the thyroid; a clinicopathologic entity. *J Clin Endocrinol Metabol.* 1959;19(1):152–161.
151. Wolfe HJ, Melvin KE, Cervi-Skinner SJ, et al. C-cell hyperplasia preceding medullary thyroid carcinoma. *N Engl J Med.* 1973;289(9):437–441.
152. Hogan AR, Zhuge Y, Perez EA, Koniaris LG, Lew JI, Sola JE. Pediatric thyroid carcinoma: incidence and outcomes in 1753 patients. *J Surg Res.* 2009;156(1):167–172.
153. Raval MV, Sturgeon C, Bentrem DJ, et al. Influence of lymph node metastases on survival in pediatric medullary thyroid cancer. *J Pediatr Surg.* 2010;45(10):1947–1954.
154. Donis-Keller H, Dou S, Chi D, et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Hum Mol Genet.* 1993;2(7):851–856.
155. Mulligan LM, Kwok JB, Healey CS, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature.* 1993;363(6428):458–460.
156. Hofstra RM, Landsvater RM, Ceccherini I, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature.* 1994;367(6461):375–376.
157. Carlson KM, Dou S, Chi D, et al. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. *Proc Natl Acad Sci U S A.* 1994;91(4):1579–1583.
158. Eng C, Clayton D, Schuffenecker I, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA.* 1996;276(19):1575–1579.
159. Wells Jr SA. Advances in the management of MEN2: from improved surgical and medical treatment to novel kinase inhibitors. *Endocr Relat Cancer.* 2018;25(2):T1–T13.
160. Frank-Raue K, Raue F. Hereditary medullary thyroid cancer genotype-phenotype correlation. [Recent results in cancer research Fortschritte der Krebsforschung Progres dans les recherches sur le cancer]. *Medullary Thyroid Carcinoma.* 2015;204:139–156.
161. Romei C, Ciampi R, Elisei R. A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. *Nat Rev Endocrinol.* 2016;12(4):192–202.
162. Baloh RH, Enomoto H, Johnson Jr EM, Milbrandt J. The GDNF family ligands and receptors – implications for neural development. *Curr Opin Neurobiol.* 2000;10(1):103–110.
163. Wagner SM, Zhu S, Nicolescu AC, Mulligan LM. Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2. *Clinics (Sao Paulo).* 2012;67(Suppl 1):77–84.
164. Drosten M, Putzer BM. Mechanisms of disease: cancer targeting and the impact of oncogenic RET for medullary thyroid carcinoma therapy. *Nat Clin Pract Oncol.* 2006;3(10):564–574.

165. Wells Jr SA, Chi DD, Toshima K, et al. Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. *Ann Surg.* 1994;220(3):237–247. discussion 247–250.
166. Machens A. Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med.* 2004;350(9):943.
167. Mete O, Asa SL. Precursor lesions of endocrine system neoplasms. *Pathology.* 2013;45(3):316–330.
168. Machens A, Lorenz K, Weber F, Dralle H. Genotype-specific progression of hereditary medullary thyroid cancer. *Hum Mutat.* 2018;39(6):860–869.
169. Wray CJ, Rich TA, Waguespack SG, Lee JE, Perrier ND, Evans DB. Failure to recognize multiple endocrine neoplasia 2B: more common than we think? *Ann Surg Oncol.* 2008;15(1):293–301.
170. Brauckhoff M, Machens A, Hess S, et al. Premonitory symptoms preceding metastatic medullary thyroid cancer in MEN 2B: an exploratory analysis. *Surgery.* 2008;144(6):1044–1050. discussion 1050–1043.
171. Guillem JG, Wood WC, Moley JF, et al. ASCO/SSO review of current role of risk-reducing surgery in common hereditary cancer syndromes. *J Clin Oncol.* 2006;24(28):4642–4660.
172. National Comprehensive Cancer Network. Thyroid Carcinoma (Version 3.2018). https://www.nccn.org/professionals/physician_gls/default.aspx#site; 2018. (Accessed 19 January 2019).
173. Raue F, Frank-Raue K. Update on multiple endocrine neoplasia type 2: focus on medullary thyroid carcinoma. *J Endocr Soc.* 2018;2(8):933–943.
174. Sosa JA, Tuggle CT, Wang TS, et al. Clinical and economic outcomes of thyroid and parathyroid surgery in children. *J Clin Endocrinol Metabol.* 2008;93(8):3058–3065.
175. Youngwirth LM, Adam MA, Thomas SM, Roman SA, Sosa JA, Scheri RP. Pediatric thyroid cancer patients referred to high-volume facilities have improved short-term outcomes. *Surgery.* 2018;163(2):361–366.
176. Rohmer V, Vidal-Trecan G, Bourdelot A, et al. Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Français d'Etude des Tumeurs Endocrines. *J Clin Endocrinol Metabol.* 2011;96(3):E509–E518.
177. Machens A, Lorenz K, Dralle H. Constitutive RET tyrosine kinase activation in hereditary medullary thyroid cancer: clinical opportunities. *J Intern Med.* 2009;266(1):114–125.
178. Machens A, Lorenz K, Dralle H. Individualization of lymph node dissection in RET (rearranged during transfection) carriers at risk for medullary thyroid cancer: value of pretherapeutic calcitonin levels. *Ann Surg.* 2009;250(2):305–310.
179. Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK, Wells Jr SA. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. *N Engl J Med.* 2005;353(11):1105–1113.
180. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. *Nat Rev Clin Oncol.* 2018;15(3):151–167.
181. Fox E, Widemann BC, Chuk MK, et al. Vandetanib in children and adolescents with multiple endocrine neoplasia type 2B associated medullary thyroid carcinoma. *Clin Cancer Res.* 2013;19(15):4239–4248.
182. Kraft IL, Akshintala S, Zhu Y, et al. Outcomes of children and adolescents with advanced hereditary medullary thyroid carcinoma treated with vandetanib. *Clin Cancer Res.* 2018;24(4):753–765.
183. Chuk MK, Widemann BC, Minard CG, et al. A phase 1 study of cabozantinib in children and adolescents with recurrent or refractory solid tumors, including CNS tumors: Trial ADVL1211, a report from the Children's Oncology Group. *Pediatr Blood Cancer.* 2018;65(8). e27077.
184. Golpanian S, Tashiro J, Sola JE, et al. Surgically treated pediatric nonpapillary thyroid carcinoma. *Eur J Pediatr Surg.* 2016;26(6):524–532.
185. de Groot JW, Plukker JT, Wolffenbuttel BH, Wiggers T, Sluiter WJ, Links TP. Determinants of life expectancy in medullary thyroid cancer: age does not matter. *Clin Endocrinol.* 2006;65(6):729–736.
186. Mathiesen JS, Kroustrup JP, Vestergaard P, et al. Survival and long-term biochemical cure in medullary thyroid carcinoma in Denmark 1997–2014: a nationwide study. *Thyroid.* 2019;29(3):368–377.
187. Cupisti K, Wolf A, Raffel A, et al. Long-term clinical and biochemical follow-up in medullary thyroid carcinoma: a single institution's experience over 20 years. *Ann Surg.* 2007;246(5):815–821.
188. Machens A, Niccoli-Sire P, Hoegel J, et al. Early malignant progression of hereditary medullary thyroid cancer. *N Engl J Med.* 2003;349(16):1517–1525.
189. Zenaty D, Aigrain Y, Peuchmaur M, et al. Medullary thyroid carcinoma identified within the first year of life in children with hereditary multiple endocrine neoplasia type 2A (codon 634) and 2B. *Eur J Endocrinol.* 2009;160(5):807–813.
190. Meijer JA, le Cessie S, van den Hout WB, et al. Calcitonin and carcinoembryonic antigen doubling times as prognostic factors in medullary thyroid carcinoma: a structured meta-analysis. *Clin Endocrinol.* 2010;72(4):534–542.
191. Costante G, Durante C, Francis Z, Schlumberger M, Filetti S. Determination of calcitonin levels in C-cell disease: clinical interest and potential pitfalls. *Nat Clin Pract Endocrinol Metab.* 2009;5(1):35–44.
192. Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL. The complex of myxomas, spotty pigmentation, and endocrine overactivity. *Medicine (Baltimore).* 1985;64(4):270–283.
193. Shenoy BV, Carpenter PC, Carney JA. Bilateral primary pigmented nodular adrenocortical disease. Rare cause of the Cushing syndrome. *Am J Surg Pathol.* 1984;8(5):335–344.
194. Saggini A, Brandi ML. Skin lesions in hereditary endocrine tumor syndromes. *Endocr Pract.* 2011;17(Suppl 3):47–57.
195. Kamilaris CDC, Fauz FR, Vouretakis A, Stratakis CA. Carney complex. *Exp Clin Endocrinol Diabetes.* 2019;127(2–03):156–164.
196. Stratakis CA. Carney complex: a familial lentiginosis predisposing to a variety of tumors. *Rev Endocr Metab Disord.* 2016;17(3):367–371.
197. Kirschner LS, Carney JA, Pack SD, et al. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat Genet.* 2000;26(1):89–92.
198. Casey M, Vaughan CJ, He J, et al. Mutations in the protein kinase A RIalpha regulatory subunit cause familial cardiac myxomas and Carney complex. *J Clin Invest.* 2000;106(5):R31–R38.
199. Stratakis CA, Carney JA, Lin JP, et al. Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. *J Clin Invest.* 1996;97(3):699–705.
200. Bertherat J, Horvath A, Groussin L, et al. Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *J Clin Endocrinol Metabol.* 2009;94(6):2085–2091.
201. Lowe KM, Young Jr WF, Lyssikatos C, Stratakis CA, Carney JA. Cushing syndrome in Carney complex: clinical, pathologic, and molecular genetic findings in the 17 affected Mayo clinic patients. *Am J Surg Pathol.* 2017;41(2):171–181.
202. Stratakis CA, Sarlis N, Kirschner LS, et al. Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. *Ann Intern Med.* 1999;131(8):585–591.
203. Doppman JL, Travis WD, Nieman L, et al. Cushing syndrome due to primary pigmented nodular adrenocortical disease: findings at CT and MR imaging. *Radiology.* 1989;172(2):415–420.
204. Stratakis CA. Adrenocortical tumors, primary pigmented adrenocortical disease (PPNAD)/Carney complex, and other bilateral hyperplasias: the NIH studies. *Horm Metab Res.* 2007;39(6):467–473.
205. Kirschner LS. PRKAR1A and the evolution of pituitary tumors. *Mol Cell Endocrinol.* 2010;326(1–2):3–7.
206. Stratakis CA, Kirschner LS, Carney JA. Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metabol.* 2001;86(9):4041–4046.
207. Pepe S, Korbonits M, Iacovazzo D. Germline and mosaic mutations causing pituitary tumours: genetic and molecular aspects. *J Endocrinol.* 2019;240(2):R21–R45.
208. Handley J, Carson D, Sloan J, et al. Multiple lentiginosis, myxoid tumours and endocrine overactivity; four cases of Carney's complex. *Br J Dermatol.* 1992;126(4):367–371.

209. Hernandez-Ramirez LC, Tatsi C, Lodish MB, et al. Corticotropinoma as a component of Carney complex. *J Endocr Soc.* 2017;1(7):918–925.
210. Carney JA, Lyssikatos C, Seethala RR, et al. The spectrum of thyroid gland pathology in Carney complex: the importance of follicular carcinoma. *Am J Surg Pathol.* 2018;42(5):587–594.
211. Ulbright TM, Amin MB, Young RH. Intratubular large cell hyalinizing sertoli cell neoplasia of the testis: a report of 8 cases of a distinctive lesion of the Peutz-Jeghers syndrome. *Am J Surg Pathol.* 2007;31(6):827–835.
212. Daly AF, Jaffrain-Rea ML, Ciccarelli A, et al. Clinical characterization of familial isolated pituitary adenomas. *J Clin Endocrinol Metabol.* 2006;91(9):3316–3323.
213. Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev.* 2013;34(2):239–277.
214. Vierimaa O, Georgitsi M, Lehtonen R, et al. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science.* 2006;312(5777):1228–1230.
215. Daly AF, Vanbellinghen JF, Khoo SK, et al. Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. *J Clin Endocrinol Metabol.* 2007;92(5):1891–1896.
216. Jaffrain-Rea ML, Daly AF, Angelini M, Petrossians P, Bours V, Beckers A. Genetic susceptibility in pituitary adenomas: from pathogenesis to clinical implications. *Expert Rev Endocrinol Metab.* 2011;6(2):195–214.
217. Daly AF, Tichomirowa MA, Petrossians P, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metabol.* 2010;95(11):E373–E383.
218. Caimari F, Hernandez-Ramirez LC, Dang MN, et al. Risk category system to identify pituitary adenoma patients with AIP mutations. *J Med Genet.* 2018;55(4):254–260.
219. Tichomirowa MA, Barlier A, Daly AF, et al. High prevalence of AIP gene mutations following focused screening in young patients with sporadic pituitary macroadenomas. *Eur J Endocrinol.* 2011;165(4):509–515.
220. Salenave S, Ancelle D, Bahouge T, et al. Macroprolactinomas in children and adolescents: factors associated with the response to treatment in 77 patients. *J Clin Endocrinol Metabol.* 2015;100(3):1177–1186.
221. Korbonsits M, Storr H, Kumar AV. Familial pituitary adenomas - who should be tested for AIP mutations? *Clin Endocrinol.* 2012;77(3):351–356.
222. Baysal BE, Ferrell RE, Willett-Brozick JE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science.* 2000;287(5454):848–851.
223. Astuti D, Latif F, Dallol A, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet.* 2001;69(1):49–54.
224. Schiffman JD. No child left behind in SDHB testing for paragangliomas and pheochromocytomas. *J Clin Oncol.* 2011;29(31):4070–4072.
225. Neumann HP, Pawlu C, Peczkowska M, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA.* 2004;292(8):943–951.
226. Benn DE, Gimenez-Roqueplo AP, Reilly JR, et al. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. *J Clin Endocrinol Metabol.* 2006;91(3):827–836.
227. Burnichon N, Rohmer V, Amar L, et al. The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. *J Clin Endocrinol Metabol.* 2009;94(8):2817–2827.
228. Bausch B, Schiavi F, Ni Y, et al. Clinical Characterization of the Pheochromocytoma and Paraganglioma Susceptibility Genes SDHA, TMEM127, MAX, and SDHAF2 for Gene-Informed Prevention. *JAMA oncology.* 2017;3(9):1204–1212.
229. Baysal BE. Genomic imprinting and environment in hereditary paraganglioma. *Am J Med Genet C Semin Med Genet.* 2004;129C(1):85–90.
230. Burnichon N, Mazzella JM, Drui D, et al. Risk assessment of maternally inherited SDHD paraganglioma and pheochromocytoma. *J Med Genet.* 2017;54(2):125–133.
231. Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. *J Intern Med.* 2009;266(1):43–52.
232. Vanharanta S, Buchta M, McWhinney SR, et al. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet.* 2004;74(1):153–159.
233. Xekouki P, Brennand A, Whitelaw B, Pacak K, Stratakis CA. The 3PAs: an update on the association of pheochromocytomas, paragangliomas, and pituitary tumors. *Horm Metab Res.* 2018;51(7):419–436.
234. Xekouki P, Szarek E, Bullova P, et al. Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in humans and mice. *J Clin Endocrinol Metabol.* 2015;100(5):E710–E719.
235. Petr EJ, Else T. Genetic predisposition to endocrine tumors: Diagnosis, surveillance and challenges in care. *Semin Oncol.* 2016;43(5):582–590.
236. Tufton N, Sahdev A, Akker SA. Radiological surveillance screening in asymptomatic succinate dehydrogenase mutation carriers. *J Endocr Soc.* 2017;1(7):897–907.
237. Carpten JD, Robbins CM, Villablanca A, et al. HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nat Genet.* 2002;32(4):676–680.
238. Hyde SM, Rich TA, Waguespack SG, Perrier ND, Hu MI. CDC73-related disorders. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*((R)); 2008. Seattle (WA), [updated 2018 Apr 26].
239. Pichardo-Lowden AR, Manni A, Saunders BD, Baker MJ. Familial hyperparathyroidism due to a germline mutation of the CDC73 gene: implications for management and age-appropriate testing of relatives at risk. *Endocr Pract.* 2011;17(4):602–609.
240. Tan MH, Teh BT. Renal neoplasia in the hyperparathyroidism-jaw tumor syndrome. *Curr Mol Med.* 2004;4(8):895–897.
241. Bradley KJ, Hobbs MR, Buley ID, et al. Uterine tumours are a phenotypic manifestation of the hyperparathyroidism-jaw tumour syndrome. *J Intern Med.* 2005;257(1):18–26.
242. Giusti F, Marini F, Brandi ML. Multiple endocrine neoplasia type 1. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*((R)); 2005. Seattle (WA), [updated 2017 Dec 14].
243. Brandi ML, Gagel RF, Angeli A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metabol.* 2001;86(12):5658–5671.
244. Marx SJ. Molecular genetics of multiple endocrine neoplasia types 1 and 2. *Nat Rev Cancer.* 2005;5(5):367–375.
245. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). *Best Pract Res Clin Endocrinol Metab.* 2010;24(3):355–370.
246. Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metabol.* 2012;97(9):2990–3011.
247. Pieterman CR, Vriens MR, Dreijerink KM, van der Luijt RB, Valk GD. Care for patients with multiple endocrine neoplasia type 1: the current evidence base. *Familial Cancer.* 2011;10(1):157–171.
248. Trump D, Faren B, Wooding C, et al. Clinical studies of multiple endocrine neoplasia type 1 (MEN1). *QJM.* 1996;89(9):653–669.
249. Goudet P, Dalac A, Le Bras M, et al. MEN1 disease occurring before 21 years old: a 160-patient cohort study from the Groupe d'étude des Tumeurs Endocrines. *J Clin Endocrinol Metabol.* 2015;100(4):1568–1577.
250. Frost M, Lines KE, Thakker RV. Current and emerging therapies for PNETs in patients with or without MEN1. *Nat Rev Endocrinol.* 2018;14(4):216–227.
251. van Leeuwen RS, de Laat JM, Pieterman CRC, Dreijerink K, Vriens MR, Valk GD. The future: medical advances in MEN1 therapeutic approaches and management strategies. *Endocr Relat Cancer.* 2017;24(10):T179–T193.
252. Wermer P. Genetic aspects of adenomatosis of endocrine glands. *Am J Med.* 1954;16(3):363–371.

253. Chandrasekharappa SC, Guru SC, Manickam P, et al. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science*. 1997;276(5311):404–407.
254. Lemmens I, Van de Ven WJ, Kas K, et al. Identification of the multiple endocrine neoplasia type 1 (MEN1) gene. The European Consortium on MEN1. *Hum Mol Genet*. 1997;6(7):1177–1183.
255. Agarwal SK, Kester MB, Debelenko LV, et al. Germline mutations of the MEN1 gene in familial multiple endocrine neoplasia type 1 and related states. *Hum Mol Genet*. 1997;6(7):1169–1175.
256. Weber F, Mulligan LM. Happy 20th anniversary MEN1: from positional cloning to gene function restoration. *Endocr Relat Cancer*. 2017;24(10):E7–E11.
257. Lemos MC, Thakker RV. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. *Hum Mutat*. 2008;29(1):22–32.
258. Agarwal SK. The future: genetics advances in MEN1 therapeutic approaches and management strategies. *Endocr Relat Cancer*. 2017;24(10):T119–T134.
259. Bassett JH, Forbes SA, Pannett AA, et al. Characterization of mutations in patients with multiple endocrine neoplasia type 1. *Am J Hum Genet*. 1998;62(2):232–244.
260. Goncalves TD, Toledo RA, Sekiya T, et al. Penetrance of functioning and nonfunctioning pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1 in the second decade of life. *J Clin Endocrinol Metabol*. 2014;99(1):E89–E96.
261. Knudson Jr AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*. 1971;68(4):820–823.
262. Hyde SM, Cote GJ, Grubbs EG. Genetics of Multiple Endocrine Neoplasia Type 1/Multiple Endocrine Neoplasia Type 2 Syndromes. *Endocrinol Metab Clin North Am*. 2017;46(2):491–502.
263. Vannucci L, Marini F, Giusti F, Ciuffi S, Tonelli F, Brandi ML. MEN1 in children and adolescents: Data from patients of a regional referral center for hereditary endocrine tumors. *Endocrine*. 2018;59(2):438–448.
264. Machens A, Schaaf L, Karges W, et al. Age-related penetrance of endocrine tumours in multiple endocrine neoplasia type 1 (MEN1): a multicentre study of 258 gene carriers. *Clin Endocrinol*. 2007;67(4):613–622.
265. Manoharan J, Raue F, Lopez CL, et al. Is routine screening of young asymptomatic MEN1 patients necessary? *World J Surg*. 2017;41(8):2026–2032.
266. Goudet P, Murat A, Binquet C, et al. Risk factors and causes of death in MEN1 disease. A GTE (Groupe d'Etude des Tumeurs Endocrines) cohort study among 758 patients. *World J Surg*. 2010;34(2):249–255.
267. Dean PG, van Heerden JA, Farley DR, et al. Are patients with multiple endocrine neoplasia type I prone to premature death? *World J Surg*. 2000;24(11):1437–1441.
268. Nilubol N, Weinstein L, Simonds WF, et al. Preoperative localizing studies for initial parathyroidectomy in MEN1 syndrome: is there any benefit? *World J Surg*. 2012;36(6):1368–1374.
269. Udelsman R, Pasiaka JL, Sturgeon C, Young JE, Clark OH. Surgery for asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metabol*. 2009;94(2):366–372.
270. Hunter GJ, Schellingerhout D, Vu TH, Perrier ND, Hamberg LM. Accuracy of four-dimensional CT for the localization of abnormal parathyroid glands in patients with primary hyperparathyroidism. *Radiology*. 2012;264(3):789–795.
271. Civelek AC, Ozalp E, Donovan P, Udelsman R. Prospective evaluation of delayed technetium-99m sestamibi SPECT scintigraphy for preoperative localization of primary hyperparathyroidism. *Surgery*. 2002;131(2):149–157.
272. Nobecourt PF, Zagzag J, Asare EA, Perrier ND. Intraoperative decision-making and technical aspects of parathyroidectomy in young patients with MEN1 related hyperparathyroidism. *Front Endocrinol (Lausanne)*. 2018;9:618.
273. Schreinemakers JM, Pieterman CR, Scholten A, Vriens MR, Valk GD, Rinkes IH. The optimal surgical treatment for primary hyperparathyroidism in MEN1 patients: a systematic review. *World J Surg*. 2011;35(9):1993–2005.
274. Waldmann J, Lopez CL, Langer P, Rothmund M, Bartsch DK. Surgery for multiple endocrine neoplasia type 1-associated primary hyperparathyroidism. *Br J Surg*. 2010;97(10):1528–1534.
275. Tonelli F, Marini F, Giusti F, Brandi ML. Total and subtotal parathyroidectomy in young patients with multiple endocrine neoplasia type 1-related primary hyperparathyroidism: potential post-surgical benefits and complications. *Front Endocrinol (Lausanne)*. 2018;9:558.
276. Tonelli F, Marcucci T, Fratini G, Tommasi MS, Falchetti A, Brandi ML. Is total parathyroidectomy the treatment of choice for hyperparathyroidism in multiple endocrine neoplasia type 1? *Ann Surg*. 2007;246(6):1075–1082.
277. Goudet P, Murat A, Cardot-Bauters C, et al. Thymic neuroendocrine tumors in multiple endocrine neoplasia type 1: a comparative study on 21 cases among a series of 761 MEN1 from the GTE (Groupe des Tumeurs Endocrines). *World J Surg*. 2009;33(6):1197–1207.
278. Moyes VJ, Monson JP, Chew SL, Akker SA. Clinical use of cinacalcet in MEN1 hyperparathyroidism. *Int J Endocrinol*. 2010;2010:906163.
279. Verges B, Boureille F, Goudet P, et al. Pituitary disease in MEN type 1 (MEN1): data from the France-Belgium MEN1 multicenter study. *J Clin Endocrinol Metabol*. 2002;87(2):457–465.
280. Scheithauer BW, Kovacs K, Nose V, et al. Multiple endocrine neoplasia type 1-associated thyrotropin-producing pituitary carcinoma: report of a probable de novo example. *Hum Pathol*. 2009;40(2):270–278.
281. Syro LV, Scheithauer BW, Kovacs K, et al. Pituitary tumors in patients with MEN1 syndrome. *Clinics (Sao Paulo)*. 2012;67(Suppl 1):43–48.
282. Beckers A, Betea D, Valdes Socin H, Stevenaert A. The treatment of sporadic versus MEN1-related pituitary adenomas. *J Intern Med*. 2003;253(6):599–605.
283. Trouillas J, Labat-Moleur F, Sturm N, et al. Pituitary tumors and hyperplasia in multiple endocrine neoplasia type 1 syndrome (MEN1): a case-control study in a series of 77 patients versus 2509 non-MEN1 patients. *Am J Surg Pathol*. 2008;32(4):534–543.
284. Dickson PV, Rich TA, Xing Y, et al. Achieving eugastrinemia in MEN1 patients: both duodenal inspection and formal lymph node dissection are important. *Surgery*. 2011;150(6):1143–1152.
285. Borson-Chazot F, Garby L, Raverot G, et al. Acromegaly induced by ectopic secretion of GHRH: a review 30 years after GHRH discovery. *Ann Endocrinol (Paris)*. 2012;73(6):497–502.
286. Hayes AR, Grossman AB. The ectopic adrenocorticotrophic hormone syndrome: rarely easy, always challenging. *Endocrinol Metab Clin North Am*. 2018;47(2):409–425.
287. Kulke MH, Benson 3rd AB, Bergsland E, et al. Neuroendocrine tumors. *J Natl Compr Canc Netw*. 2012;10(6):724–764.
288. Tomassetti P, Migliori M, Lalli S, Campana D, Tomassetti V, Corinaldesi R. Epidemiology, clinical features and diagnosis of gastroenteropancreatic endocrine tumours. *Ann Oncol*. 2001;12(Suppl 2):S95–S99.
289. van Treijen, M.J.C., van Beek, D.J., van Leeuwen, R.S., Vriens, M.R., Valk, G.D. Diagnosing nonfunctional pancreatic NETs in MEN1: the evidence base. *J Endocr Soc*, 2(9), 1067–1088.
290. Marx SJ, Goltzman D. Evolution of our understanding of the hyperparathyroid syndromes: a historical perspective. *J Bone Miner Res*. 2019;34(1):22–37.
291. Lee L, Ito T, Jensen RT. Imaging of pancreatic neuroendocrine tumors: recent advances, current status, and controversies. *Expert Rev Anticancer Ther*. 2018;18(9):837–860.
292. Jackson JE. Angiography and arterial stimulation venous sampling in the localization of pancreatic neuroendocrine tumours. *Best Pract Res Clin Endocrinol Metab*. 2005;19(2):229–239.
293. Sadowski SM, Cadiot G, Dansin E, Goudet P, Triponez F. The future: surgical advances in MEN1 therapeutic approaches and management strategies. *Endocr Relat Cancer*. 2017;24(10):T243–T260.
294. Sadowski SM, Millo C, Cottle-Delisle C, et al. Results of (68) Gallium-DOTATATE PET/CT Scanning in Patients with Multiple Endocrine Neoplasia Type 1. *J Am Coll Surg*. 2015;221(2):509–517.

295. Cryer PE, Axelrod L, Grossman AB, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metabol.* 2009;94(3):709–728.
296. Grant CS. Insulinoma. *Best Pract Res Clin Gastroenterol.* 2005;19(5):783–798.
297. Jensen RT, Berna MJ, Bingham DB, Norton JA. Inherited pancreatic endocrine tumor syndromes: advances in molecular pathogenesis, diagnosis, management, and controversies. *Cancer.* 2008;113(7 Suppl):1807–1843.
298. Berna MJ, Hoffmann KM, Serrano J, Gibril F, Jensen RT. Serum gastrin in Zollinger-Ellison syndrome: I. Prospective study of fasting serum gastrin in 309 patients from the National Institutes of Health and comparison with 2229 cases from the literature. *Medicine (Baltimore).* 2006;85(6):295–330.
299. Norton JA, Cornelius MJ, Doppman JL, Maton PN, Gardner JD, Jensen RT. Effect of parathyroidectomy in patients with hyperparathyroidism, Zollinger-Ellison syndrome, and multiple endocrine neoplasia type I: a prospective study. *Surgery.* 1980;102(6):958–966.
300. McGuigan JE, Wolfe MM. Secretin injection test in the diagnosis of gastrinoma. *Gastroenterology.* 1980;79(6):1324–1331.
301. Berna MJ, Hoffmann KM, Long SH, Serrano J, Gibril F, Jensen RT. Serum gastrin in Zollinger-Ellison syndrome: II. Prospective study of gastrin provocative testing in 293 patients from the National Institutes of Health and comparison with 537 cases from the literature. evaluation of diagnostic criteria, proposal of new criteria, and correlations with clinical and tumoral features. *Medicine (Baltimore).* 2006;85(6):331–364.
302. Arnold R. Diagnosis and differential diagnosis of hypergastrinemia. *Wien Klin Wochenschr.* 2007;119(19-20):564–569.
303. Goldman JA, Blanton WP, Hay DW, Wolfe MM. False-positive secretin stimulation test for gastrinoma associated with the use of proton pump inhibitor therapy. *Clin Gastroenterol Hepatol.* 2009;7(5):600–602.
304. Triponez F, Goudet P, Dossèh D, et al. Is surgery beneficial for MEN1 patients with small (≤ 2 cm), nonfunctioning pancreaticoduodenal endocrine tumor? An analysis of 65 patients from the GTE. *World J Surg.* 2006;30(5):654–662. discussion 663–654.
305. Marx SJ. Recent topics around multiple endocrine neoplasia type 1. *J Clin Endocrinol Metabol.* 2018;103(4):1296–1301.
306. Goudet P, Bonithon-Kopp C, Murat A, et al. Gender-related differences in MEN1 lesion occurrence and diagnosis: a cohort study of 734 cases from the Groupe d'étude des Tumeurs Endocrines. *Eur J Endocrinol.* 2011;165(1):97–105.
307. Sakurai A, Imai T, Kikumori T, et al. Thymic neuroendocrine tumor in multiple endocrine neoplasia type 1: female patients are not rare exceptions. *Clin Endocrinol.* 2012;78(2):248–254.
308. Dias AR, Azevedo BC, Alban LBV, et al. Gastric neuroendocrine tumor: review and update. *Arq Bras Cir Dig.* 2017;30(2):150–154.
309. Norton JA, Melcher ML, Gibril F, Jensen RT. Gastric carcinoid tumors in multiple endocrine neoplasia-1 patients with Zollinger-Ellison syndrome can be symptomatic, demonstrate aggressive growth, and require surgical treatment. *Surgery.* 2004;136(6):1267–1274.
310. Gatta-Cherifi B, Chabre O, Murat A, et al. Adrenal involvement in MEN1. Analysis of 715 cases from the Groupe d'étude des Tumeurs Endocrines database. *Eur J Endocrinol.* 2012;166(2):269–279.
311. Langer P, Cupisti K, Bartsch DK, et al. Adrenal involvement in multiple endocrine neoplasia type 1. *World J Surg.* 2002;26(8):891–896.
312. Manoharan J, Albers MB, Bartsch DK. The future: diagnostic and imaging advances in MEN1 therapeutic approaches and management strategies. *Endocr Relat Cancer.* 2017;24(10):T209–T225.
313. Casey RT, Saunders D, Challis BG, et al. Radiological surveillance in multiple endocrine neoplasia type 1: a double-edged sword? *Endocr Connect.* 2017;6(3):151–158.
314. Sipple JH. The association of pheochromocytoma with carcinoma of the thyroid gland. *Am J Med.* 1961;31:163–166.
315. Cushman Jr P. Familial endocrine tumors; report of two unrelated kindred affected with pheochromocytomas, one also with multiple thyroid carcinomas. *Am J Med.* 1962;32:352–360.
316. Steiner AL, Goodman AD, Powers SR. Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: multiple endocrine neoplasia, type 2. *Medicine (Baltimore).* 1968;47(5):371–409.
317. Simpson NE, Kidd KK. Where is the locus for multiple endocrine neoplasia type 2A? *Henry Ford Hosp Med J.* 1987;35(2-3):168–171.
318. Mathew CG, Chin KS, Easton DF, et al. A linked genetic marker for multiple endocrine neoplasia type 2A on chromosome 10. *Nature.* 1987;328(6130):527–528.
319. Williams ED, Pollock DJ. Multiple mucosal neuromata with endocrine tumours: a syndrome allied to von Recklinghausen's disease. *J Pathol Bacteriol.* 1966;91:71–80.
320. Chong GC, Beahrs OH, Sizemore GW, Woolner LH. Medullary carcinoma of the thyroid gland. *Cancer.* 1975;35(3):695–704.
321. Lora MS, Waguespack SG, Moley JF, Walvoord EC. Adrenal ganglioneuromas in children with multiple endocrine neoplasia type 2: a report of two cases. *J Clin Endocrinol Metabol.* 2005;90(7):4383–4387.
322. Decker RA, Peacock ML, Watson P. Hirschsprung disease in MEN 2A: increased spectrum of RET exon 10 genotypes and strong genotype-phenotype correlation. *Hum Mol Genet.* 1998;7(1):129–134.
323. Gagel RF. When "The 7-Year Itch" is indicative of an endocrine malignant condition. *Endocr Pract.* 2002;8(1):72–74.
324. Romei C, Mariotti S, Fugazzola L, et al. Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. *Eur J Endocrinol.* 2010;163(2):301–308.
325. Romei C, Tacito A, Molinaro E, et al. Twenty years of lesson learning: how does the RET genetic screening test impact the clinical management of medullary thyroid cancer? *Clin Endocrinol.* 2015;82(6):892–899.
326. Loveday C, Josephs K, Chubb D, et al. p.Val804Met, the Most Frequent Pathogenic Mutation in RET, Confers a Very Low Lifetime Risk of Medullary Thyroid Cancer. *J Clin Endocrinol Metabol.* 2018;103(11):4275–4282.
327. Margraf RL, Crockett DK, Krautscheid PM, et al. Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. *Hum Mutat.* 2009;30(4):548–556.
328. Prete FP, Abdel-Aziz T, Morkane C, Brain C, Kurzawinski TR, Group MENiCUC. Prophylactic thyroidectomy in children with multiple endocrine neoplasia type 2. *Br J Surg.* 2018;105(10):1319–1327.
329. Febrero B, Rodriguez JM, Rios A, et al. Prophylactic thyroidectomy in multiple endocrine neoplasia 2 (MEN2) patients with the C634Y mutation: A long-term follow-up in a large single-center cohort. *Eur J Surg Oncol.* 2018;45(4):625–630.
330. Wohlhll N, Cote GJ, Bugalho MM, et al. Relevance of RET protooncogene mutations in sporadic medullary thyroid carcinoma. *J Clin Endocrinol Metabol.* 1966;81(10):3740–3745.
331. Schuffenecker I, Ginet N, Goldgar D, et al. Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Le Groupe d'Etude des Tumeurs a Calcitonine. *Am J Hum Genet.* 1997;60(1):233–237.
332. Machens A, Gimm O, Hinze R, Hoppner W, Boehm BO, Dralle H. Genotype-phenotype correlations in hereditary medullary thyroid carcinoma: oncological features and biochemical properties. *J Clin Endocrinol Metabol.* 2001;86(3):1104–1109.
333. Long KL, Etzel C, Rich T, et al. All in the family? Analyzing the impact of family history in addition to genotype on medullary thyroid carcinoma aggressiveness in MEN2A patients. *Familial Cancer.* 2017;16(2):283–289.
334. Rich TA, Feng L, Busaidy N, et al. Prevalence by age and predictors of medullary thyroid cancer in patients with lower risk germline RET proto-oncogene mutations. *Thyroid.* 2014;24(7):1096–1106.
335. Voss RK, Feng L, Lee JE, et al. Medullary thyroid carcinoma in MEN2A: ATA moderate or high-risk RET mutations do not predict disease aggressiveness. *J Clin Endocrinol Metabol.* 2017;102(8):2807–2813.
336. Modigliani E, Vasen HM, Raue K, et al. Pheochromocytoma in multiple endocrine neoplasia type 2: European study. The European Study Group. *J Intern Med.* 1995;238(4):363–367.

337. DeLellis RA, Wolfe HJ, Gagel RF, et al. Adrenal medullary hyperplasia. A morphometric analysis in patients with familial medullary thyroid carcinoma. *Am J Pathol.* 1976;83(1):177–196.
338. Carney JA, Sizemore GW, Tyce GM. Bilateral adrenal medullary hyperplasia in multiple endocrine neoplasia, type 2: the precursor of bilateral pheochromocytoma. *Mayo Clin Proc.* 1975;50(1):3–10.
339. Korpershoek E, Petri BJ, Post E, et al. Adrenal medullary hyperplasia is a precursor lesion for pheochromocytoma in MEN2 syndrome. *Neoplasia.* 2014;16(10):868–873.
340. Yip L, Cote GJ, Shapiro SE, et al. Multiple endocrine neoplasia type 2: evaluation of the genotype-phenotype relationship. *Arch Surg.* 2003;138(4):409–416. discussion 416.
341. Frank-Raue K, Rybicki LA, Erlic Z, et al. Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. *Hum Mutat.* 2011;32(1):51–58.
342. Machens A, Brauckhoff M, Holzhausen HJ, Thanh PN, Lehnert H, Dralle H. Codon-specific development of pheochromocytoma in multiple endocrine neoplasia type 2. *J Clin Endocrinol Metabol.* 2005;90(7):3999–4003.
343. Thosani S, Ayala-Ramirez M, Palmer L, et al. The characterization of pheochromocytoma and its impact on overall survival in multiple endocrine neoplasia type 2. *J Clin Endocrinol Metabol.* 2013;98(11):E1813–E1819.
344. Machens A, Lorenz K, Dralle H. Peak incidence of pheochromocytoma and primary hyperparathyroidism in multiple endocrine neoplasia 2: need for age-adjusted biochemical screening. *J Clin Endocrinol Metabol.* 2013;98(2):E336–E345.
345. Schuffenecker I, Virally-Monod M, Brohet R, et al. Risk and penetrance of primary hyperparathyroidism in multiple endocrine neoplasia type 2A families with mutations at codon 634 of the RET proto-oncogene. Groupe D'étude des Tumeurs a Calcitonine. *J Clin Endocrinol Metabol.* 1998;83(2):487–491.
346. Carling T, Udelsman R. Parathyroid surgery in familial hyperparathyroid disorders. *J Intern Med.* 2005;257(1):27–37.
347. Magalhaes PK, Antonini SR, de Paula FJ, de Freitas LC, Maciel LM. Primary hyperparathyroidism as the first clinical manifestation of multiple endocrine neoplasia Type 2A in a 5-year-old child. *Thyroid.* 2011;21(5):547–550.
348. Castinetti F, Moley J, Mulligan L, Waguespack SG. A comprehensive review on MEN2B. *Endocr Relat Cancer.* 2018;25(2):T29–T39.
349. Mathiesen JS, Habra MA, Bassett JH, et al. Risk profile of the RET A883F germline mutation: an international collaborative study. *J Clin Endocrinol Metabol.* 2017;102(6):2069–2074.
350. Brauckhoff M, Machens A, Lorenz K, Bjoro T, Varhaug JE, Dralle H. Surgical curability of medullary thyroid cancer in multiple endocrine neoplasia 2B: a changing perspective. *Ann Surg.* 2014;259(4):800–806.
351. Raue F, Dralle H, Machens A, Bruckner T, Frank-Raue K. Long-term survivorship in multiple endocrine neoplasia Type 2B diagnosed before and in the new Millennium. *J Clin Endocrinol Metabol.* 2017;103(1):235–243.
352. Brauckhoff M, Gimm O, Weiss CL, et al. Multiple endocrine neoplasia 2B syndrome due to codon 918 mutation: clinical manifestation and course in early and late onset disease. *World J Surg.* 2004;28(12):1305–1311.
353. Leboulleux S, Travagli JP, Caillou B, et al. Medullary thyroid carcinoma as part of a multiple endocrine neoplasia type 2B syndrome: influence of the stage on the clinical course. *Cancer.* 2002;94(1):44–50.
354. O'Riordain DS, O'Brien T, Weaver AL, et al. Medullary thyroid carcinoma in multiple endocrine neoplasia types 2A and 2B. *Surgery.* 1994;116(6):1017–1023.
355. Makri A, Akshintala S, Derse-Anthony C, et al. Pheochromocytoma in children and adolescents with multiple endocrine neoplasia Type 2B. *J Clin Endocrinol Metabol.* 2019;104(1):7–12.
356. Grubbs EG, Waguespack SG, Rich TA, et al. Do the recent American Thyroid Association (ATA) Guidelines accurately guide the timing of prophylactic thyroidectomy in MEN2A? *Surgery.* 2010;148(6):1302–1310.
357. Machens A, Elwerr M, Lorenz K, Weber F, Dralle H. Long-term outcome of prophylactic thyroidectomy in children carrying RET germline mutations. *Br J Surg.* 2018;105(2):e150–e157.
358. Morris LF, Waguespack SG, Edeiken-Monroe BS, et al. Ultrasonography should not guide the timing of thyroidectomy in pediatric patients diagnosed with multiple endocrine neoplasia syndrome 2A through genetic screening. *Ann Surg Oncol.* 2013;20(1):53–59.
359. Opsahl EM, Brauckhoff M, Schlichting E, et al. A Nationwide study of multiple endocrine neoplasia Type 2A in Norway: predictive and prognostic factors for the clinical course of medullary thyroid carcinoma. *Thyroid.* 2016;26(9):1225–1238.
360. Pellegata NS. MENX and MEN4. *Clinics (Sao Paulo).* 2012;67 (Suppl 1):13–18.
361. Pellegata NS, Quintanilla-Martinez L, Siggelkow H, et al. Germline mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci U S A.* 2006;103 (42):15558–15563.
362. Alrezk R, Hannah-Shmouni F, Stratakis CA. MEN4 and CDKN1B mutations: the latest of the MEN syndromes. *Endocr Relat Cancer.* 2017;24(10):T195–T208.
363. Iacovazzo D, Korbonits M. X-linked acrogigantism. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R))*; 2018. Seattle (WA).
364. Trivellin G, Daly AF, Faucz FR, et al. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med.* 2014;371(25):2363–2374.
365. Iacovazzo D, Caswell R, Bunce B, et al. Germline or somatic GPR101 duplication leads to X-linked acrogigantism: a clinicopathological and genetic study. *Acta Neuropathol Commun.* 2016;4(1):56.
366. Jaspersion KW, Patel SG, Ahnen DJ. APC-associated polyposis conditions. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R))*; 1998. Seattle (WA), [updated 2017 Feb 2].
367. Marchesa P, Fazio VW, Church JM, McGannon E. Adrenal masses in patients with familial adenomatous polyposis. *Dis Colon Rectum.* 1997;40(9):1023–1028.
368. Shiroky JS, Lerner-Ellis JP, Govindarajan A, Urbach DR, Devon KM. Characteristics of adrenal masses in familial adenomatous polyposis. *Dis Colon Rectum.* 2018;61(6):679–685.
369. Else T. Association of adrenocortical carcinoma with familial cancer susceptibility syndromes. *Mol Cell Endocrinol.* 2012;351(1):66–70.
370. Cetta F. FAP associated papillary thyroid carcinoma: a peculiar subtype of familial nonmedullary thyroid cancer. *Patholog Res Int.* 2015;2015:309348.
371. Pradhan D, Sharma A, Mohanty SK. Cribriform-morular variant of papillary thyroid carcinoma. *Pathol Res Pract.* 2015;211(10):712–716.
372. Achatz MI, Porter CC, Brugieres L, et al. Cancer screening recommendations and clinical management of inherited gastrointestinal cancer syndromes in childhood. *Clin Cancer Res.* 2017;23 (13):e107–e114.
373. Shuman C, Beckwith JB, Weksberg R. Beckwith-Wiedemann syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R))*; 2000. Seattle (WA), [updated 2016 Aug 11].
374. Brioude F, Kalish JM, Mussa A, et al. Expert consensus document: clinical and molecular diagnosis, screening and management of Beckwith-Wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol.* 2018;14(4):229–249.
375. Mussa A, Molinatto C, Baldassarre G, et al. Cancer risk in Beckwith-Wiedemann syndrome: a systematic review and meta-analysis outlining a novel (epi)genotype specific histotype targeted screening protocol. *J Pediatr.* 2016;176(142–149). e141.
376. Lapunzina P. Risk of tumorigenesis in overgrowth syndromes: a comprehensive review. *Am J Med Genet C Semin Med Genet.* 2005;137C(1):53–71.
377. MacFarland SP, Mostoufi-Moab S, Zolley K, et al. Management of adrenal masses in patients with Beckwith-Wiedemann syndrome. *Pediatr Blood Cancer.* 2017;64(8).
378. Carney JA, Sheps SG, Go VL, Gordon H. The triad of gastric leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma. *N Engl J Med.* 1977;296(26):1517–1518.
379. Carney JA. Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney Triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin Proc.* 1999;74(6):543–552.

380. Carney JA. Carney triad: a syndrome featuring paraganglionic, adrenocortical, and possibly other endocrine tumors. *J Clin Endocrinol Metabol.* 2009;94(10):3656–3662.
381. Boikos SA, Xekouki P, Fumagalli E, et al. Carney triad can be (rarely) associated with germline succinate dehydrogenase defects. *Eur J Hum Genet.* 2016;24(4):569–573.
382. Settas N, Fauz FR, Stratakis CA. Succinate dehydrogenase (SDH) deficiency, Carney triad and the epigenome. *Mol Cell Endocrinol.* 2018;469:107–111.
383. Schultz KAP, Williams GM, Kamihara J, et al. DICER1 and associated conditions: identification of at-risk individuals and recommended surveillance strategies. *Clin Cancer Res.* 2018;24(10):2251–2261.
384. Schultz KAP, Rednam SP, Kamihara J, et al. PTEN, DICER1, FH, and their associated tumor susceptibility syndromes: clinical features, genetics, and surveillance recommendations in childhood. *Clin Cancer Res.* 2017;23(12):e76–e82.
385. Foulkes WD, Priest JR, Duchaine TF. DICER1: mutations, microRNAs and mechanisms. *Nat Rev Cancer.* 2014;14(10):662–672.
386. Hill DA, Ivanovich J, Priest JR, et al. DICER1 mutations in familial pleuropulmonary blastoma. *Science.* 2009;325(5943):965.
387. Slade I, Bacchelli C, Davies H, et al. DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. *J Med Genet.* 2011;48(4):273–278.
388. de Kock L, Sabbaghian N, Plourde F, et al. Pituitary blastoma: a pathognomonic feature of germ-line DICER1 mutations. *Acta Neuropathol.* 2014;128(1):111–122.
389. de Kock L, Wang YC, Revil T, et al. High-sensitivity sequencing reveals multi-organ somatic mosaicism causing DICER1 syndrome. *J Med Genet.* 2016;53(1):43–52.
390. Khan NE, Bauer AJ, Schultz KAP, et al. Quantification of thyroid cancer and multinodular goiter risk in the DICER1 syndrome: a family-based cohort study. *J Clin Endocrinol Metabol.* 2017;102(5):1614–1622.
391. Rio Frio T, Bahubeshi A, Kanellopoulou C, et al. DICER1 mutations in familial multinodular goiter with and without ovarian Sertoli-Leydig cell tumors. *JAMA.* 2011;305(1):68–77.
392. Caimari F, Kumar AV, Kurzawinski T, et al. A novel DICER1 mutation in familial multinodular goitre. *Clin Endocrinol.* 2018;89(1):110–112.
393. Khan NE, Bauer AJ, Doros L, et al. Macrocephaly associated with the DICER1 syndrome. *Genet Med.* 2017;19(2):244–248.
394. van der Tuin K, de Kock L, Kamping EJ, et al. Clinical and molecular characteristics may alter treatment strategies of thyroid malignancies in DICER1 syndrome. *J Clin Endocrinol Metabol.* 2019;104(2):277–284.
395. van Engelen K, Villani A, Wasserman JD, et al. DICER1 syndrome: Approach to testing and management at a large pediatric tertiary care center. *Pediatr Blood Cancer.* 2018;65(1).
396. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science.* 1990;250(4985):1233–1238.
397. Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol.* 2009;27(8):1250–1256.
398. Valdez JM, Nichols KE, Kesserwan C. Li-Fraumeni syndrome: a paradigm for the understanding of hereditary cancer predisposition. *Br J Haematol.* 2017;176(4):539–552.
399. Kratz CP, Achatz MI, Brugieres L, et al. Cancer Screening Recommendations for Individuals with Li-Fraumeni Syndrome. *Clin Cancer Res.* 2017;23(11):e38–e45.
400. Masciari S, Van den Abbeele AD, Diller LR, et al. F18-fluorodeoxyglucose-positron emission tomography/computed tomography screening in Li-Fraumeni syndrome. *JAMA.* 2008;299(11):1315–1319.
401. Formiga M, de Andrade KC, Kowalski LP, Achatz MI. Frequency of thyroid carcinoma in Brazilian TP53 p.R337H carriers With Li Fraumeni Syndrome. *JAMA Oncology.* 2017;3(10):1400–1402.
402. Mastellaro MJ, Seidinger AL, Kang G, et al. Contribution of the TP53 R337H mutation to the cancer burden in southern Brazil: Insights from the study of 55 families of children with adrenocortical tumors. *Cancer.* 2017;123(16):3150–3158.
403. Wasserman JD, Novokmet A, Eichler-Jonsson C, et al. Prevalence and functional consequence of TP53 mutations in pediatric adrenocortical carcinoma: a children's oncology group study. *J Clin Oncol.* 2015;33(6):602–609.
404. Ribeiro RC, Sandrini F, Figueiredo B, et al. An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A.* 2001;98(16):9330–9335.
405. Mastellaro MJ, Ribeiro RC, Oliveira-Filho AG, et al. Adrenocortical tumors associated with the TP53 p.R337H germline mutation can be identified during child-care consultations. *J Pediatr (Rio J).* 2018;94(4):432–439.
406. Kohlmann W, Gruber SB. Lynch Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R))*; 2004. Seattle (WA), [updated 2018 Apr 12].
407. Raymond VM, Everett JN, Furtado LV, et al. Adrenocortical carcinoma is a lynch syndrome-associated cancer. *J Clin Oncol.* 2013;31(24):3012–3018.
408. Challis BG, Kandasamy N, Powlson AS, et al. Familial adrenocortical carcinoma in association with Lynch syndrome. *J Clin Endocrinol Metabol.* 2016;101(6):2269–2272.
409. Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. *Nat Rev Dis Primers.* 2017;3:17004.
410. Walther MM, Herring J, Enquist E, Keiser HR, Linehan WM. von Recklinghausen's disease and pheochromocytomas. *J Urol.* 1999;162(5):1582–1586.
411. Moramarco J, El Ghorayeb N, Dumas N, et al. Pheochromocytomas are diagnosed incidentally and at older age in neurofibromatosis type 1. *Clin Endocrinol.* 2017;86(3):332–339.
412. Eisenhofer G, Pacak K, Huynh TT, et al. Catecholamine metabolic and secretory phenotypes in pheochromocytoma. *Endocr Relat Cancer.* 2011;18(1):97–111.
413. Relles D, Baek J, Witkiewicz A, Yeo CJ. Periampullary and duodenal neoplasms in neurofibromatosis type 1: two cases and an updated 20-year review of the literature yielding 76 cases. *J Gastrointest Surg.* 2010;14(6):1052–1061.
414. Perren A, Wiesli P, Schmid S, et al. Pancreatic endocrine tumors are a rare manifestation of the neurofibromatosis type 1 phenotype: molecular analysis of a malignant insulinoma in a NF-1 patient. *Am J Surg Pathol.* 2006;30(8):1047–1051.
415. Lee WS, Koh YS, Kim JC, et al. Zollinger-Ellison syndrome associated with neurofibromatosis type 1: a case report. *BMC Cancer.* 2005;5:85.
416. Favere AM, Tsukumo DM, Matos PS, Santos SL, Lalli CA. Association between atypical parathyroid adenoma and neurofibromatosis. *Arch Endocrinol Metab.* 2015;59(5):460–466.
417. Menon RK, Ferrau F, Kurzawinski TR, et al. Adrenal cancer in neurofibromatosis type 1: case report and DNA analysis. *Endocrinol Diabetes Metab Case Rep.* 2014;2014:140074.
418. Josefson J, Listernick R, Fangusaro JR, Charrow J, Habiby R. Growth hormone excess in children with neurofibromatosis type 1-associated and sporadic optic pathway tumors. *J Pediatr.* 2011;158(3):433–436.
419. McGarrity TJ, Amos CI, Baker MJ. Peutz-Jeghers syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews((R))*; 2001. Seattle (WA), [updated 2016 Jul 14].
420. Beggs AD, Latchford AR, Vasen HF, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut.* 2010;59(7):975–986.
421. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am J Gastroenterol.* 2010;105(6):1258–1264 author reply 1265.
422. Triggiani V, Guastamacchia E, Renzulli G, et al. Papillary thyroid carcinoma in Peutz-Jeghers syndrome. *Thyroid.* 2011;21(11):1273–1277.
423. Dreyer L, Jacyk WK, du Plessis DJ. Bilateral large-cell calcifying Sertoli cell tumor of the testes with Peutz-Jeghers syndrome: a case report. *Pediatr Dermatol.* 1994;11(4):335–337.
424. Crocker MK, Gourgari E, Lodish M, Stratakis CA. Use of aromatase inhibitors in large cell calcifying sertoli cell tumors: effects on gynecomastia, growth velocity, and bone age. *J Clin Endocrinol Metabol.* 2014;99(12):E2673–E2680.
425. Young RH, Welch WR, Dickersin GR, Scully RE. Ovarian sex cord tumor with annular tubules: review of 74 cases including 27 with

- Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. *Cancer*. 1982;50(7):1384–1402.
426. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*. 1997;16(1):64–67.
 427. Yehia L, Eng C. 65 years of the double helix: One gene, many endocrine and metabolic syndromes: PTEN-opathies and precision medicine. *Endocr Relat Cancer*. 2018;25(8):T121–T140.
 428. Hansen-Kiss E, Beinkampen S, Adler B, et al. A retrospective chart review of the features of PTEN hamartoma tumour syndrome in children. *J Med Genet*. 2017;54(7):471–478.
 429. Harach HR, Soubeyran I, Brown A, Bonneau D, Longy M. Thyroid pathologic findings in patients with Cowden disease. *Ann Diagn Pathol*. 1999;3(6):331–340.
 430. Smith JR, Marqusee E, Webb S, et al. Thyroid nodules and cancer in children with PTEN hamartoma tumor syndrome. *J Clin Endocrinol Metabol*. 2011;96(1):34–37.
 431. Laury AR, Bongiovanni M, Tille JC, Kozakewich H, Nose V. Thyroid pathology in PTEN-hamartoma tumor syndrome: characteristic findings of a distinct entity. *Thyroid*. 2011;21(2):135–144.
 432. Ngeow J, Mester J, Rybicki LA, Ni Y, Milas M, Eng C. Incidence and clinical characteristics of thyroid cancer in prospective series of individuals with Cowden and Cowden-like syndrome characterized by germline PTEN, SDH, or KLLN alterations. *J Clin Endocrinol Metabol*. 2011;96(12):E2063–E2071.
 433. Northrup H, Koenig MK, Pearson DA, Au KS. Tuberous sclerosis complex. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*(R); 1999. Seattle (WA), [updated 2018 Jul 12].
 434. van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science*. 1997;277(5327):805–808.
 435. Dworakowska D, Grossman AB. Are neuroendocrine tumours a feature of tuberous sclerosis? A systematic review. *Endocr Relat Cancer*. 2009;16(1):45–58.
 436. Boronat S, Barber I. Less common manifestations in TSC. *Am J Med Genet C Semin Med Genet*. 2018;178(3):348–354.
 437. Flader M, Kurzawa P, Maldyk J, et al. Papillary thyroid carcinoma in a boy with familial tuberous sclerosis complex attributable to a TSC2 deletion—a case report. *Curr Oncol*. 2017;24(5):e423–e428.
 438. Larson AM, Hedgire SS, Deshpande V, et al. Pancreatic neuroendocrine tumors in patients with tuberous sclerosis complex. *Clin Genet*. 2012;82(6):558–563.
 439. Latif F, Tory K, Gnara J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science*. 1993;260(5112):1317–1320.
 440. van Leeuwen RS, Ahmad S, Links TP, Giles RH. Von Hippel-Lindau syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*(R); 2000. Seattle (WA), [updated 2018 Sep 6].
 441. Crespigio J, Berbel LCL, Dias MA, et al. Von Hippel-Lindau disease: a single gene, several hereditary tumors. *J Endocrinol Invest*. 2018;41(1):21–31.
 442. Maher ER, Neumann HP, Richard S. von Hippel-Lindau disease: a clinical and scientific review. *Eur J Hum Genet*. 2011;19(6):617–623.
 443. Launbjerg K, Bache I, Galanakis M, Bisgaard ML, Binderup MLM. von Hippel-Lindau development in children and adolescents. *Am J Med Genet A*. 2017;173(9):2381–2394.
 444. Sovinz P, Urban C, Uhrig S, et al. Pheochromocytoma in a 2.75-year-old-girl with a germline von Hippel-Lindau mutation Q164R. *Am J Med Genet A*. 2010;152A(7):1752–1755.
 445. Kruizinga RC, Sluiter WJ, de Vries EG, et al. Calculating optimal surveillance for detection of von Hippel-Lindau-related manifestations. *Endocr Relat Cancer*. 2014;21(1):63–71.
 446. Gaal J, van Nederveen FH, Erlic Z, et al. Parasympathetic paragangliomas are part of the Von Hippel-Lindau syndrome. *J Clin Endocrinol Metabol*. 2009;94(11):4367–4371.
 447. Charlesworth M, Verbeke CS, Falk GA, Walsh M, Smith AM, Morris-Stiff G. Pancreatic lesions in von Hippel-Lindau disease? A systematic review and meta-synthesis of the literature. *J Gastrointest Surg*. 2012;16(7):1422–1428.
 448. O'Toole SM, Sahdev A, Bhattacharya S, Feakins R, Gevers EF, Drake WM. Paediatric pancreatic neuroendocrine tumours in von Hippel-Lindau disease. *Endocr Relat Cancer*. 2018;25(9):L43–L47.
 449. Krauss T, Ferrara AM, Links TP, et al. Preventive medicine of von Hippel-Lindau disease-associated pancreatic neuroendocrine tumors. *Endocr Relat Cancer*. 2018;25(9):783–793.
 450. Blansfield JA, Choyke L, Morita SY, et al. Clinical, genetic and radiographic analysis of 108 patients with von Hippel-Lindau disease (VHL) manifested by pancreatic neuroendocrine neoplasms (PNETs). *Surgery*. 2007;142(6):814–818. discussion 818 e811–812.
 451. Corcos O, Couvelard A, Giraud S, et al. Endocrine pancreatic tumors in von Hippel-Lindau disease: clinical, histological, and genetic features. *Pancreas*. 2008;37(1):85–93.
 452. Tirosh A, Sadowski SM, Linehan WM, et al. Association of VHL genotype with pancreatic neuroendocrine tumor phenotype in patients with von Hippel-Lindau disease. *JAMA Oncology*. 2018;4(1):124–126.
 453. Tirosh A, Journy N, Folio LR, et al. Cumulative radiation exposures from CT screening and surveillance strategies for von Hippel-Lindau-associated solid pancreatic tumors. *Radiology*. 2019;290(1):116–124.
 454. Wagner, M., Browne, H.N., Marston Linehan, W., Merino, M., Babar, N., Stratton, P. Lipid cell tumors in two women with von Hippel-Lindau syndrome. *Obstet Gynecol*, 116 Suppl 2, 535–539.
 455. Rowland KJ, Chernock RD, Moley JF. Pheochromocytoma in an 8-year-old patient with multiple endocrine neoplasia type 2A: implications for screening. *J Surg Oncol*. 2013;108(4):203–206.
 456. Hernandez FC, Sanchez M, Alvarez A, et al. A five-year report on experience in the detection of pheochromocytoma. *Clin Biochem*. 2000;33(8):649–655.
 457. Schiavi F, Boedeker CC, Bausch B, et al. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. *JAMA*. 2005;294(16):2057–2063.
 458. Ricketts CJ, Forman JR, Rattenberry E, et al. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in SDHB and SDHD. *Hum Mutat*. 2010;31(1):41–51.
 459. van der Tuin K, Mensenkamp AR, Tops CMJ, et al. Clinical aspects of SDHA-related pheochromocytoma and paraganglioma: a nationwide study. *J Clin Endocrinol Metabol*. 2018;103(2):438–445.
 460. Stratakis CA, Schussheim DH, Freedman SM, et al. Pituitary macroadenoma in a 5-year-old: an early expression of multiple endocrine neoplasia type 1. *J Clin Endocrinol Metabol*. 2000;85(12):4776–4780.
 461. Newey PJ, Jayabalan J, Walls GV, et al. Asymptomatic children with multiple endocrine neoplasia type 1 mutations may harbor nonfunctioning pancreatic neuroendocrine tumors. *J Clin Endocrinol Metabol*. 2009;94(10):3640–3646.

16

Puberty in the Female and Its Disorders

Robert L. Rosenfield, David W. Cooke, Sally Radovick

CHAPTER OUTLINE

DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM 528

Maturation of the Neuroendocrine-Ovarian Axis 528

Regulation of the Neuroendocrine-Ovarian Axis 539

Adrenarche and the Regulation of Adrenal Androgen Secretion 546

Hormonal Secretion, Transport, Metabolism, and Action 547

Maturation of Sex Hormone Target Organs 554

NORMAL HORMONAL AND SEXUAL DEVELOPMENTAL STAGES 557

The Fetus and Neonate 557

Childhood 558

Adolescence 558

Adult Menstrual Cycle 560

Normal Variations in Pubertal Development 560

ABNORMAL PUBERTY 564

Abnormal Development 564

Other Dysgenetic Disorders 565

Precocious Puberty 566

Hypogonadism 573

Nonhypoestrogenic Menstrual Disturbances 585

Hyperandrogenism in Adolescence 588

FUTURE DIRECTIONS 600

References 600

Puberty is the stage of development during which secondary sexual characteristics appear and there is a transition from the sexually immature to the sexually mature stage. Adolescence is widely used as a generally synonymous term for puberty, but it is often used to convey an added cultural connotation as a psychosocial coming of age.

By the mid-1960s a general concept of the major factors involved in the initiation of puberty was established (Fig. 16.1).^{1,2} A decrease in sensitivity of the brain “gonadostat” to sex hormone negative feedback was thought to be the primary event. This signaled the hypothalamus to discharge neurohumors (then unidentified), which in turn stimulated the pituitary to release gonadotropins. The resultant rise in secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), was thought to account directly for increased estrogen production by the ovary. A mature relationship was thought to develop in which the blood levels of estrogen and gonadotropins were regulated reciprocally via the gonadostat,³ much as a furnace is regulated by a thermostat. The pineal was identified as having gonadal suppressive properties. The increased adrenocortical secretion of 17-ketosteroids (17-KS), which becomes apparent at about the time of puberty (“adrenarche”), was thought to be caused by a pituitary factor stimulating adrenal androgens in synergism with adrenocorticotrophic hormone (ACTH).⁴

The rapid scientific advances since 1965 have permitted this concept to be tested in increasingly sophisticated ways. In the

subsequent decade, radioimmunoassay (RIA), originally developed by Yalow and Berson, was applied to the measurement of gonadotropins and sex steroids; the gonadotropin-releasing hormone (GnRH) for both LH and FSH was isolated, identified, and synthesized by Guillemin’s and Schally’s groups. Cyclic adenosine-3’,5’-monophosphate (cAMP), postulated by Sutherland to mediate the action of peptide hormones, was found to mediate gonadotropin effects on the ovarian follicle. The initial steps in the mechanism of action of steroid hormones were defined by Jensen, Gorski, and their groups. The landmark nature of many of these discoveries was recognized by the awarding of Nobel Prizes in Medicine to Sutherland in 1971 and to Yalow, Schally, and Guillemin in 1977.

Our present view of the mechanisms controlling puberty is more refined and complex than it once was, although the earlier schema is correct in a general sense. The gonadostat is a patently oversimplistic concept for a complex system that regulates the activity of the hypothalamic GnRH pulse generator, a functionally interconnected and synchronized network of GnRH neurons.⁵ The gonadostat setting seems to change throughout childhood in a biphasic manner. This concept is illustrated in Fig. 16.2.^{6,7} During most of fetal and perinatal life, the gonadostat is insensitive to negative feedback by sex steroid hormones; at this time the nascent neuroendocrine-gonadal axis functions at a pubertal level. The gonadostat becomes increasingly sensitive to negative feedback during infancy but does not become highly sensitive until midchildhood, at which time GnRH pulse generator activity is minimal. During late prepuberty, the gonadostat begins to relinquish its inhibition. This permits the onset of puberty. The changing set-point initially permits increasing, episodic secretion of GnRH. Increasing sensitivity of the pituitary gonadotropic cells to GnRH follows. The change in LH and FSH secretion is first detectable during sleep. Gradually, the gonads become increasingly sensitized to gonadotropin stimulation, grow at an increased rate, and bring about sustained rises in plasma sex steroid hormone levels. Some of these phenomena synergize with others, so that autoamplification occurs and the pace of change accelerates. Eventually, the set-point for gonadotropin release comes to vary sufficiently to encompass a positive feedback mechanism.

The data on which this model is based are presented later. The most recent data on the hormonal milieu and accompanying physical stages of normal puberty are then presented. Abnormal puberty is subsequently discussed: the causes, differential diagnosis, and management.

DEVELOPMENT OF THE FEMALE REPRODUCTIVE SYSTEM

Maturation of the Neuroendocrine-Ovarian Axis

Fetus

Neuroendocrine Unit. The anterior lobe of the pituitary gland, of stromal ectodermal origin, and the posterior lobe, of neural origin, differentiate by 11 weeks’ gestational age.⁸ By this time, GnRH neurons have migrated from the olfactory placode into place in the medial basal hypothalamus.⁹ Hypothalamic GnRH subsequently rises in parallel with fetal pituitary and serum LH and FSH.¹⁰ All peak at about 20 to

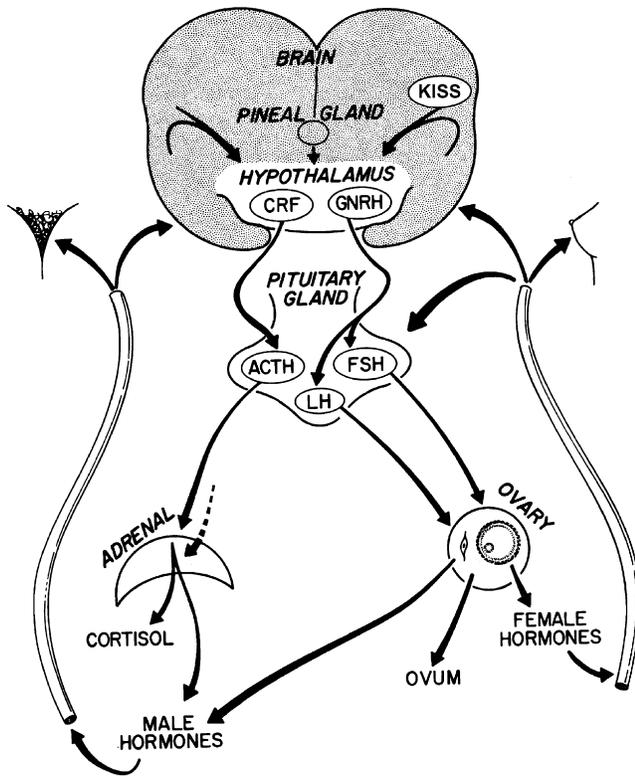


Fig. 16.1 Schematic representation of the neuroendocrine-ovarian axis involved in normal pubertal development. *ACTH*, Adrenocorticotropic hormone; *CRF*, corticotropin-releasing factor; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *KISS*, kisspeptin; *LH*, luteinizing hormone.

24 weeks, as the connections of the pituitary portal system become complete, to levels not again seen until menopause.¹¹ Serum LH and FSH levels are higher in human female than male fetuses.¹¹ In rats, GnRH-containing neurons develop earlier in females than in males,¹² and there are sexual dimorphisms in the degree of synapsing of specific tracts with

dendritic spines in the preoptic nucleus, one of the major GnRH-containing areas of the hypothalamus.^{13,14} These differences may be determined by gonadal sex steroid hormone output. In all species studied, fetal secretion of LH, particularly LH pulse frequency, is permanently desensitized to estradiol-progesterone negative feedback by fetal virilization.¹⁵ In the rat, this has been demonstrated to be mediated by permanent impairment of estradiol-induced progesterone receptor (PR) gene expression.¹⁶

In late gestation, fetal hypothalamic GnRH and pituitary gonadotropin secretion fall to low levels. These changes are likely explicable by the negative feedback effect of the high sex steroids produced by the fetoplacental unit. Meanwhile, maturation of the central nervous system (CNS) tracts that inhibit hypothalamic GnRH secretion and mediate gonadal negative feedback signals appears to progress throughout gestation.^{17,18}

The production of gonadotropins by the fetal pituitary seems to facilitate normal ovarian development. Hypophysectomy of rhesus fetuses has been reported to reduce the number of germ cells and oocytes, as well as the integrity of the rete ovarii.¹⁹ Therefore it seems that survival of gametes depends upon the secretions of the fetal pituitary.

Ovary. The ovaries differentiate in the urogenital ridge adjacent to the anlage of the adrenal cortex and the kidney. The granulosa cells are the homologues of the Sertoli cells of the testes. The theca, interstitial, and hilus cells are the homologues of the Leydig cells; hilus cells may even contain crystalloids like Leydig cells. Adrenocortical rests occasionally have been found in the hilus of the ovary.²⁰ Conversely, ovarian rests have been identified in the adrenal glands.²¹

The primitive germ cells migrate into the ovary from the yolk sac endoderm during the first month of gestation. The testes become histologically discernable by 8 weeks' gestation.²² The ovaries develop²³ in the absence of testicular development being switched on by the signaling cascade initiated by the *SRY* gene on the Y chromosome.^{24,25} Activation of the β -catenin signaling pathway by *Wnt-4* and *R-spondin1* permit forkhead (*Fox*) L2 transcription factor expression by germ cells to activate ovarian differentiation by sustaining oocyte and granulosa cell development and suppressing Sertoli and Leydig cell differentiation; they also support later aspects of follicle development.^{26,27} Steroidogenic factor-1 (*SF-1*) *WT-1*, *LIM-1*, and

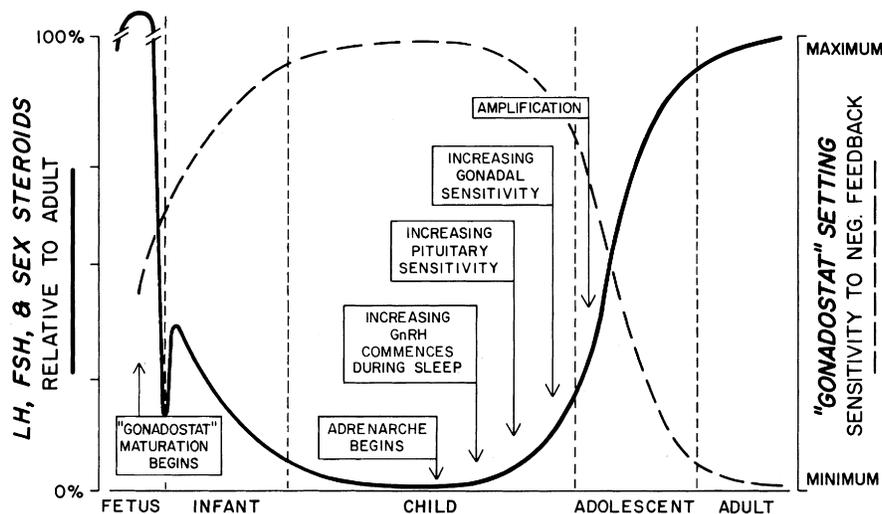


Fig. 16.2 The changing pattern of serum gonadotropins and sex hormones from fetal life to maturity in relationship to the apparent sensitivity of the central nervous system “gonadostat” to the negative feedback effect of sex hormones and the underlying hormonal events. *FSH*, Follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *LH*, luteinizing hormone. (Modified from Grumbach, M., Grave, C., Mayer, F. (Eds.) (1974), *The Control of the Onset of Puberty*. New York, John Wiley & Sons.)

possibly *DAX-1* genes play roles in the formation of the ovaries.²⁸ Germ cell bone morphogenetic proteins (BMPs) are necessary for primordial germ cell proliferation.

Primitive germ cells undergo mitotic division to become oogonia, a process that is maximum at 8 to 12 weeks. Oogonia then undergo oogenesis, entering the prophase of meiosis to become primary oocytes beginning at 12 to 16 weeks.²⁹ The number of oocytes reaches a peak at 20 weeks when there are 6.8 million germ cells, of which 80% appear to be viable (Fig. 16.3).³⁰ When oocytes enter the diplotene stage of meiotic prophase they must be furnished with granulosa cells to form a primordial follicle, or else they undergo atresia.³¹

The ovary remains histologically undifferentiated until primordial follicles appear at about 16 weeks, when the epithelium of the secondary sex cords provides granulosa cells to the oocytes.²² However, the fetal ovary has the capacity for androgen and estrogen formation and signaling, although at a far lower level than the testes, by 12 weeks.^{22,32-35} Primordial follicles become primary follicles when the encircling granulosa cell layer becomes cuboidal. Primordial and small primary follicles (Fig. 16.4)^{36,37} are resting follicles, which are the major repository of germ cells.³⁸ This stock of germ cells is depleted only very slowly during childhood (see Fig. 16.3). Residence of primordial follicles in the ovarian cortex restrains their progression partly because of cortex mechanical rigidity.³⁹ Mechanical effects are mediated by the growth-restrictive Hippopotamus signaling pathway and by vascular permeability via the vascular endothelial growth factor signaling pathway.⁴⁰

Secondary follicles and preantral follicles, characterized, respectively, by organization of a distinct theca cell layer and proliferation of granulosa cell layers, then appear successively. Preantral follicles develop at 24 to 26 weeks.^{22,31} Antral (graafian) follicles appear near term, and those granulosa cells enveloping the oocyte to become the cumulus.^{37,41,42} Ovarian estrogen production appears to be virtually unresponsive to gonadotropins until early antral follicles develop at near term gestational age.^{18,43,44} One or two antral follicles of 1 to 2 mm in diameter are present in the ovary at term.^{18,22,31} At this time, ovarian follicle development is complete,^{37,41,42} and the complement of ova is greater than at any other time during postnatal life (see Fig. 16.3), totaling 2 million, of which half appear atretic.^{30,45}

Both X-chromosomes are active in oocytes,⁴⁶ and the oocytes secrete factors, such as growth differentiation factor-9 (GDF9),⁴⁰ necessary for the induction of the granulosa cell layer that is necessary for oocyte survival.^{41,47} Oocyte-specific chemokines and transcription factors then coordinately direct the formation of primordial follicles and their subsequent development to primary follicles.⁴⁰ GDF9 interaction with growth factors, such as BMP 9 and transforming growth factor beta (TGF- β), is then critical for primary follicle granulosa cell proliferation. Then preantral follicles develop when GDF9, in coordination with other growth factors, induces the theca cell layer from fibroblast-like stem cells.^{48,49} A host of local factors then regulate further follicle growth and development; for example, the forkhead transcription factor FOXL2, expressed specifically in granulosa cells, restrains GDF9 from prematurely activating follicle growth.⁵⁰

Estrogen receptor (ER) expression is critical for development of the granulosa cell layer.⁴⁰ Insulin and androgen promote the primordial-primary follicle transition. Only upon reaching the early antral follicle stage does further follicle development become strictly dependent on FSH action.

Follicle number is determined by the balance between survival and atresia of ovarian germ cells. The endowment of ovarian germ cells has been thought to be determined during fetal life since the germ cells of the ovary, unlike those of the testes, seem to be a nonrenewing population. However, female germline stem cells can replicate,⁵¹ which suggests that local environmental factors extrinsic to the oocyte hold it in a state of suspended animation.⁵² The endowment of follicles may also be influenced by circulating factors, such as toxins⁵³ and placental insufficiency.⁵⁴ Some clinical evidence suggests that fetal undernutrition slows the rate of atresia.⁵⁵ Studies in mice indicate that puberty appears to be a critical developmental window for the regulation of the follicle population because a wave of primordial follicle depletion is triggered by gonadotropin action on the intrinsic apoptotic pathway.⁵⁶

Placenta. The fetoplacental unit becomes the major source of sex hormones in the female fetus in the latter half of pregnancy: the fetal adrenal gland provides 17-KS as substrate for the formation of potent sex steroids by the placenta. Excess androgen, from any source, in the female fetus masculinizes genital

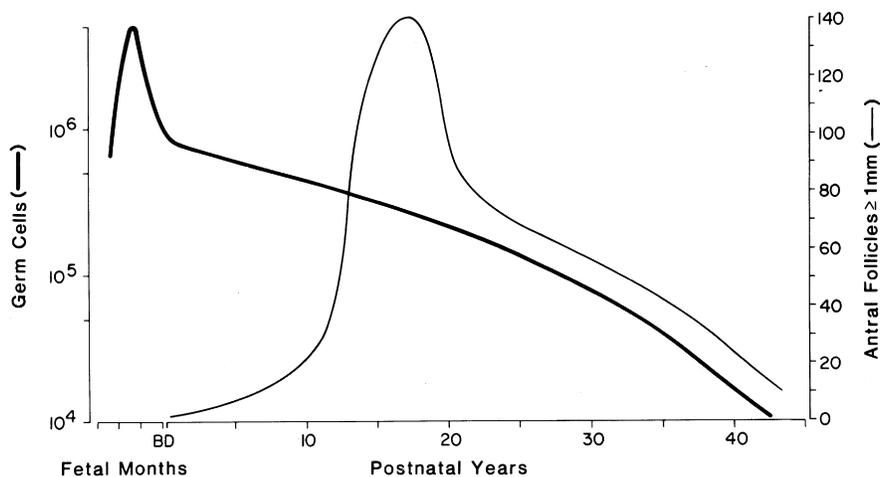


Fig. 16.3 The development of ovarian follicles from fetal life to maturity. Curves for total number of viable germ cells (*thick line*) and large antral follicles (*thin line*) smoothed from the data of Baker and Block. The number of germ cells is maximal at the fifth month of fetal life. The loss of germ cells is exponential throughout postnatal life. At puberty, a marked shift occurs in the pattern of development of follicles. An increased fraction grows to large antral size.

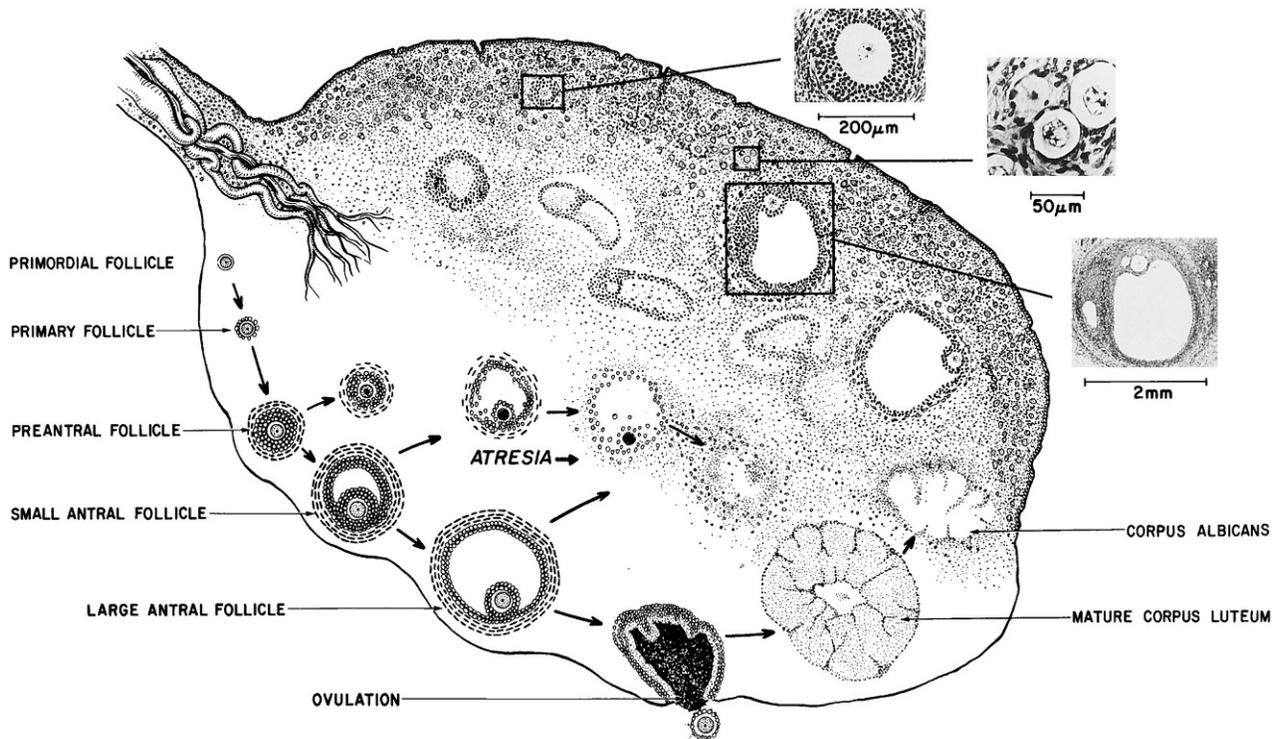


Fig. 16.4 The human ovary. The lower portion of the figure shows the classification of follicles. Preantral follicles contain as many as 300 granulosa cells, and their diameter ranges from 50 to 200 µm. The oocyte diameter increases from 25 or less to 80 µm. Antral (graafian, tertiary, or vesicular) follicles have a fluid-filled antrum and a full-grown oocyte, are lined with more than 300 granulosa cells, and have a well-developed theca. They are greater than 200 µm in diameter. The dimensions of the mature ovary are approximately 1.25 × 2.75 × 4 cm. The upper portion of the figure illustrates the histological appearance of the perimenarcheal ovary. (Photomicrographs from Peters, H. (1979). The human ovary in childhood and early maturity. *Eur J Obstet Gynecol Reprod Biol*, 9(3), 137; modified from Ross, G.T., Schreiber, J.R. (1978). The ovary. In: Yen, S.S.C., Jaffe, R. (eds.), *Reproductive Endocrinology*. Philadelphia, WB Saunders, p. 63.)

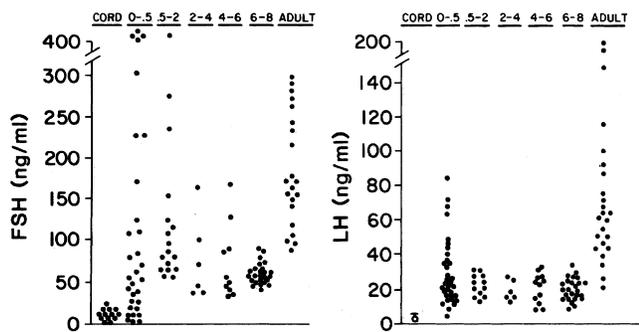


Fig. 16.5 Left: The distribution of serum gonadotropin levels according to early generation radioimmunoassays from birth to adulthood (age in years). Left: follicle-stimulating hormone (FSH). Right: luteinizing hormone (LH) levels. Umbilical cord level of LH measured by beta-subunit-specific radioimmunoassay. Standard LER-907: 100 ng equivalent to 2 mIU FSH and 6 mIU LH of the First International Reference Preparation of human pituitary gonadotropin for bioassay. (Data from Winter, J., Faiman, C., Hobson, W., Prasad, A., Reyes, F. (1975). Pituitary-gonadal relations in infancy: I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*, 40, 545; Kaplan, S., Grumbach, M., Aubert, M. (1976). The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. *Recent Prog Horm Res*, 32, 161.)

differentiation, as discussed in other chapters. This also programs for LH elevation and insulin resistance in adult life.¹⁵ Another factor predisposing to postnatal insulin resistance is placental insufficiency, via hypoxemia and resultant overactivation of fetal prostaglandin production and cortisol secretion.⁵⁷

Adolescent

The endocrinological changes of puberty actually begin in late preadolescence before secondary sex characteristics appear, as just reviewed. The underlying basic event is increasing secretion of hypothalamic GnRH. Puberty is the consequence of the hypothalamus releasing GnRH with increasing frequency and amplitude, first only at night, then gradually throughout the day.

Increased GnRH secretion in man was initially deduced when Kastin, Job, Grumbach and their collaborators demonstrated that preadolescent children had GnRH-releasable pituitary stores of LH and FSH (Figs. 16.6 and 16.9).⁸⁷ Subsequently, it was reported that in man, the output of an immunoreactive fragment of GnRH begins to rise in late childhood and increases to adult levels during puberty.^{73,88} Studies in the rat suggest that hypothalamic GnRH increases through puberty.⁸⁹

Knobil subsequently showed that puberty can be induced in the immature female rhesus monkey by administering GnRH in hourly pulses that yield blood levels of about 2000 pg/mL.⁹⁰

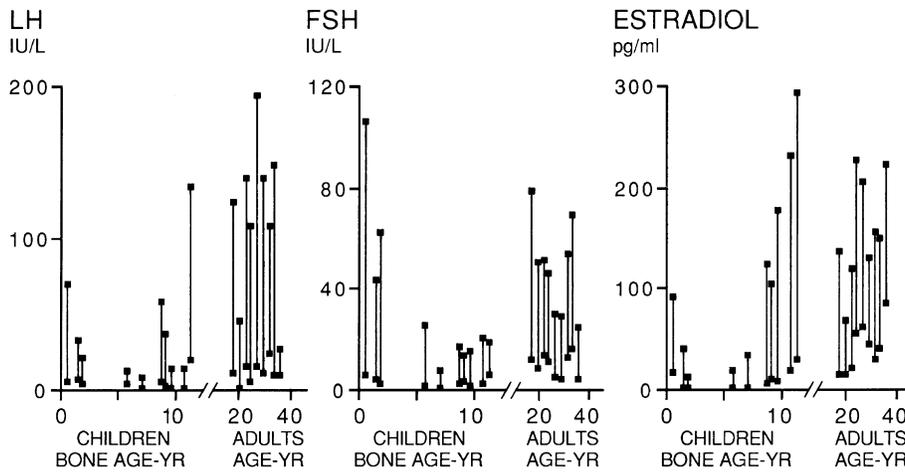


Fig. 16.6 Basal and peak responses to the gonadotropin-releasing hormone agonist nafarelin (1 mcg/kg subcutaneously) during development. Lines connect the basal and peak responses in control children. The responses are related to bone age in children and chronological age in adults. Note the biphasic pattern of the responses. They are high in infancy, lower in midchildhood, and rise again during puberty. The peak gonadotropin responses occur at approximately 4 hours, and peak estradiol responses occur at 20 hours. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone. (From Rosenfield, R.L., Burstein, S., Cuttler, L., et al. (1989). Use of nafarelin for testing pituitary-ovarian function. *J Reprod Med*, 34, 1044.)

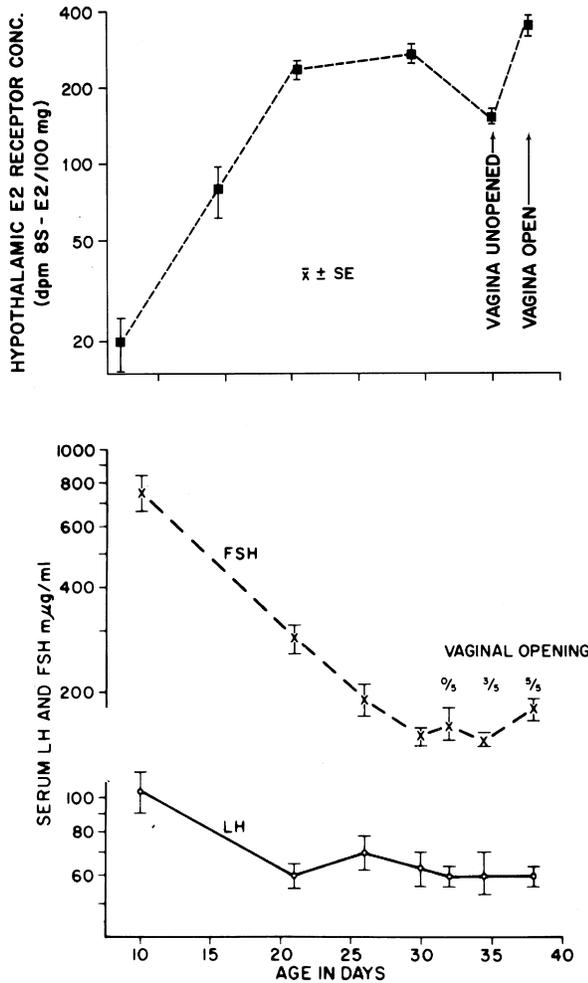


Fig. 16.7 Relationship of maturation of hypothalamic estrogen receptors (top) to serum gonadotropin levels (bottom) in the developing female rat. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone. (From Rosenfield, R.L. (1977). Hormonal events and disorders of puberty. In: Givens, J.R. (ed.), *Gynecologic Endocrinology*. Chicago, Year Book Medical. By permission of Mosby-Year Book.)

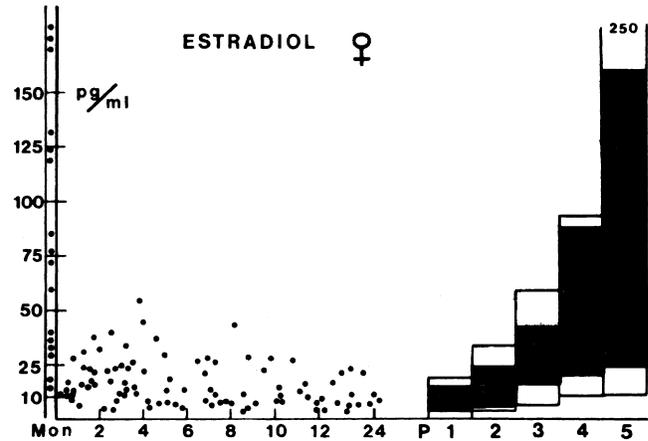


Fig. 16.8 The distribution of plasma estradiol levels in infant females compared with pubertal and adult female levels. The columns represent the normal ranges for the various stages of puberty. The area between 10th and 90th percentiles is dark. Stage P1 includes all prepubertal girls older than 2 years. The values between the ordinates were found between 2 and 5 days of age. (From Bidlingmaier, F., Knorr, D. (1978). Oestrogens: physiological and clinical aspects. *Pediatr Adolesc Endocrinol*, 4, 41-84.)

Prolonged administration of GnRH according to this regimen first gradually brings about transient increases in LH and FSH. This then induces cyclic follicular development. The resultant moderate estradiol surge is of such magnitude as to result in menarche because of withdrawal menstrual bleeding in an anovulatory cycle (Fig. 16.10). Continuation of the same GnRH regimen leads to development of normal monthly ovulatory menstrual periods. Physiological pulses of GnRH in man probably attain lower concentrations (200 pg/mL) and occur at slightly wider intervals than in monkeys.⁹¹ Consequently, LH pulses in mature women occur at intervals of approximately 1.5 hour during the follicular phases, slowing during the luteal phase.⁹²

Puberty begins in response to increased GnRH secretion. Serum LH first begins to rise disproportionately to FSH; this LH-FSH disparity is particularly evident during sleep, which is

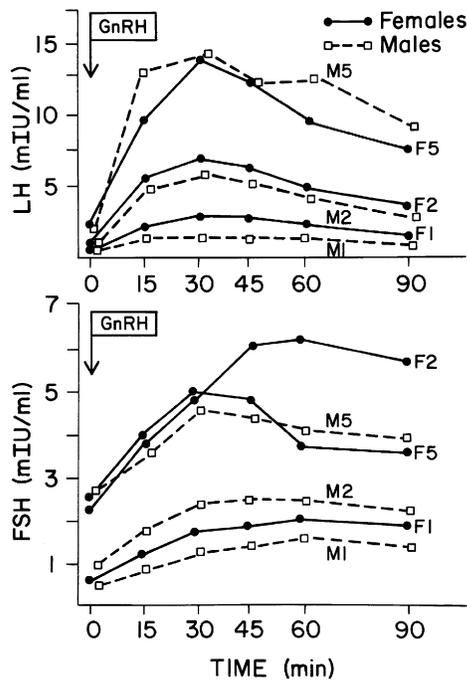


Fig. 16.9 The luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to gonadotropin-releasing hormone (GnRH) bolus (50 mcg/kg/day) in males (M) and females (F) in prepuberty (age 5–6 years: F1, M1), early puberty (F2, M2), and later puberty (F5, M5). The responses to GnRH tend to progress with advancing puberty. However, early pubertal girls have a readily releasable FSH pool that is greater than that of more advanced adolescents. The peak responses of girls tend to be somewhat greater than those of boys at comparable stages. (Modified with permission from Ehrmann et al, Polycysticovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev.* 1995;16:322–353.)

reflected in responses to GnRH or GnRH agonist (Table 16.1). Puberty becomes clinically apparent as thelarche when estradiol levels are sustained >10 pg/mL.⁹³ It seems likely that a rise in inhibin-B as increasing ovarian follicles develop plays a key negative-feedback role in limiting further increase in FSH levels during puberty. FSH levels become less GnRH-dependent during puberty.⁷⁷ The mechanisms for differential regulation of FSH and LH are discussed later in this chapter.

Pubertal gonadotropin cycles seem to develop well before menarche^{7,94} and are capable of inducing cyclic estrogen production.^{94,95} Our working model of the nature of pituitary-ovarian dynamics in early puberty is illustrated in Fig. 16.11.

Puberty progresses as LH rises. Whereas serum FSH levels rise about 2.5-fold over the course of puberty, LH levels rise 25-fold or more.⁷⁷ The initial change in LH secretion at the beginning of puberty is a nightly increase in LH secretion that begins within 20 minutes of the onset of sleep. Subsequently, LH increases more with the onset of sleep, stays up longer, and falls less during waking hours. As the child approaches menarche, the daytime LH levels continue to increase until the diurnal rhythm is typically lost. FSH levels follow a similar pattern, although the FSH changes are less striking. The gonadotropin diurnal rhythm during puberty seems entirely related to sleep, unlike the cortisol circadian rhythm.⁹⁶ There is a delay of about 12 h between the peak LH level during sleep and the estradiol zenith, such that estradiol levels are maximal between late morning and early afternoon.^{97,98} The gonadotropin and estradiol rhythms in an early pubertal girl are shown in Fig. 16.12.⁹⁸

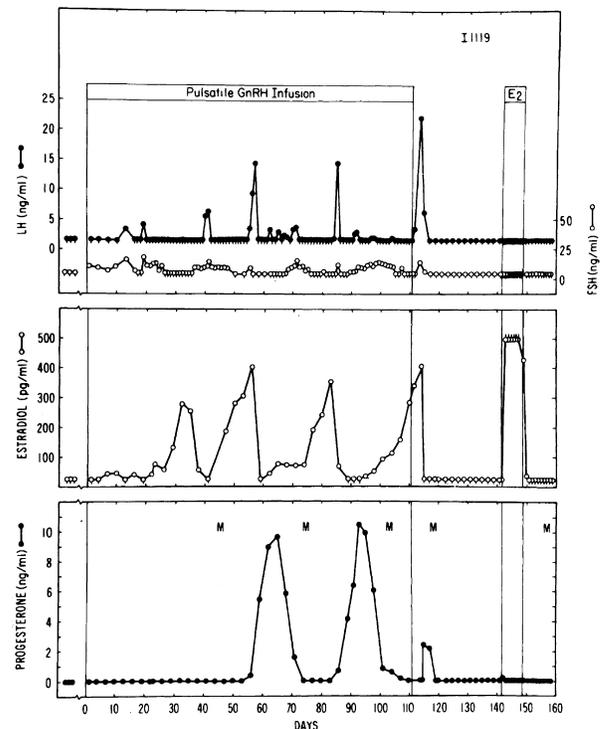


Fig. 16.10 Induction of puberty in a 13-month-old prepubertal rhesus monkey by an unvarying pulsatile gonadotropin-releasing hormone (GnRH) regimen (1 mcg/min \times 6 min hourly). Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone were undetectable before the GnRH infusion. On GnRH infusion, a rise in FSH was the first change detectable by midmorning sampling midway between GnRH pulses. A substantial E2 surge occurred approximately 1 month later. The subsequent LH surge was too modest to elicit ovulation, but menses (M) occurred a few days after subsidence of the week-long E2 surge—menarche resulting from an anovulatory cycle. Continuation of the GnRH led to the sustained occurrence of ovulatory menstrual cycles at 28-day intervals. An identical outcome results if an arcuate-lesioned adult animal undergoes this GnRH regimen. The third of the LH surges occurred 2 days after GnRH was discontinued. Progesterone secretion from the corpus luteum was blunted and transient in the absence of sustained LH secretion. A subsequent increase in plasma E2 produced by E2 implantation subcutaneously failed to elicit a gonadotropin surge, indicating that the animal had reverted to an immature state. Menarche eventually spontaneously recurred in such animals at the usual age (approximately 27 months). Small vertical lines beneath data points indicate values below the sensitivity of the assay. Note that gonadotropins and E2 were often undetectable (prepubertal range) during the induced puberty. (From Knobil, E. (1980). The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res.* 36, 53.)

Augmentation of the bioactivity of serum LH occurs during pubertal progression. Plasma LH bioactivity rises nearly fivefold more during the course of puberty than does LH as measured by polyclonal RIA.^{99,100} The change in bioactive LH is mirrored well by the “third-generation” monoclonal antibody-based immunometric (“pediatric”) assays that have very high specificity for bioactive LH epitopes. However, disparities in the ratio of bioactive to immunoreactive LH (B/I) persist with these assays, for reasons related to the molecular microheterogeneity of gonadotropins, which is discussed later. Serum FSH rises during puberty according to immunoassay more so than by bioassay.¹⁰¹

TABLE 16.1 Typical Female Normal Ranges for Luteinizing Hormone, Follicle-Stimulating Hormone, and Ovarian Steroids at Baseline and in Response to Adrenocorticotrophic Hormone and Gonadotropin-Releasing Hormone Agonist Tests^a

	LH (U/L)	FSH (U/L)	Estradiol (pg/mL)	Estrone (pg/mL)	Testosterone (ng/dL)	Androstene- dione (ng/dL)	DHEA (ng/dL)	17PROG (ng/dL)	17PREG (ng/dL)	DHEAS (mcg/dL)
BASELINE (8:00 AM)										
Preterm infants, 26–28 wk, day 4	0.1–175	2–200	—	—	<45	60–940	80–1485	100–2000	375–3550	125–880
Term infants, day 1	—	—	300–500	300–500	16–75	100–410	300–2600	150–850	110–3000	20–410
Term infants, day 3–7	—	—	<15	<20	<20	280	40–1300	<80	35–800	90–360
Term infants, maximum 1–6 mo	≤1.1	1.2–19	<7–55	≤20	<10–45	≤40	≤950	≤110	40–765	≤115
Children, 1–5 y	<0.15	<0.16–3.5	0.5	0.5	0.5	10–50	20–130	5–115	10–105	5–35
Children, 6–10 y	≤0.3	≤2.9	0.9	0.9	0.9	10–75	20–345	5–115	10–200	10–115
Premenarcheal pubertal, 9–13 y	≤7.2	1.1–9.0	≤55	10–35	10–35	40–175	40–600	16–220	35–350	35–130
Postmenarcheal, early follicular phase	1.5–5.6	3.6–7.9	20–85	20–50	20–60	50–200	100–850	≤130 ^b	55–360	75–255
PEAK AFTER ACTH_{1–24} (30–60 MINUTES AFTER ≥10 mcg/m² IV)										
Children, 1–5 y	—	—	—	—	<20	16–70	25–100	50–270	45–350	5–35
Children, 6–10 y old	—	—	—	—	<20	25–100	70–320	85–300	60–650	10–115
Premenarcheal pubertal, 9–13 y	—	—	—	—	10–35	55–230	70–725	90–400	150–750	35–130
Postmenarcheal, early follicular phase	—	—	—	—	20–60	60–250	250–1470	35–160 ^b	150–1070	75–255
PEAK AFTER GnRH AGONIST (LEUPROLIDE ACETATE 10 mcg/kg SC)										
Prepubertal, 6–9 y	1.2–8.9	9.3–37	≤55	—	<20 ^c	25–50 ^c	25–70 ^c	<25 ^c	—	—
Premenarcheal pubertal, 9–13 y	2.8–99	14–40	30–350	—	10–45 ^c	25–165 ^c	60–185 ^c	<155 ^c	—	—
Postmenarcheal, early follicular phase	30–135	16–60	65–260	—	10–60 ^c	50–180 ^c	60–450 ^c	30–135 ^c	—	—
Conversion multipliers to SI units			3.67	3.70	0.0347	0.0349	0.0347	0.0303	0.0316	0.0271
			(pmol/L)	(pmol/L)	(nmol/L)	(nmol/L)	(nmol/L)	(nmol/L)	(nmol/L)	(μmol/L)

17PREG, 17-Hydroxypregnenolone; 17PROG, 17-hydroxyprogesterone; ACTH, adrenocorticotrophic hormone; DHEA, dehydroepiandrosterone; GnRH, gonadotropin releasing hormone; FSH, follicle-stimulating hormone; IV, intravenous; LH, luteinizing hormone; SC, subcutaneous.

^a5th to 95th percentile for third-generation gonadotropin immunoassays and high-specificity steroid assays after preparatory chromatography, except for DHEAS. Values differ slightly among laboratories.

^b17-Hydroxyprogesterone early follicular phase baseline levels >130 ng/dL are found in women who are heterozygous for 21-hydroxylase deficiency, and they often have responses to ACTH greater than those shown. 17PROG begins rising during the late follicular phase and peaks as high as 400 ng/dL in the luteal phase of the cycle.

^cAt 1600hr after dexamethasone administration (0.5 mg po at 1200hr) to blunt coincidental adrenocortical secretion.

(Data from Rosenfield, R.L. (2007). Identifying children at risk of polycystic ovary syndrome. *J Clin Endocrinol Metab*, 92, 787–791; Rosenfield, R.L., Bordini, B., Yu, C. (2013). Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab*, 98, 1591–1601; Mortensen, M., Ehrmann, D.A., Littlejohn, E., Rosenfield, R.L. (2009).

Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. *J Clin Endocrinol Metab*, 94, 1579–1586; Forest, M. (1979). Function of the ovary in the neonate and infant. *Eur J Obstet Gynecol Reprod Biol*, 9, 145–160; de Peretti, E., Forest, M.G. (1982). Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. *Horm Res*, 16, 10–22;

Bidlingmaier, F., Knorr, D. (1978). Oestrogens: physiologic and clinical aspects. *Pediatr Adolesc Endocrinol*, 4, 41–84; Chellakooty, M., Schmidt, I.M., Haavisto, A.M., Boisen, K.A., Damgaard, I.N., Mau, C., et al. (2003). Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab*, 88, 3516–3520; Greaves, R.F.,

Pitkin, J., Ho, C.S., Baglin, J., Hunt, R.W., Zacharin, M.R. (2015). Hormone modeling in preterm neonates: establishment of pituitary and steroid hormone reference intervals. *J Clin Endocrinol Metab*, 100, 1097–1103; Johannsen, T.H., Main, K.M., Ljubicic, M.L., Jensen, T.K., Andersen, H.R., Andersen, M.S., et al. (2018). Sex differences in reproductive hormones during mini-puberty in infants with normal and

disordered sex development. *J Clin Endocrinol Metab*, 103, 3028–3037; Endocrine Sciences/LabCorp. 2018 Expected Values and S.I. Unit Conversion Tables. <https://www.endocrinesciences.com/sites/default/files/Endocrine%20Sciences%20Expected%20Values.pdf>.)

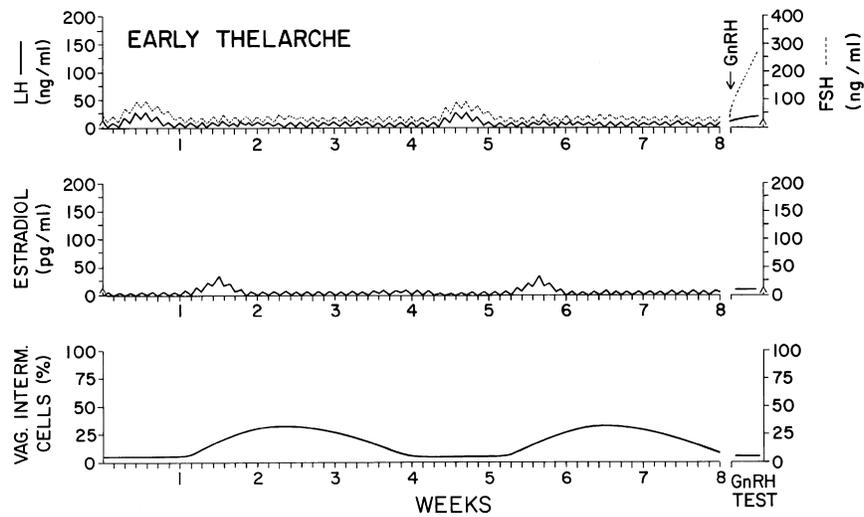


Fig. 16.11 Diagram depicting our working hypothesis of the hormonal patterns in girls during very early puberty. We conceptualize this pattern as occurring both cyclically in the earliest stage of normal puberty and occasionally in unsustained sexual precocity (i.e., most US cases of idiopathic premature thelarche). Daytime and nighttime serum concentrations of hormones (gonadotropins relative to the LER-907 standard) and the percentage of intermediate cells on vaginal smear are shown. The typical response to a gonadotropin-releasing hormone (*GnRH*) test is illustrated. Subclinical hormonal cycles lasting approximately 1 month result from a few days of increased follicle-stimulating hormone (*FSH*) and luteinizing hormone (*LH*) secretion. Because the drive to gonadotropin release is relatively weak, *FSH* and *LH* production are suppressed promptly and for long periods of time by the resultant modest amounts of estradiol (*E2*) secretion. Estradiol is detectable in plasma for only a few days a month. Maturation of the vaginal mucosa, however, is detectable for approximately 2 weeks after *E2* production has waned.

Estradiol output increases rapidly in the year approaching menarche.¹⁰² This seems to be the result of a variety of autoamplification phenomena that facilitate puberty, maturation of the dominant follicle, and ovulation. These are summarized in Box 16.1.^{103–116} These phenomena occur at all levels of the axis. The CNS is stimulated by preovulatory levels of

estradiol to increase *GnRH* pulse amplitude. At the pituitary level, there is the self-priming effect of *GnRH*, whereby a pulse of *GnRH* sensitizes the pituitary to have a greater *LH* response to a subsequent identical *GnRH* pulse. Critical patterns of estradiol and progesterone secretion enhance the pituitary *LH* and *FSH* responsiveness to *GnRH*. At the gonadal level, the cascade of events is augmented by the *FSH* induction of aromatase activity and progesterin production in granulosa cells, phenomena in which androgens play a synergistic role. Furthermore, *FSH* stimulates granulosa cell mitosis and induces *LH* receptors, phenomena in which estradiol may play a synergistic role. Subsequently, *LH* is able to further enhance the aromatase and

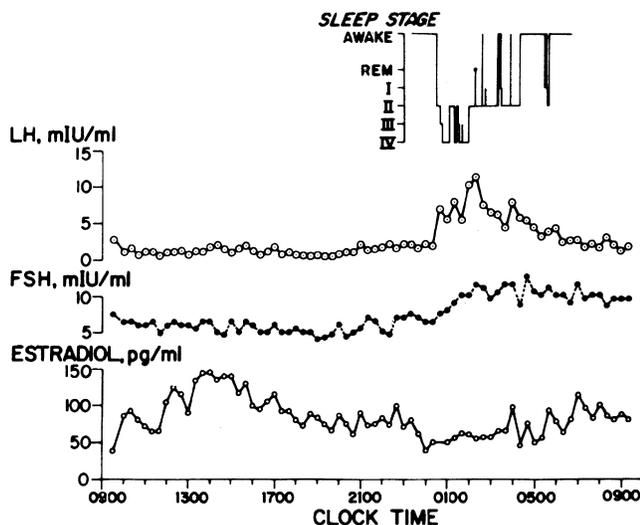


Fig. 16.12 The patterns of serum luteinizing hormone (*LH*), follicle-stimulating hormone (*FSH*), and estradiol (*E2*) typical of early female puberty. Note that daytime gonadotropin levels are in the prepubertal range. Note also the episodic nature of *LH* release at intervals of 1 to 3 hours. Estradiol levels are seen to fluctuate considerably in the course of the daytime, rising to peak levels about 12 hours after the maximum nocturnal gonadotropin surges. (From Boyar, R.M., Wu, R.H.K., Roffwarg, H., et al. (1976). Human puberty: 24-hour estradiol patterns in pubertal girls. *J Clin Endocrinol Metab*, 43, 1418.)

BOX 16.1 Autoamplification Processes Involved in Pubertal Progression, Follicle Maturation, and Ovulation^{38,99, 103–116}

Central nervous system *KISS1* and *GnRH* secretion increases^{103–104} via:

- *E2*-inducing progesterone receptors¹⁰⁵
- Progesterone synergization with *E2*¹⁰⁶

Pituitary *LH* and *FSH* responsiveness to *GnRH* increases via:

- *GnRH* self-priming¹⁰⁷
- Critical patterns of *E2* secretion-stimulating *LH/FSH* responsiveness^{108,109}
- Progesterone synergization with *E2*^{109–111}
- *LH* bioactivity increases⁹⁹

Gonadal responsiveness to *FSH* and *LH* increases via:

- *FSH*-inducing aromatase and progesterone in granulosa cells: androgens and progesterone synergization with this effect^{112–114}
- *FSH*-stimulated granulosa meiosis³⁸ and *FSH*-inducing granulosa *LH* receptors; *IGF-1* synergization^{115,116}

E2, Estradiol; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *IGF-1*, insulin-like growth factor-1; *KISS1*, kisspeptin; *LH*, luteinizing hormone.

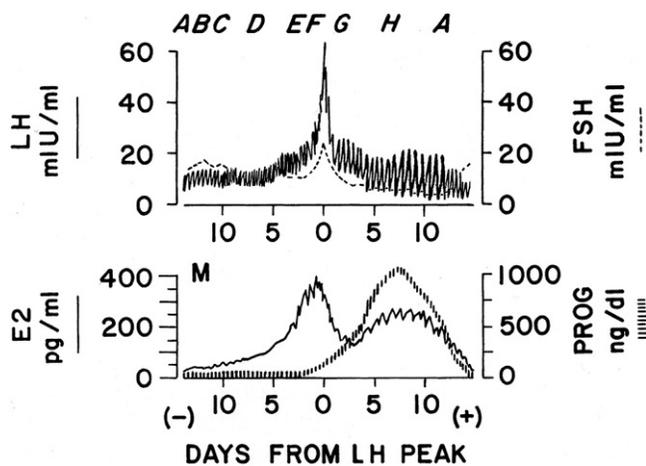


Fig. 16.13 Diagram of gonadotropin and female hormone levels during the normal menstrual cycle. The levels are centered in reference to the day of the midcycle luteinizing hormone (LH) peak (day 0). Letters A through F above the top panel correspond to stages of follicular development in Fig. 16.14. G and H are discussed in the text. M (bottom panel) shows time of menses. E2, Estradiol; FSH, follicle-stimulating hormone; PROG, progesterone. (Data from Abraham, G.E. (1974). Ovarian and adrenal contributions to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*, 39, 340; Ross, G.T., Cargille, C.M., Lipsett, M.B., Rayford, P.L., Marshall, J.R., Strott, C.A., Rodbard, D. (1970). Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Recent Prog Horm Res*, 26, 1; and Soules, M., Steiner, R., Clifton, D., Cohen, N., Aksel, S., Bremner, W. (1984). Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab*, 58, 378.)

progesterone effects. Progesterone itself plays a synergistic role in stimulating granulosa cell progesterone and prostaglandin synthesis in concert with both FSH and LH. In the rat, ovarian GnRH receptor sites also diminish just before ovulation,¹¹⁷ and at about this time the ovary changes its pattern of metabolism so that the secretion of androstenediol-3 β -monosulfate decreases to levels that are no longer inhibitory to LH secretion.¹¹⁸

The preovulatory gonadotropin surge occurs when all these cascading processes culminate in activation of the positive feedback mechanism, a unique feature of the female neuroendocrine system.^{119,120} Positive feedback refers to the neuroendocrine system acquiring the ability to secrete a midcycle surge of LH in response to the increasing estrogen secretion by a preovulatory follicle, that is, when the ovary signals via increasing estrogen secretion that it is prepared for ovulation.

Menarche does not necessarily indicate full maturation of the neuroendocrine-ovarian axis. As the studies of Knobil illustrate (see Fig. 16.10), menarche can be caused by estrogen-withdrawal bleeding—and it is about half of the time—but ovulatory cycles may follow in short order. General characteristics of the mature ovary are shown in Fig. 16.4.

The morphology of the normal adolescent ovary has long been considered polycystic, and histological examination typically has shown thecal luteinization.^{80,121} In the perimenarcheal period, the combination of a high number of follicles and mature gonadotropin stimulation leads to a greater number of 2 to 9 mm antral follicles within a year after menarche than at any other stage (see Fig. 16.3).³⁰ This often leads to a “multifollicular” ultrasonographic appearance^{82,122,123} that overlaps adult criteria for polycystic ovary morphology in one-third to one-half of normal adolescents (see section on polycystic ovary syndrome).¹²⁴

Adult

The follicular phase of each menstrual cycle recapitulates puberty in many respects. Gonadotropin and sex hormone levels are low during the premenstrual phase of the mature cycle (Fig. 16.13A).^{125,126} Gonadotropin concentrations then increase at the time of menstruation, FSH predominating in the early follicular phase, whereas nocturnal LH pulsation is slow¹²⁷ (Fig. 16.13B). LH pulsation increases to a circadian pattern around a stable baseline, and estradiol production slowly begins as antral follicles develop (Fig. 16.13C). Estradiol levels gradually increase and serum FSH levels fall reciprocally (Fig. 16.13D). Upon formation of a dominant follicle, serum estradiol concentrations increase geometrically. This selectively begins to amplify the pituitary’s LH response to GnRH as estradiol reaches about 90 pg/mL for over 3 days (Fig. 16.13E).

When the serum estradiol rises to over 200 to 300 pg/mL for 36 hours, the positive feedback mechanism is activated and the midcycle gonadotropin surge commences (Fig. 16.13F). Estradiol then appears to induce PR expression in the hypothalamus and pituitary.¹²⁹ An increase in progesterone to 100 ng/dL facilitates the LH surge, shortens the duration of time over which estradiol is required for the surge to 24 hours, and brings about an FSH surge. The mechanism of progesterone action involves inhibition of GnRH cleavage.¹¹¹ Androgens may also play a role in facilitating FSH and GnRH release.^{130,131} The LH surge is then primarily responsible for luteinizing the preovulatory ovarian follicle (see Fig. 16.13F). At this time, LH pulses become larger in amplitude but slower in frequency and their apparent bioactivity increases. Ovulation then results.

As the follicle is disrupted by ovulation, estrogen levels fall (Fig. 16.13G). Meanwhile, serum progesterone increases steadily as the corpus luteum begins to form, and comes to be sustained at very high levels for several days, along with a lesser increase in 17-hydroxyprogesterone (17-OHP) and a return of estradiol to late follicular phase levels (Fig. 16.13H).^{125,126,132} In response to the high progesterone level, LH pulses become slow and large.^{127,132} In the absence of increasing human chorionic gonadotropin (hCG) from a conceptus, the corpus luteum’s life span is exhausted and its production of progesterone and estradiol wanes. Subsequently, FSH begins to rise out of proportion to LH. Shortly after the sex steroids withdraw from the scene, the endometrium sloughs, giving rise to menstrual flow. Meanwhile, the follicular growth induced earlier by FSH begins to gain momentum and the next cycle begins.

Follicular (Proliferative) Phase Ovary. The hormonal functions of the follicle have dual purposes that must be closely coordinated: to change the milieu of the ovum to prepare for ovulation and to signal the pituitary to send the signal to ovulate, that is, the LH surge. Thus the ovary is the zeitgeber for the cycle; the normal cyclic pattern of ovarian hormone secretion induces the midcycle surge of pituitary gonadotropins, even in the presence of unchanging circadian pulses of GnRH.⁹⁰ Ovarian hormones also augment the amplitude of the GnRH response,^{103–106} which is a “fail-safe” mechanism that “guarantees” a preovulatory gonadotropin surge.

Ovarian follicular development and steroid secretion in relationship to changing gonadotropin levels are illustrated in Fig. 16.14.^{38,133–135} FSH and LH play major roles in granulosa and thecal cell differentiation, respectively, whereas a host of local factors modulate gonadotropin action. For example, follicular maturation in response to gonadotropins is enhanced by insulin-like growth factors (IGFs), TGF- β , and fibroblast growth factor, whereas it is inhibited by TGF- α .

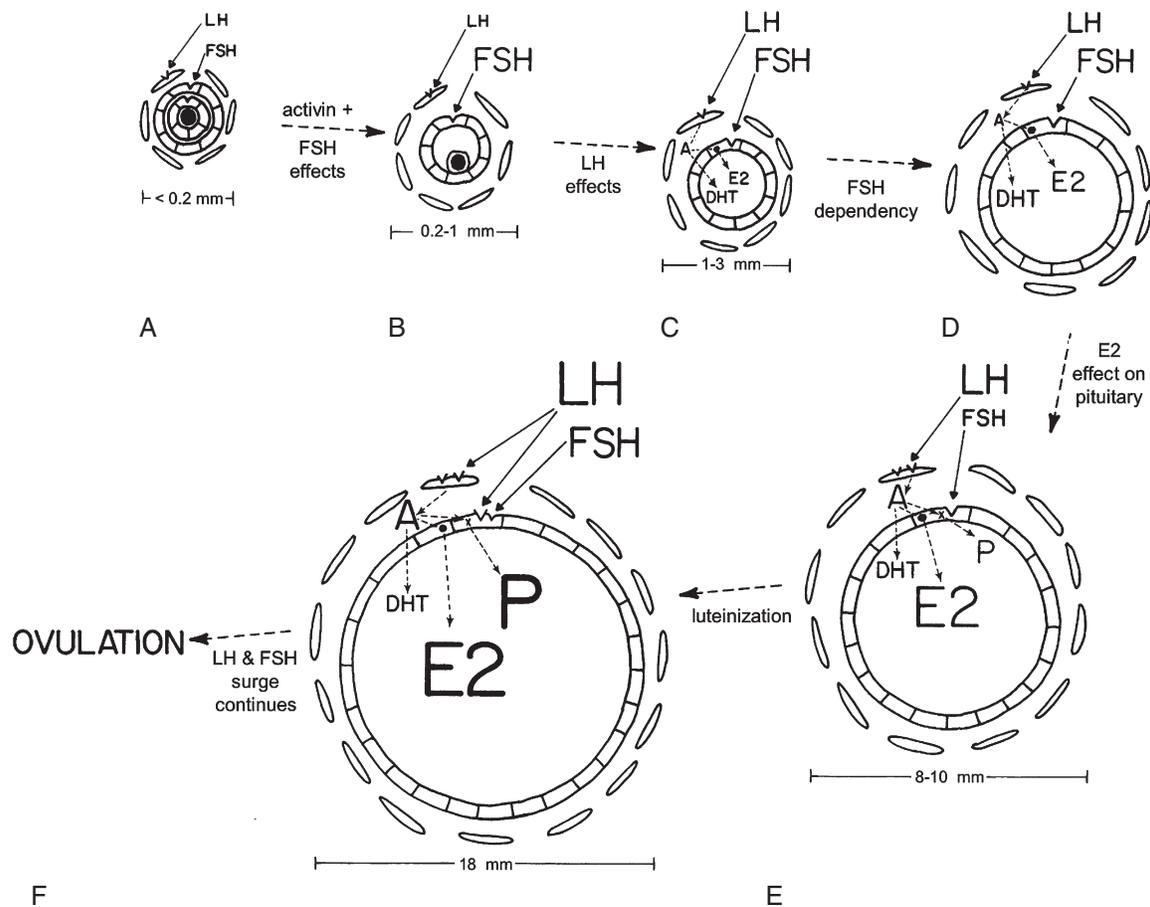


Fig. 16.14 Relationships among gonadotropins, the ovarian follicle, and ovarian steroids according to the two-cell two-gonadotropin model of ovarian steroidogenesis. A through F: Stages of ovarian follicular development found during the times of the menstrual cycle designated by the corresponding letters on Fig. 16.13. The size of the letters designating hormones relates to the magnitude of their serum and/or follicular concentrations. (A) Preantral follicle with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) receptors in theca and granulosa cells, respectively. There is no antrum surrounding the ovum (stippled in center). (B) Small antral follicle. Activin upregulates FSH receptors, and FSH receptor activation is required to initiate antrum formation. (C) Larger antral follicle (≥ 1 mm). Aromatase activity (.) has been induced in granulosa cells. Interactions between theca and granulosa cells, the former producing androgens (androstenedione [A], and dihydrotestosterone [DHT] synthesis. (D) FSH-dependent granulosa cell multiplication (not shown) is responsible for more follicular growth and more E2 synthesis. (E) Estradiol enhances pituitary LH secretion in response to GnRH, while at the same time inhibiting pituitary FSH secretion. The increased LH induces more theca LH receptors and stimulates androgen production. Androgens serve as a substrate for E2 formation and synergize with FSH to stimulate progesterone (P) secretion. (F) In the preovulatory follicle, FSH induces LH receptors on the granulosa cell, which completes luteinization. Steroid secretion is augmented further. Then, increasing progesterone amplifies the positive feedback effect of E2 to initiate the preovulatory gonadotropin surge.

Primordial follicle growth and development is gonadotropin independent. Subsequently, granulosa cells of preantral follicles develop FSH receptors, and theca cells, which encircle granulosa cells, develop LH receptors (see Fig. 16.14A). Activin causes FSH-independent upregulation of FSH receptors in preantral follicles,¹¹⁶ although it opposes FSH stimulation of antral follicle development.³⁸ Primordial follicle growth is constitutively repressed by nuclear forkhead transcription factor Foxo3; when Foxo3 is released in response to stimulation of the PTEN-PI3K-Akt pathway, follicular growth progresses to the point where follicles become responsive to FSH.¹³⁶

Antrum formation requires a trace (prepubertal) amount of FSH receptor activation (see Fig. 16.14B).^{38,137-140} FSH stimulates androgen receptor expression in primary follicles, and androgens in turn stimulate further expression of FSH receptors and the early stages of follicular growth.¹⁴¹ Androgen action is also necessary for the development of a full complement of follicles, and androgen excess stimulates excessive follicle number.^{142,143} LH stimulates the appearance in theca cells of the enzymes necessary for androgen biosynthesis.¹⁴⁴ Evidence that theca cells of small antral follicles form estradiol is meager.¹⁴⁵

As antral follicles grow over 2.5 mm in diameter, their granulosa cells begin to form estradiol from androgen supplied by theca cells (see Fig. 16.14C).¹⁴⁶⁻¹⁵⁰ Androgen production at low levels may synergize with FSH to stimulate aromatase activity within the granulosa cells.^{112,151,152}

At this stage, follicles are increasingly FSH dependent and consequently uniformly FSH responsive.^{38,137} IGF-1 is required for follicular growth beyond the early antral stage in response to FSH.¹⁵³ Antral follicles do not grow over 5 mm in diameter without a pubertal degree of FSH stimulation.¹³⁹ By the midfollicular phase, the proliferation of FSH-responsive granulosa cells results in an accelerating rate of estradiol production and preferential conversion of androstenedione to estradiol rather than dihydrotestosterone (DHT) by these cells (see Fig. 16.14D).^{146-148,150,154,155} Estradiol itself clearly stimulates proliferation of granulosa cells and oocyte survival in rodents.¹⁵⁶ In humans, estradiol appears to promote antral growth independently of LH¹⁵⁷ and is synergistic with FSH in bringing about the development of the dominant follicle.^{158,159}

A dominant follicle is selected at the beginning of the menstrual cycle from a crop of follicles that were recruited

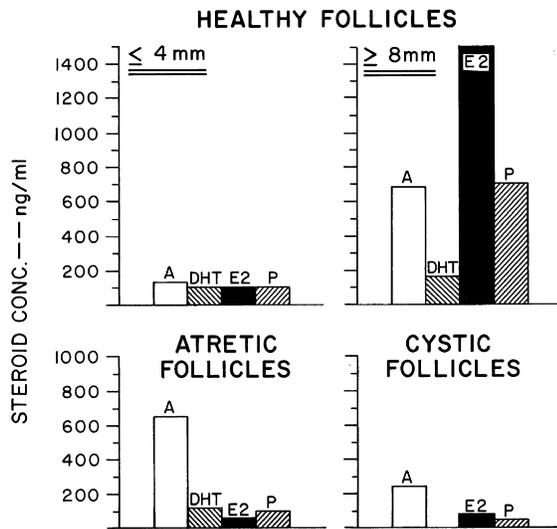


Fig. 16.15 Normal human antral fluid steroid concentrations. Healthy follicles are well populated by granulosa cells ($\geq 50\%$ of maximal complement). Healthy follicles seem capable of further development because many of them (75%) contain healthy-appearing oocytes (histologically intact germinal vesicles), 96% of which are viable in culture. Moderately large follicles (8 mm or larger in diameter) make their appearance only in the midfollicular phase of the cycle and contain follicle-stimulating hormone. Data are shown only for those large follicles well populated by granulosa cells, only one of which usually arises in the follicular phase of each menstrual cycle. Atretic follicles are small follicles beginning to show degenerative changes in the number of granulosa cells and appearance of the oocyte. Cystic follicles tend to be larger follicles with only a sparse granulosa cell lining. The testosterone content of antral fluid is about a third that of dihydrotestosterone (DHT) owing to the pattern of granulosa cell metabolism of androstenedione (A). E₂, Estradiol; P, progesterone. (Data from McNatty, K.P., Makris, A., Reinhold, V.N., De Grazia, C., Osathanondh, R., Ryan, K.J. (1979). Metabolism of androstenedione by human ovarian tissues in vitro with particular reference to reductase and aromatase activity. *Steroids*, 34, 429–443.)

2.5 months prior.³⁸ Recruitment of a group of follicles is normally promoted by the midcycle FSH surge and regresses with increasing corpus luteum progesterone secretion. Another wave of follicle growth in the late luteal phase is promoted by the rise of FSH as luteal progesterone and estradiol secretion wanes. The selected follicle is the one that is the most sensitive to FSH (lowest "FSH threshold"). FSH is critically important during the follicular phase for optimal development of this dominant follicle. By the midfollicular phase of the cycle this follicle becomes virtually the sole source of estradiol (see Fig. 16.14E). Typically, there is only one such follicle. Only this follicle continues to grow so as to reach a diameter of 10 mm or more. All other gonadotropin-dependent follicles undergo atresia.

At this stage, the rising estradiol level is suppressing FSH secretion and augmenting pituitary LH responsiveness to GnRH. FSH is more bioactive in the dominant follicle because it is more efficiently concentrated¹⁵⁴ and because local factors increase ovarian responsiveness to FSH. The increased LH causes further proliferation of thecal cells and an increase in their LH receptor content.¹¹³ Androgen production is consequently increased. This synergizes with FSH to both augment aromatase activity and bring about increasing progesterone secretion by the well-estrogenized granulosa cells of these follicles. Progesterone then enhances the synthesis of both itself and estradiol.^{113,115} The increased thecal androstenedione production is diverted much more to estradiol than to DHT biosynthesis. Antral fluid steroid concentrations reflect these changes (see Fig. 16.15).^{146,147,154}

Activin acts to prevent premature luteinization of granulosa cells, and activin tone seems to wane as the preovulatory phase approaches.^{38,116}

FSH next induces LH receptors in the granulosa cells, luteinizing them (see Fig. 16.14F).⁴⁰ Androgen and insulin synergize with FSH in this induction of LH receptors. LH then joins FSH in acting on luteinized granulosa cells to augment estradiol and progesterone production.

The LH and FSH surge then occurs in response to the positive feedback action of estradiol at both the CNS and pituitary levels, an effect amplified by the rising levels of progesterone. The final steps in follicle maturation ensue rapidly: the LH surge induces granulosa cell PR and prostaglandin synthase while inhibiting cyclin gene transcription,^{26,160} and the FSH surge upregulates vascular endothelial growth factor.¹⁶¹ In the absence of these critical steps, ovulation and follicular rupture do not occur. Then the follicle promptly becomes desensitized to LH and FSH and ceases to grow.¹⁶² This is followed by an inflammatory-type response. Protease activity, prostaglandin production, and vascular permeability increase; cell junctions loosen; and cumulus cells form a mucopolysaccharide envelope around the oocyte (cumulus expansion).

Oocyte meiotic maturation resumes in response to a specific phosphodiesterase,¹⁶³ forming the haploid gamete (secondary oocyte) and the first polar body in response to the LH surge.¹⁶⁴ Ovulation of the cumulus-oocyte complex then occurs. The presence of a favorable follicular steroidal milieu is necessary both for ovulation (a premature LH surge in a subject with an unripe follicle will not result in ovulation) and subsequent developmental competence of the oocyte.^{165,166} Meiosis will go to completion and the second polar body will be extruded only in response to contact with a sperm.

The processes stimulating dominant follicle emergence are delicately balanced by those preventing it. It seems critical that the intraovarian concentration of androgens not become excessive. Androgen excess interferes with follicle viability beyond about the 8-mm stage¹⁴⁸ and synergizes with FSH to cause premature luteinization.⁴⁰ These interfere with the emergence of dominant follicles. Follicles arrested in their growth become atretic, and atretic follicles contain relatively high concentrations of androgens (see Fig. 16.15). Progesterone also suppresses further differentiation of nondominant follicles¹⁶⁷ by some of the same mechanisms.¹⁶⁸ High concentrations of estrogen play a critical role in inhibiting selection of the dominant follicles in primates.¹⁶⁹ If there is interference with estrogenization, multiple large cystic follicles develop that are impaired in their ability to ovulate and undergo androgen-dependent atresia.^{170–172}

Anti-Müllerian hormone (AMH) and inhibins have emerged as other granulosa cell factors important in the regulation of follicular development. AMH is the major hormonal paracrine inhibitor of primordial follicle progression.⁴⁰ It is produced by the granulosa cells of small growing follicles. As follicles grow, intrafollicular AMH levels rise sufficiently to inhibit recruitment of primordial follicles to the primary follicle stage; it also inhibits P450c17 activity, GnRH release, and FSH stimulation of aromatase activity.^{40,173} Because estradiol inhibits AMH production,^{174,175} there exists an intrafollicular short negative feedback loop confining AMH expression to follicles up to about 8 mm in diameter. Thus AMH appears to act as a follicular gatekeeper, ensuring that each small antral follicle produces little estradiol before selection of the dominant follicle, which allows a direct ovarian-pituitary dialogue regulating the development of the follicle selected to undergo ovulation.¹⁷⁶

Inhibin-B is the predominant form of inhibin.^{177,178} It arises from granulosa cells in small follicles before aromatase is expressed¹⁷⁹ and is regulated by FSH in a sluggish negative feedback loop. It upregulates thecal steroidogenesis, as discussed later. Inhibin-A is a product of the preovulatory follicle (and corpus luteum) that responds to both LH and FSH.

Atresia is the fate of all except the few hundred follicles chosen for ovulation during an individual's life span. Most follicles beyond the primordial stage become atretic. Atresia occurs by the process of programmed cell death.³⁸ This apoptotic process has diverse determinants, including cell death inducer and repressor genes.^{135,180} FSH support becomes increasingly necessary for survival as the follicle matures, and it is normally only the follicle that has the lowest FSH threshold that escapes atresia.

Luteal (Secretory) Phase Ovary. Ovulatory rupture of the dominant follicle (see Fig. 16.13G) is followed by invasive proliferation of capillaries and fibroblasts from the theca that breaks down the separating basement membrane. The luteinized granulosa and theca cells then intermingle and complete the luteinization process by forming the corpus luteum.¹⁸¹

Histologically, luteinization is a process of lipid droplet accumulation that begins as the dominant follicle forms. The biochemical hallmark of the luteinized granulosa cell is the acquisition of LH receptors, with the subsequent capacity to form progesterone, 17-OHP, and estrogen in response to LH/hCG.^{182–184}

During its functional life span, the corpus luteum is normally the major source of the sex hormones secreted by the ovary. Corpus luteum function reaches its peak about 4 days after ovulation and begins to wane about 4 days before menstruation (see Fig. 16.13H). Loss of sensitivity to LH and estradiol heralds luteal senescence. Regression of the corpus luteum—luteolysis—occurs if pregnancy does not provide hCG. Luteolysis is probably mediated by prostaglandins. Transformation of the corpus luteum into an avascular scar, the corpus albicans, then occurs.

Early luteal phase increases in secretion of both estradiol and progesterone cause secretory transformation and hyperplasia of the endometrium, which is necessary for implantation of the fertilized egg. Later falloff in secretion of female hormones to a level insufficient to maintain the endometrium results in menstruation (see Fig. 16.13A). Withdrawal of progesterone is specifically responsible for constriction of spiral arteries, local prostaglandin accumulation, and subsequent ischemic necrosis of the endometrium. Normal menstrual flow then results from a complete slough of the secretory endometrium.

Documentation of ovulation can be accomplished by demonstrating collapse of the dominant follicle by ultrasonographic monitoring¹⁸⁵ or by assessing the luteal transition in the estradiol/progesterone ratio,¹⁸⁶ detecting the LH surge,¹⁸⁷ or demonstrating a normal midluteal phase rise of serum progesterone either directly¹⁸⁵ or indirectly by a rise in basal body temperature.¹⁸⁸ A significant rise in basal body temperature, averaging 0.55° C, usually occurs when serum progesterone reaches 400 ng/dL or more and continues as long as that level is maintained. While the results of these methods are correlated, LH surges are sometimes inadequate to stimulate a follicle sufficiently mature to develop into a normally functioning corpus luteum, particularly during adolescence^{124,189} (see Luteal Phase Defects).

Regulation of the Neuroendocrine-Ovarian Axis

Factors Controlling the Onset of Puberty

Pubertal onset is under the control of a complex regulatory network that is able to dynamically respond to numerous endogenous and environmental signals. GnRH neurons play a critical hierarchical role in the direct and indirect integration of these central and peripheral signals. Reproductive development is coupled with metabolic cues that may disrupt the maturational process. The mechanisms by which neuroendocrine and genetic factors control pubertal development remain unknown. Epidemiological studies indicate that nutrition, ethnicity, and genetic factors, are normally important in the pubertal process.¹⁹⁰ Environmental chemicals and chronic inflammatory disease can disrupt the process.^{190–193}

Evidence that there are genetic factors involved in the timing of puberty comes from multiple studies.^{194–204} It has been estimated that between 50% and 80% of the variation in the timing of puberty is genetically determined. Several large genome-wide association studies (GWAS) of age at menarche, examined pubertal timing in healthy females^{205–207} to identify the genes responsible. These studies demonstrated that there is significant genetic heterogeneity in pubertal timing in the general population that is likely to involve hundreds of common variants. The gene Lin-28 homolog B (*LIN28B*)²⁰⁸ was the first locus associated with age of menarche. *LIN28B* is the human ortholog of the *Caenorhabditis elegans* gene that controls developmental timing through micro ribonucleic acid (microRNA). Mutations in *LIN28B* have not been identified in humans with disorders of puberty.^{209,210} The 1000 Genomes Project studied genotype data in about 370,000 women and identified 389 independent signals ($P < 5 \times 10^{-8}$) for age at menarche,²¹¹ with effect sizes per allele ranging from 1 week to 5 months. These signals explain only about 7.4% of the population variance in age at menarche. Genes implicated in GnRH signaling, pituitary development, hormonal regulation, fatty acid biosynthesis, and energy homeostasis have been implicated.^{207,212–215} Although mutations in these genes have been shown to cause physiological interruptions in development, their role in the initiation of puberty remains unknown. Specifically, single nucleotide polymorphisms (SNPs) in the GnRH and GnRH receptor genes have not been associated with variations in the timing of puberty in the general population.²¹⁶

The key in the initiation of puberty is the activation of the hypothalamic GnRH pulse generator. The molecular events that control the pulse generator include a complex interplay between both inhibitory and stimulatory factors. The mechanism of central activation of puberty first appears to be a consequence of a removal of a restraint mechanism, with a rise in gonadotropin secretion (initially during sleep).²¹⁷ This restraint in the GnRH pulse generator is independent of the presence of gonads⁷⁰ and more intense in males.²¹⁸ A targeted gene approach in mice has confirmed that ER α (also termed ESR1) in Kiss1 neurons mediates feedback suppression of both Kiss1 expression and gonadotropin secretions during the prepubertal period.²¹⁹

However, the high levels of testosterone to which the male fetus was exposed during the period of sexual differentiation may be responsible for the more prolonged suppression of GnRH release in males than females. A role for decreased estrogen feedback sensitivity by the hypothalamic pulse generator near the time of puberty has also been shown.²²⁰

Recent evidence points to an important role for the kisspeptin 1 receptor (*KISS1R*), a G-protein-coupled receptor (previously known as GPR54), and its ligand, kisspeptin, an excitatory neuropeptide, as a signal for pubertal GnRH release. Expression of both proteins has been found to increase before pubertal onset in association with the increase in GnRH pulse generator activity in the hypothalamus.²²¹ Kisspeptin binding to its receptor on GnRH neurons stimulates GnRH secretion. Mice with knockout of *Kiss1r* were found to be infertile despite having normal GnRH neurons.^{222,223} Leptin and androgen synergistically upregulate this system, and estrogen antagonizes it.²²⁴ Mutations in *KISS1R* result in hypogonadotropic hypogonadism.^{213,222,225} However, mutations in *KISS1R* have not been found in boys with pubertal delay, nor have polymorphic sequences been associated with delay of pubertal development.²²⁶ Elegant studies in primates have demonstrated an increase in kisspeptin during pubertal development with a corresponding increase in *KISS1R* associated with an increase in LH. The maximum level of expression of kisspeptin and *KISS1R* in the hypothalamus in both males and females occurs at puberty.^{227,228} For each Tanner stage, girls tend to have higher kisspeptin levels than boys, potentially explaining their earlier onset of puberty.²²⁹

Chronic administration of kisspeptin to immature female rats induces precocious activation of the central axis.²²⁷ In addition, chronic treatment with kisspeptin restores pubertal development in a rat model of undernutrition.²³⁰ Kisspeptin may thus not only influence the priming of puberty, but also the integration of nutritional and energy status.²³¹ Although it is clear that kisspeptin activation of GnRH neurons occurs at puberty and that GnRH is increasingly sensitive to kisspeptin activation during development,^{232,233} other pathways contribute to GnRH activation since the hypogonadism associated with deficiency of *KISS1* or *KISS1R* is not complete.²³⁴

Neurokinin B (NKB) signaling seems to be critical for the initiation of puberty.²³⁵ Some kisspeptin neurons, KNDy neurons, coexpress NKB, dynorphin A, and their receptors (TAC3R and KOR), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility.²³⁶ Receptors for NKB are also located on GnRH neurons, where they seem to modulate GnRH release or transport.²³⁷ Loss-of-function mutations in *TAC3* and its receptor *TACR3* in patients with normosmic GnRH deficiency and pubertal failure²³⁸ have identified a role for this neuropeptide in the control of GnRH secretion. Although kisspeptin directly regulates GnRH expression and secretion, NKB agonists failed to stimulate GnRH release in rodents. It appears most likely that a collaborative mechanism that includes both kisspeptin and NKB signaling to GnRH neurons is necessary for reproductive function in females.^{239,240} To investigate the interactions of kisspeptin and NKB in humans,

the effects of the coadministration of kisspeptin-54, NKB, and an opioid receptor antagonist, naltrexone, on LH pulsatility were studied. Subjects receiving kisspeptin and naltrexone increased LH and LH pulsatility, whereas NKB alone did not affect gonadotropins. NKB and kisspeptin given together had significantly lower increases in gonadotropins compared with kisspeptin alone. These results suggest significant interactions between the KNDy neuropeptides on GnRH pulse generation in humans.²⁴¹ Further, *Tacr3* knockout mice are infertile,^{242,243} although they appear to have reversible central hypogonadism.²⁴⁴ Interestingly, a mutation in *TAC3R* was found in one patient with constitutional delay of growth and pubertal development (CDGP) in a study of 50 patients,²⁴⁵ whereas none have been reported in *TAC3*.

Disrupting mutations in makorin ring finger protein 3 (MKRN3), a paternally expressed, imprinted gene located in the Prader-Willi syndrome locus, are associated with central precocious puberty.²⁴⁶ This indicates the presence of a GnRH release-inhibiting pathway centered in the arcuate nucleus.

Initiation of puberty involves coordinated changes in transsynaptic and glial-neuronal communication.²⁴⁷ Mediating pubertal restraint are the major inhibitory systems: gamma-aminobutyric acid (GABA)ergic,²⁴⁸ some opioidergic contribution,²⁴⁹ and gonadotrophin-inhibiting hormone (GnIH), an RFamide-related peptide (RFRP).²⁵⁰ The major excitatory systems involve glutamate and kisspeptin signaling, with glial cells facilitating GnRH secretion in diverse ways (Fig. 16.16).^{236,247}

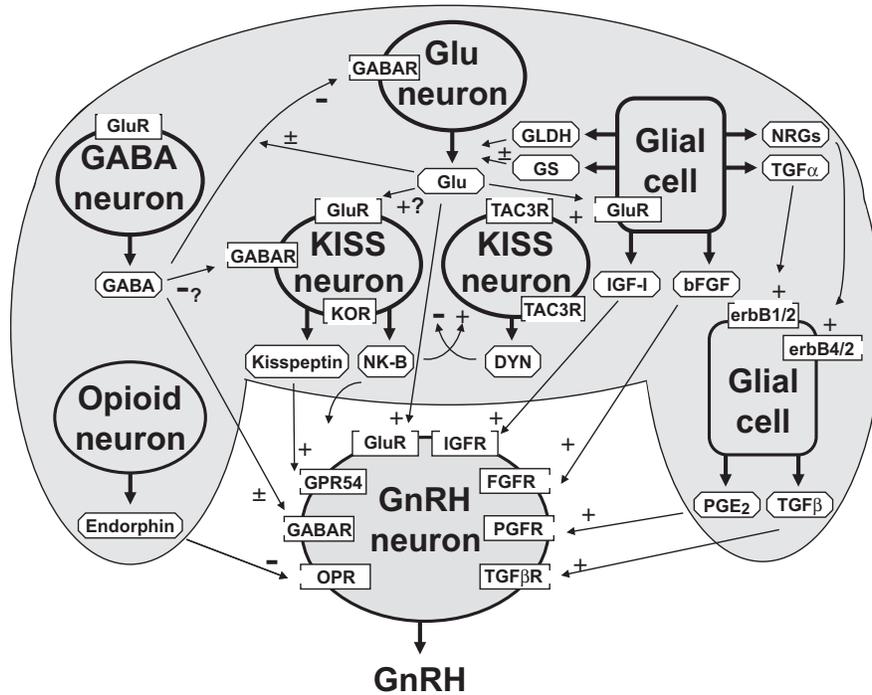


Fig. 16.16 The molecular biological basis for the major known proximate hypothalamic pathways regulating gonadotropin-releasing hormone (*GnRH*) secretion. The left-hand column depicts the major inhibitory pathways, which involve gamma-aminobutyric acid (*GABA*) signaling through the *GABA* receptor and opioidergic signaling through the endorphin receptor (*OPR*). The central column depicts the major excitatory pathways, which involve glutamate (*Glu*) signaling through the family of glutamate receptors and kisspeptin (*KISS*) signaling through *GPR54*. The right column shows the major glial factors that facilitate *GnRH* release. These include the elaboration of the enzymes glutamic dehydrogenase (*GLDH*) and glutamine synthase (*GS*), which regulate the concentration of glutamate and the elaboration of a variety of growth factors. Most kisspeptin neurons coexpress neurokinin B (*NK-B*), dynorphin A (*DYN*), and their receptors (*TAC3R* and *KOR*), the primary function of which seems to be synchronizing kisspeptin neuron pulsatility; receptors for NKB are also located on *GnRH* neurons. +, Positive stimulation; -, inhibition; ±, either; ?, unknown; *bFGF*, basic fibroblast growth factor; *erbB 1-4*, subunits for the *TGF-β* and *NRG* receptors; *IGF-1*, insulin-like growth factor 1; *NRG*, neuroregulins; *PGE*, prostaglandin E; *R*, receptor; *TGFβ*, tumor growth factor β. (Modified from Ojeda, S.R., Lomniczi, A., Mastronardi, C., et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology*, 147, 1166–1174).

It appears that GABA receptor signaling develops in advance of glutamate signaling.²⁵¹ Increased signaling via glutamate receptors of several types (ionotropic and metabotropic) appears to be the major proximate change in neurotransmission involved in puberty onset.^{217,218,247} At puberty, however, seemingly as a consequence of glutamate receptor signaling, GABA-A receptor signaling on GnRH neurons increases GnRH secretion.^{217,221,252,253} Glial cells facilitate the process through elaboration of TGFs (especially TGF- β 1), IGF-1, neuregulins, prostaglandin E₂, and the elaboration of enzymes that control the concentration of glutamate (glutamic dehydrogenase, which catalyzes the synthesis of glutamate, and glutamine synthase, which converts glutamate to glutamine).²⁵⁴

The basis of the change in neurotransmitter balance is becoming clearer. A second tier of control seems to be modulation of these processes by increased hypothalamic expression at puberty of tumor-suppressor genes that act to integrate glial-neuronal interactions. A yet higher echelon of candidate hypothalamic genes have been identified that are transcriptional regulators of the second-tier genes. These genes include *Oct-2*, a regulator of the POU-domain homeobox genes, enhanced at puberty 1 (*EAP1*), knock-out of which delays puberty and decreases fertility of mice, thyroid transcription factor I (*TTF1*), yin yang 1 (*YY1*), and *CUX1*.²⁵⁵ Genes contiguous to elastin appear to be involved in the pace of puberty: deletion of chromosome 7q11.23 in Williams syndrome typically leads to an early normal onset but rapid pace of puberty with an abbreviated pubertal growth spurt.²⁵⁶ Substantial redundancy of these networks and the signaling neurochemicals exists since the onset of puberty is dependent on the expression of many genes, likely arranged in a coordinated network. The gene products may function as activators or repressors of targets important for pubertal onset and progression. Sex steroids have been implicated as important modulators in pubertal onset.²⁵¹

MicroRNAs, specifically the miR-200/429 family and miR-155, have been shown to be important in the epigenetic regulation of puberty by regulating GnRH gene transcription.²⁵⁷ miR-7a2 is critical for normal pituitary development and deficiency results in gonadotropin deficiency.²⁵⁸

Thus the onset of puberty is controlled by an opposing increase in excitatory and a corresponding decrease in inhibitory signaling from neural networks targeting the GnRH neuron. Lesioning studies indicate that inhibitory tracts mainly seem to be routed through the posterior hypothalamus and stimulatory ones through the anterior hypothalamic preoptic area.^{1,259} These studies have been complemented by studies in genetically engineered mouse models. In one such model, the anteroventral periventricular nucleus (AVPV) population of neurons was shown to be the site of estrogen positive feedback in the control of pubertal progression, and kisspeptin cells in the arcuate nucleus of the hypothalamus were shown to be critical for estradiol negative feedback.²⁶⁰ Indeed, it appears that KNDy neurons integrate negative feedback of sex steroids to regulate GnRH secretion.^{261,262} Postmortem hypothalamic tissues were collected by The Netherlands Brain Bank, and sections were stained for kisspeptin by immunohistochemistry to determine the number of kisspeptin-immunoreactive neurons within the infundibular nucleus. This study showed that the number of kisspeptin neurons is greater in the infant/prepubertal and elderly periods compared with the adult period. In MTF transsexuals, but not homosexual men, female-typical kisspeptin expression was observed. The authors suggest that infundibular kisspeptin neurons are sensitive to circulating sex steroid hormones and that the sex reversal observed in MTF transsexuals might in part reflect an atypical brain sexual

differentiation.²⁶³ Neonatal androgenization, which ablates the ability to generate a midcycle LH surge, was shown to selectively inhibit development of the AVPV population of kisspeptin neurons.²³⁶

An overview of the systems involved in regulating the initiation of puberty is shown in Fig. 16.17. Pubertal maturation and skeletal maturation seem to have common determinants.

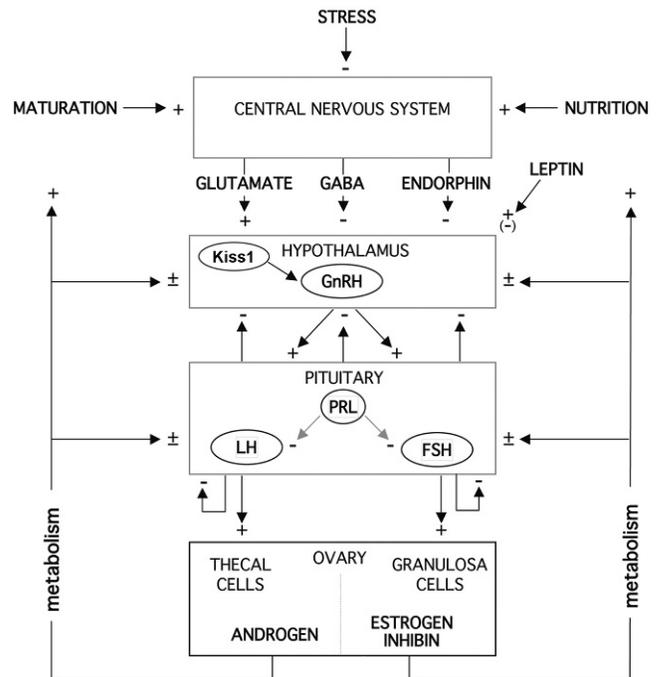


Fig. 16.17 Diagram of the major mechanisms controlling the development and function of sex hormone secretion by the unripe antral follicle. Regulation may be either stimulatory (+) or inhibitory (-). The central nervous system (CNS) influences kisspeptin (KISS1) and gonadotropin-releasing hormone (*GnRH*) secretion both negatively and positively. For the CNS to relinquish its inhibitory control over *GnRH* secretion, it must achieve a high level of maturity. Even after this is achieved, psychological or physical stress may negatively influence the system. Nutrition must be optimal. Leptin is a critical mediator of the nutrition effect. Sex steroids have a maturing effect. Whether efferent tracts from the hypothalamus to the cerebrum play a role in reproductive function is unknown. Pineal secretion of melatonin and other substances are known to exert inhibitory influences on *GnRH* in lower animals (not shown). Kisspeptin stimulates *GnRH*, which in turn stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Paracrine and autocrine feedback of the gonadotropins on *GnRH* release and on their own release, respectively, are shown. Prolactin (PRL) has multiple effects on gonadotropin secretion. In unripe antral follicles, LH acts on thecal and interstitial cells and FSH acts on granulosa cells. Androstenedione and testosterone secreted by the theca cells are aromatized by the granulosa cell, under the influence of FSH, to estradiol. The granulosa cell is also the site of production of the FSH inhibitor inhibin B. Estradiol has a biphasic effect on the mature pituitary and on hypothalamic *GnRH* release as well. Androgens seem normally to be of minor importance in regulating gonadotropin release in females. Intraovarian mechanisms seem to modulate LH action so as to coordinate thecal formation of androgens with granulosa cell formation of estrogens. Paracrine and autocrine factors, including insulin-like growth factors, are involved. GABA, Gamma-aminobutyric acid.

Abundant clinical evidence indicates that sex steroid hormones are among these determinants.^{264,265} Thus genes involved in sex steroid hormone metabolism and action are candidate regulators of the onset of puberty. There is limited and inconsistent data on the role of endocrine-disrupting chemicals on the timing of puberty, although some animal and epidemiological evidence supports the potential for some compounds to accelerate the time of pubertal onset and for others to delay the timing.^{190–192,266} Experience with diethylstilbestrol indicates that fetal exposures can have epigenetic effects.²⁶⁷ The growth hormone (GH)-IGF system is another determinant. GH facilitates the onset and tempo of puberty.²⁶⁸ Experimental studies suggest that this occurs through GH or IGF actions at all levels of the neuroendocrine-ovarian axis.^{269,270} Girls generally enter puberty when they achieve a pubertal bone age. Pubertal stage normally correlates better with the bone age ($r = 0.82$) than with the chronological age ($r = 0.72$, RLR unpublished data), particularly as menarche approaches.²⁷¹ Skeletal age correlates better with menarche than chronological age, height, or weight, and its variance at menarche is half that of chronological age.²⁷² The bone age at the onset of breast development averages about 10.75 years, and that at menarche averages about 13.0 years. Disorders that accelerate bone maturation, such as congenital adrenal hyperplasia (CAH) or hyperthyroidism, tend to advance the age of onset of true puberty.²⁷³ Disorders that retard skeletal maturation, such as GH deficiency, hypothyroidism, or anemia, tend to delay the onset of puberty.²⁷⁴ On the other hand, some data suggest that factors linked to intrauterine growth retardation, although not necessarily the growth retardation itself, predispose to sexual precocity.¹⁹⁰

Optimal nutrition is clearly necessary for initiation and maintenance of normal menstrual cycles. The hypothesis that body fat is the weight-related trigger for pubertal development originated with the discovery by Frisch and coworkers that weight correlated with initiation of the pubertal growth spurt, peak growth velocity, and menarche better than chronological age or height.²⁷⁵ Midchildhood may be a critical period for weight to influence the onset of puberty.¹⁹⁰ Suboptimal nutrition related to socioeconomic factors is an important factor in the later onset of puberty in underdeveloped than in developed countries.¹⁹⁰ Conversely, obesity appears to be an important factor in advancing the onset of puberty in the United States.²⁷⁶ Some of the obesity effect may be mediated by IGF-1 and adrenal androgen.²⁷⁷

Leptin appears to be an important link between nutrition and the attainment and maintenance of reproductive competence.^{218,278,279} Leptin deficiency causes obesity and gonadotropin deficiency. Paradoxically, prolonged leptin excess can downregulate the leptin receptor and GnRH release.²⁸⁰ Leptin is secreted by white adipose cells, acting on the hypothalamus to reduce appetite and stimulate gonadotropin secretion.²⁸¹ A critical threshold level appears to signal that nutritional stores are sufficient for mature function of the GnRH pulse-generator and, thus to be permissive for puberty. Blood leptin levels rise throughout childhood and puberty to reach higher levels in girls than boys²⁸² and are positively related to adiposity and negatively related to testosterone levels.²⁸³ Leptin binding protein, a truncated form of the leptin receptor, falls as puberty begins, which suggests that circulating leptin becomes more bioavailable.²⁸² Whether leptin has a direct role in the pubertal activation of the GnRH pulse generator is unknown. In models of leptin insufficiency, the administration of kisspeptin induced LH secretion.²²⁷ Conversely, leptin's effect on puberty did not require signaling in kisspeptin neurons in other mouse models.²⁸⁴

Other factors also link nutrition and gonadotropic function. Part of the leptin effect is mediated by inhibition of

hypothalamic neuropeptide Y (NPY) formation.²⁸⁵ NPY is a potent appetite-stimulating member of the pancreatic polypeptide family that directly inhibits GnRH release during food deprivation.²⁸⁵ However, in the preovulatory state, it stimulates GnRH release,²⁸⁶ an effect mediated by a different neural network acting on a different NPY receptor subtype on the GnRH neuron.²⁸⁷ NPY is also inhibited by the anorexigenic peptide YY (PYY), a gut hormone secreted in response to food and inhibited by GH; the pubertal fall in PYY has been postulated to permit the coordinated pubertal rise in appetite and gonadotropins.²⁸⁸ Insulin may also signal nutritional status to KNDy neurons, since deletion of the insulin receptor in KNDy neurons in genetically modified mice resulted in pubertal delay and reduced serum LH levels in both sexes. Interestingly, adult fertility was not affected.²⁸⁹

GWAS studies of pubertal timing implicated several genes associated with body weight other than leptin and leptin receptor and include fat mass and obesity-associated protein (*FTO*), SEC16 homolog B (*SEC16B*), transmembrane protein 18 (*TMEM18*), and neuronal growth regulator 1 (*NEGR1*).²¹¹ Rare heterozygous variants in *FTO* have been identified in pedigrees with CDGP associated with low body mass index (BMI) and growth and pubertal delay.²⁹⁰ Mice made heterozygous for the *FTO* gene knockout displayed delayed puberty, but did not manifest low body mass. Other mediators linking nutrition and puberty include melanocortin (MC)3/4 receptors, signaling from alpha-melanocyte-stimulating hormone (MSH) to increase *Kiss1* expression and mediate the permissive effects of leptin on puberty,²⁹¹ and ghrelin and mutations in the ghrelin receptor growth hormone secretagogue receptor (*GHSR*).^{292,293} A small cohort of 31 CDGP patients was analyzed for mutations in *GHSR*, and 5 patients were found to have point mutations in this gene.²⁹⁴

Other cues that provide information on nutritional status to the central reproductive axis may include glucose,²⁹⁵ ghrelin,²⁹⁶ and insulin.²⁹⁷ The effect of these factors on LH pulsatility may be mediated directly at the level of the gonadotroph or indirectly by changes in GnRH secretion. There is little evidence for the role of pineal secretions in human reproduction that is found in lower animals.^{298,299}

The essential element for the onset of puberty is an increase in pulsatile hypothalamic GnRH secretion that is regulated by a complex interplay of excitatory and inhibitory signals that have yet to be fully understood or elucidated.²⁴⁷ During childhood the activity of the GnRH pulse generating system is restrained, an awakening of the pulse generator occurs gradually during late childhood, and the tempo of GnRH neuronal activation increases during puberty. The underlying mechanisms for all these changes are unclear. The pubertal diminution in tone of the CNS centers that inhibit hypothalamic GnRH secretion during childhood has traditionally been considered to result from decreasing sensitivity of a "gonadostat" to negative feedback by sex steroids.^{6,300} However, this now seems an overly simplistic concept for a mechanism that seems to involve a change in the balance of neural inhibitory and stimulatory signals that impinge upon the GnRH neuron.

Many studies have been performed to help understand the initiating developmental events or the "trigger" for pubertal onset. In fact, it is becoming increasingly clear that there is no single "trigger" for puberty, but a gradual increase in GnRH pulsatility associated with a complex interplay of factors and hypothalamic developmental programs. Thus the apparent "sensitivity of the gonadostat" seems increasingly likely to reflect the degree of activity of the GnRH neuron. That is, when GnRH secretory activity is attenuated, the pulse generator is easily inhibited; when the GnRH neuron is active, the pulse generator is relatively insensitive to negative feedback.

The integration of hypothalamic signaling systems along with the developmental changes in the control of GnRH neuronal function seem to converge to trigger the onset of puberty. In the rat, structural remodeling of the GnRH neuron was demonstrated during pubertal progression by an increase in the density of dendritic and somal spines; the percentage of total neurons with spines being lowest at birth and increased gradually postnatally until puberty.³⁰¹ The spiny processes of neurons are the location of excitatory synapses important in neuronal plasticity. The greatest percentage of complex neurons is in the peripubertal period, with the percentage decreasing after completion of puberty.³⁰² These developmental changes are correlated with an increase in excitatory synaptic input to the GnRH neuron triggering the onset of puberty in mice.^{302,303} Which excitatory synaptic input (e.g., glutamatergic, kisspeptinergic, or yet unknown neurochemical signals) plays a role in the pubertal increase in GnRH secretion is unknown. Whether primate or human GnRH neurons undergo synaptic excitatory remodeling during development is also unknown.³⁰⁴

Since its discovery, numerous studies have demonstrated the expression of the kisspeptin-signaling system in several peripheral sites implicating it in biological processes, such as the regulation of ovarian function, embryo implantation, placentation, angiogenesis, and insulin secretion. However, whether kisspeptin is secreted from sites of peripheral expression and the impact on the reproductive axis are currently unclear.^{305,306}

Regulation of Gonadotropin Secretion

An essential feature of the mature HPG axis is the long-loop, negative-feedback control of gonadotropin secretion by gonadal secretory products, as depicted in Fig. 16.17. The generally tonic nature of gonadotropin secretion is punctuated by two prominent types of periodicity: two- to threefold pulsations of LH above trough levels at 1.5-hour intervals and, in the sexually mature female, by a transient, midcycle, preovulatory gonadotropin surge. The latter is characterized by a greater than 10-fold, rapid rise of LH and a lesser rise of FSH. This surge

is brought about by positive feedback when a critical level of estradiol, facilitated by a modest rise in progesterone, is achieved for a critical period of time, as discussed in relation to Fig. 16.13.

Estradiol, in concert with inhibin, reciprocally regulates FSH in a sensitive, log-dose, negative-feedback loop.³⁰⁷ Progesterone in high (luteal phase) concentrations is a major negative regulator of GnRH-LH pulse frequency.¹²⁹ Androgens have a biphasic long-loop feedback relationship with gonadotropins: at modest elevations they stimulate gonadotropin release and at very high levels they inhibit it.³⁰⁸

Estradiol exerts triphasic, and progesterone biphasic, effects on gonadotropin secretion. As estradiol rises after the midpoint of the follicular phase it selectively reduces the FSH response to GnRH, and when it reaches preovulatory levels it transiently exerts positive feedback effects on LH and, to a lesser extent, FSH.³⁰⁹ At sustained high levels estradiol suppresses both gonadotropins. As progesterone reaches a preovulatory level, it enhances the estradiol positive feedback effect, but at the higher levels that ensue during the luteal phase, it suppresses LH pulse frequency while enhancing LH pulse amplitude.¹²⁹

The GnRH neurons primarily responsible for maintenance of the reproductive cycle are those of the arcuate (infundibular) nucleus (Fig. 16.18).⁹⁰ GnRH neurons are inherently pulsatile.³¹⁰ Synchrony is promoted by fluxes of ionic calcium into these cells and autocrine GnRH inhibitory feedback. GnRH secretion is modulated by the variety of neurotransmitters and growth factors involved in initiating puberty.²⁴⁷ Synchrony of the network of GnRH neurons that accounts for pulsatility is conferred when the hypothalamic concentration of GABA periodically falls from levels inhibitory to GABA receptors in the presence of an excitatory neurotransmitter.^{311,312} EAP1, a hypothalamic protein previously shown to be important for pubertal onset, has also been implicated in the control of menstrual cyclicity in primates.³¹³

Sex steroid signals are in part conveyed to GnRH neurons indirectly. Regulation of GnRH secretion by estrogen involves

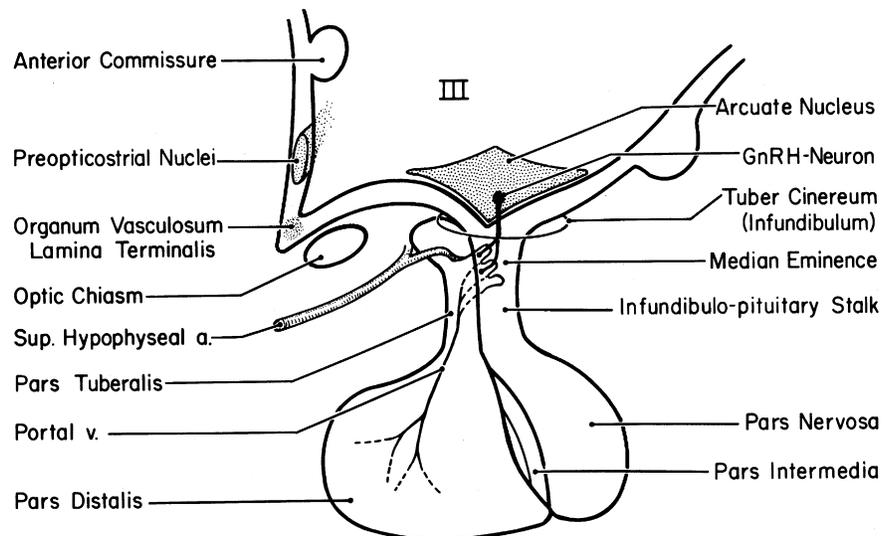


Fig. 16.18 The location of major gonadotropin-releasing hormone (GnRH)-containing neurons (shaded) in relation to the hypothalamus and pituitary gland. The neurons are of greatest density in the arcuate nuclei and in the periventricular wall of the medial basal hypothalamus. These neurons project to the adjacent median eminence, the second most dense population of GnRH neurons lies in the preopticostrual area. The development of some is altered by early androgenization. Some are connected by the stria terminalis to the amygdalae. Other projections from this area appear to connect indirectly with the median eminence, perhaps via the organum vasculosum lamina terminalis—a midline structure that resembles the median eminence. The pituitary portal veins transport blood rich in releasing factors to sinusoids engulfing anterior pituitary cells.

in part induction of PRs in the hypothalamus.^{129,314} GnRH neuronal cell lines have been studied in which estradiol directly stimulates and inhibits GnRH gene expression under different experimental conditions.^{315,316} Although progesterone exerts its main inhibitory effect on GnRH secretion, it has effects at higher CNS levels and at the pituitary level.^{317–319} Prolactin suppresses both hypothalamic and gonadotropin GnRH receptor expression.^{320,321}

Other clinically relevant factors affecting GnRH release are sleep, endorphins (endogenous opioids), and interleukins. In sexually mature women, sleep inhibits GnRH pulse frequency and this effect seems to be amplified by female hormones.³²² Endorphins are important physiological regulators of GnRH release after puberty has begun. Hypothalamic β -endorphin suppresses oophorectomy-initiated GnRH secretion, and opiate antagonists reverse this effect, as well as the sleep effect. The inhibitory effect of stress on gonadotropin release appears to be mediated by β -endorphin released from proopiomelanocortin in response to corticotropin releasing hormone (CRH).³²³ Interleukins also inhibit gonadotropin release.³²⁴ Serotonin seems to modulate LH pulsatility and facilitate the LH surge.³²⁵

GnRH receptors on the gonadotroph are maintained in an optimally active state only when GnRH is delivered in pulses approximately 1 to 2 hours apart in man.^{90,326} Pulses substantially less frequent result in a hypogonadotropic state. Paradoxically, continuous administration of an initially stimulatory dose of GnRH results in downregulation of gonadotropin production, after an initial burst of gonadotropin release.³²⁷ This is the physiological basis for the success of long-acting gonadotropin agonists in suppressing puberty in children with true central precocious puberty. However, while gonadotropins are downregulated, free alpha-subunit production is elevated and responsive to GnRH.

Hypothalamic GnRH receptor function is modulated by autocrine and paracrine factors, including GnRH itself and kisspeptin.⁵ Pituitary GnRH receptors appear to be directly and indirectly downregulated by GnRH, gonadotropins, and inhibins, as well as sex steroids.³²⁸ LH and FSH themselves inhibit GnRH release (short-loop feedback) and inhibit their own release (autocrine feedback).^{328,329}

How is differential regulation of gonadotrope LH and FSH release accomplished in response to a single GnRH pulse? The frequency of the GnRH pulse is one determinant. An increased frequency of this signal stimulates LH β -subunit gene expression, whereas slowing this signal stimulates FSH β -subunit and suppresses follistatin gene expression, altering the FSH/LH ratio.³³⁰ Pituitary adenylate cyclase activating polypeptide amplifies LH responses to GnRH while blocking its effect on FSH.³³¹

The sex hormone milieu is also clearly a major differential modulator of gonadotrope LH and FSH release.^{90,319,332–334} FSH is more sensitive than LH to inhibition by estrogen; this effect of modest levels of estradiol is of rapid onset and sustained. LH is the more sensitive to the stimulatory effects of higher estradiol levels; this effect is of later onset and short-lived. Similar relationships pertain in aromatase null mice. ER null mice have revealed ER-alpha as the predominant receptor isoform that conveys negative feedback regulation to the gonadotroph.³³⁵ Progesterone exerts both negative and positive feedback effects at the pituitary level, and these effects are antagonized by androgen. The progesterone metabolite 3α -hydroxyprogesterone suppresses FSH release.³³⁶

Androgens have complex effects on gonadotropin dynamics. Normal androgen action facilitates the midcycle gonadotropin surge in response to positive feedback.^{337,338} Elevated testosterone increases baseline LH pulse amplitude and frequency^{64,339,340} while inhibiting the capacity to mount the

gonadotropin surge.³³⁷ These actions appear to result from antagonizing progesterone action.^{16,339,340}

Inhibins of gonadal origin seem to be the major nonsteroidal-specific negative feedback regulator of pituitary FSH synthesis and secretion.^{341,342} Inhibins inhibit FSH release at the pituitary level, but they may act at a higher level as well.³⁴³ Serum levels of both inhibins rise upon FSH stimulation.^{177,178} Inhibin-B, produced by small antral follicles in response to FSH, is virtually the only inhibin moiety in blood during puberty. Its blood levels rise during the early follicular phase and then fall thereafter except for a small postovulatory peak, generally paralleling the changes in serum FSH; the latter peak may function to attenuate the FSH surge. Serum inhibin-A, a marker of the preovulatory follicle and corpus luteum, begins to rise in the late follicular phase and thereafter parallels levels of progesterone; its fall late in the luteal phase appears to contribute to the early follicular phase rise in the FSH level.

The structurally related activins seem to be important as regulators of both pituitary and ovary function.³⁴⁴ Activin is formed by gonadotropes themselves and its primary role is to stimulate FSH release. It also upregulates the activin binding protein follistatin, which arises within folliculostellate cells of the anterior pituitary.²⁸⁵ Follistatin, by competitively inhibiting binding of activin to its receptor, specifically inhibits activin stimulation of FSH secretion.³⁰⁷

Infant and Child

Neuroendocrine Unit. The hypothalamic-pituitary-gonadal (HPG) axis is transiently active during the neonatal period. This is sometimes termed the *minipuberty of the newborn*; unlike true puberty, the clinical manifestations are only nascent and do not progress. The regulation of neonatal gonadotropin secretion, like that during puberty, is incompletely known.

Serum FSH and LH are low in cord blood and remain low until estrogen concentrations fall from inhibitory levels upon disruption of the fetoplacental unit at birth. Then the LH and FSH levels of neonates promptly begin to rise in pulsatile fashion to early pubertal levels in the first week of life (see Fig. 16.5).^{7,11,58–61}

Serum LH and FSH levels rise higher in female than in male premature infants, reaching into the postmenopausal range.^{17,62} This sexual dimorphism seems to be related to lack of negative feedback because of lagging ovarian follicular development: antral follicle development begins near term gestational age.¹⁸ There is parallel hyperprolactinemia without sexual dimorphism.⁶³

At their peak between term and 4 months of age, serum gonadotropins and LH/FSH ratios are lower in girls than in boys,¹⁷ apparently because girls lack androgen-programmed accentuation of GnRH pulsatility.^{16,64,65} Responses to GnRH and GnRH agonist are similar to those of early puberty (see Fig. 16.6).^{55,66–69} In congenital agonadism, gonadotropins reach postmenopausal levels during the neonatal period.⁷⁰

After about 4 months postterm, gonadotropin and prolactin levels begin to gradually fall into the prepubertal range (see Fig. 16.5). FSH is higher in girls than in boys, a tendency that tends to persist into early childhood.^{58,71} This appears in part related to negative feedback by the higher activin-A and lower inhibin-B serum levels of girls than boys.⁷² GnRH secretion also appears to be greater in girls than in boys at this time.⁷³

The decline in gonadotropins may in part be related to the maturation of neural tracts that conduct inhibitory signals from the CNS and/or to an increase in hypothalamic sex steroid receptors. Hypothalamic ERs increase in a pattern reciprocal to the fall in serum gonadotropins in the rat (see Fig. 16.7),⁷⁴ as do hypothalamic DHT receptors.⁷⁵ Increasing sensitivity of the hypothalamus to sex steroid hormone negative feedback

could account for the inhibitory effect of the small amounts of circulating estradiol and testosterone.

A nadir in both serum gonadotropins occurs by about 6 years of age (see Figs. 16.2 and 16.5). At this age, the LH and FSH response to GnRH is minimal, apparently from lack of GnRH stimulation. Furthermore, at this stage, agonadism is seldom reflected in a rise in serum gonadotropins or gonadotropin reserve.⁷⁰

However, gonadotropin production is not completely suppressed in midchildhood. Gonadotropins have been detected in the urine of young prepubertal children, at the limits of sensitivity of classic bioassays: LH excretion averaged 3% and FSH 15% of the adult amounts.⁷⁶ Specific monoclonal antibody-based assays have revealed that LH falls to less than 0.2 U/L during the day whereas FSH remains detectable and that the gonadotropins produced at this stage are secreted in micro-pulses that approximately double in association with sleep.⁷⁷ The gonadotropins also appear to be bioactive judging from their sensitivity to estradiol negative feedback in the primate⁷⁸ and the active formation of antral follicles during childhood, which indicates gonadotropin stimulation, as discussed in the following section on the adult.

Between 7 and 10 years of age, even prepubertal girls experience subtle but significant increases in gonadotropin levels.⁷⁹ This change corresponds with rising secretion of GnRH.⁷³ These data indicate that the hormonal secretory pattern of the prepubertal 10-year-old child is different from that of the 7-year-old and indicate that the hormonal changes signaling the development of puberty are found late in the first decade of life, antedating by some time the development of secondary sex characteristics.

Ovary. The ovary of the infant and child is not quiescent. Initiation of growth and development of resting follicles occurs throughout childhood. The neonatal ovary typically contains an antral follicle with thecal luteinization,^{80,81} and the number of antral follicles approximately doubles over that in infancy by 7 years and quadruples by 9 years (see Fig. 16.3).³¹ All these antral follicles normally undergo atresia in childhood, and this augments the amount of stroma.³¹ As a result, by midchildhood, the ovaries of normal girls have up to five antral follicles 4 to 9 mm in diameter, and ovarian volume increases up to approximately 3.5 cc. Ovarian follicular development begins to accelerate just before the onset of clinical signs of puberty.^{31,82–86}

During the first few months of life, early pubertal blood levels of ovarian hormones are found as part of the transient activation of the HPG axis that occurs in the newborn. Serum estradiol and inhibin-B levels parallel those of FSH. In the neonatal period they begin rising to early pubertal levels, remain there for the first few months of life, and fall to low levels during childhood (see Fig. 16.8).^{66,69} Specifics about the hormonal changes are discussed later (see Normal Hormonal and Sexual Developmental Stages).

Regulation of Ovarian Secretion

Ovarian secretion results from the combined actions of LH and FSH, as discussed earlier with regards to Figs. 16.13 and 16.14. The early follicular phase follicle functions according to the two-cell, two-gonadotropin model illustrated in Fig. 16.19.^{144,345,346} In response to LH, androstenedione, the most abundant steroid formed in the ovary, is secreted by the theca-interstitial-stromal (thecal) cell compartment. In response to FSH regulation, aromatase then forms estrogen from precursor androstenedione in granulosa cells. FSH also stimulates granulosa cells to secrete inhibins. As by-products of the secretion of both ovarian estradiol and adrenal cortisol, androgens do not normally contribute to negative feedback regulation of gonadotropins. However,

they have a biphasic effect on gonadotropin secretion: modest elevations increase GnRH pulse frequency by interfering with progesterone negative feedback and very high levels directly inhibit gonadotropin secretion.³⁰⁸

The regulation of the intraovarian androgen concentration is critical to ovarian function.^{40,144} Androgens are important for ovarian function. Androgens are obligate substrates for estradiol biosynthesis. Androgens also increase recruitment of primordial follicles into the growing follicle pool¹⁴² and then act in conjunction with gonadotropins on granulosa cells to stimulate preantral follicle development into small antral follicles,³⁴⁷ which enhances FSH upregulation of aromatase activity.¹⁴¹ Androgens also synergize with FSH to luteinize follicles by inducing LH receptors. However, in excess androgens impair selection of the dominant follicle of women; this appears likely to result from premature luteinization of follicles, thus committing the follicle to atresia. Therefore androgen synthesis must be kept to the minimum necessary to optimize follicular development. This means that the synthesis of ovarian androgens must be coordinated with the needs of the follicle. This is achieved by intraovarian intracrine, autocrine, and paracrine modulation of LH action (see Fig. 16.19).

LH stimulates theca cell development and steroidogenesis and is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion. However, once adult LH levels are achieved, further LH increase normally has little further effect on androgen levels because excess LH causes homologous desensitization of theca cells.^{144,348} Desensitization involves downregulation of LH receptor expression and steroidogenesis. Because steroidogenic downregulation is primarily exerted on 17,20-lyase activity, which converts 17-hydroxycorticoids to 17-KS, 17-OHP levels rise in response to increased LH levels, but the rise in androgens is limited.³⁰⁸

A model of the intraovarian interaction among the major regulators of steroidogenesis is shown in Fig. 16.19.¹⁴⁴ Stimulation of androgen secretion by LH appears to be augmented by specific intraovarian FSH-dependent factors, such as inhibins and IGFs. These processes seem to normally be counterbalanced by other FSH-dependent processes that downregulate androgen formation as LH stimulation increases. Androgens and estrogens themselves seem to mediate at least a portion of this desensitization to LH, with estrogens being critical through an ER α -dependent mechanism.^{335,349}

Insulin and IGFs are important coregulators of ovarian function. Insulin upregulates theca cell LH receptor sites and action⁴⁰ and to a lesser extent estrogen biosynthesis in response to FSH.³⁵⁰ The entire IGF system is represented in the ovary: IGF-1 augments FSH receptor expression and action.^{153,351} and appears to mediate GH promotion of granulosa cell steroidogenesis.^{352,353} Insulin is equipotent with IGF-1 in stimulating thecal androgen biosynthesis,⁴⁰ and, although insulin can act through hybrids of the insulin and IGF-1 receptors³⁵⁴ and at very high levels interacts with the IGF-1 receptor,^{40,355} it appears to primarily act through its own receptor.

Androgen-expressing steroidogenic cells express a previously unrecognized protein variant, DENND (differentially expressed in normal and neoplastic development) 1A.V2, that facilitates steroidogenesis: it upregulates basal and cAMP-stimulated cytochrome P450c17 and side chain cleavage activities.^{356,357} The mechanism by which DENND1A.V2 regulates steroidogenesis is currently unknown. DENND1A is a member of the connectenn family of proteins, which are involved in protein trafficking, endocytotic processes, and receptor recycling. Thus it is tempting to speculate that it acts by upregulating LH receptor signaling.

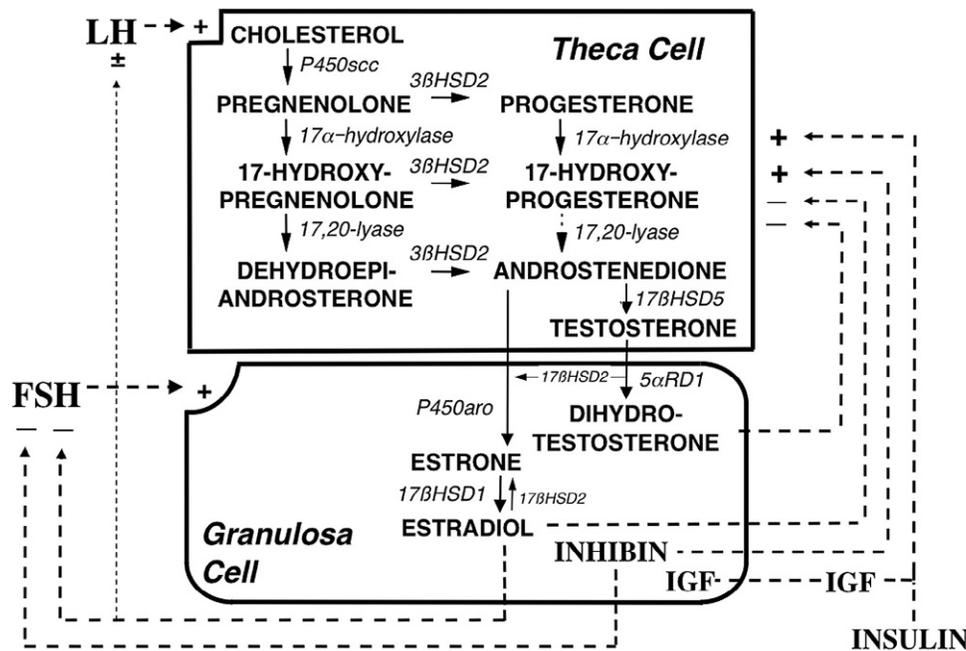


Fig. 16.19 Depiction of the organization and regulation of the major steroid biosynthetic pathways in the small antral follicle of the ovary according to the two-gonadotropin, two-cell model of ovarian steroidogenesis. Luteinizing hormone (*LH*) stimulates androgen formation within theca cells via the steroidogenic pathway common to the gonads and adrenal glands. Follicle-stimulating hormone (*FSH*) regulates estradiol biosynthesis from androgen by granulosa cells. Long-loop negative feedback of estradiol on gonadotropin secretion does not readily suppress *LH* at physiological levels of estradiol and stimulates *LH* under positive feedback circumstances. Androgen formation in response to *LH* appears to be modulated by intraovarian feedback at the levels of 17-hydroxylase and 17,20-lyase, both of which are activities of cytochrome P450c17 that is expressed only in theca cells. The relative quantity of androstenedione formation via 17-OHP (dotted arrow) in the intact follicle is probably small, as is the amount of progesterone formed from granulosa cell P450_{scc} activity in response to *FSH* (not shown). 17βHSD2 activity is minor in the ovary, and estradiol is primarily formed from androstenedione. Androgens and estradiol inhibit (–) and inhibin, insulin, and insulin-like growth factor-I (IGF) stimulate (+) 17-hydroxylase and 17,20-lyase activities. The sites of aromatase and IGF gene expression appear to vary with the stage of follicular development. Other peptides also modulate the steroidogenic response to *LH*. Pertinent enzyme activities are italicized: the 17-hydroxylase and 17,20-lyase activities of P450c17 are shown, otherwise enzyme abbreviations are as in the text. *5α-R*, 5α-Reductase; *17β-HSD5*, type 5 17β-HSD; *HSD*, hydroxysteroid dehydrogenase. (Modified with permission from Ehrmann et al, Polycysticovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*. 1995;16:322–353. Reproduced with permission from Rosenfield, R.L., Ehrmann, D.A. (2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*, 37, 467–520.)

Many other peptides modulate ovarian cell growth or function in response to gonadotropins.^{144,348} Inhibin stimulates ovarian androgen production, whereas androgens reciprocally stimulate ovarian inhibin production. Activin opposes the inhibin effect. A variety of other ovarian peptides are also capable of modulating thecal androgen synthesis.¹⁴⁴ Stimulators include catecholamines, for which an intraovarian system exists,³⁵⁸ prostaglandin, and angiotensin. Inhibitors include leptin, CRH, epidermal growth factor (EGF), tumor necrosis factor, TGF-β, and GDF9.³⁵⁹ Leptin antagonizes IGF-1 effects.³⁶⁰ TGF-β is particularly interesting because it suppresses androgen biosynthesis and stimulates aromatase activity; it also stimulates meiotic maturation of the oocyte.³⁶¹ Other peptides acting on granulosa cells include cytokines, which have diverse effects, and AMH, which inhibits aromatase.³⁶² GnRH is also capable of modulating thecal steroidogenesis. A GnRH-like protein has been described in the ovary that may act through ovarian GnRH receptors to suppress steroidogenesis in the human ovary.^{363,364} It inhibits *FSH* induction of progesterone secretion, aromatase activity, and *LH* receptors in granulosa cells, downregulates *LH* receptors, and inhibits the *hCG* stimulation of progesterone secretion by luteal cells.^{115,134}

Prolactin has complex effects on steroidogenesis. In low concentrations, it enhances ovarian estradiol and progesterone secretion by increasing *LH* receptors.³⁶⁵ On the other hand, high levels of prolactin inhibit ovarian estradiol and progesterone biosynthesis.³⁶⁶ Prolactin also stimulates adrenal androgen production.³⁶⁷

Adrenarche and the Regulation of Adrenal Androgen Secretion

Adrenarche denotes the onset of the increase in adrenal androgen production that gradually begins in midchildhood well before the pubertal maturation of the neuroendocrine-gonadal axis.^{40,368} Adrenarchal androgens contribute to the appearance of pubic hair (pubarche) and sebaceous gland and apocrine gland development.

Adrenarche results from a change in the pattern of adrenal secretory response to ACTH (Fig. 16.20). It is characterized by disproportionate rises in the responses to ACTH of the Δ⁵-3β-hydroxysteroids 17-hydroxypregnenolone and dehydroepiandrosterone (DHEA), whereas cortisol secretion does not change. Dehydroepiandrosterone sulfate (DHEAS) is the predominant marker for adrenarche. A DHEAS level over 40 mcg/dL is usually considered adrenarchal. Other serum androgens and precursors are ordinarily at the upper end of the prepubertal range at the onset of adrenarche.

Adrenarche reflects the development of the adrenocortical zona reticularis.⁴⁰ Humans and some higher primates are unique in having an adrenal zone with similar structure-function-developmental stage relationships.^{369,370} Although the zona reticularis resembles the fetal zone of the adrenal cortex in its location and function, it appears to originate from stem cells located in the outer definitive zone of the fetal adrenal gland.^{371,372} This zone becomes continuous at about 5 years of age and enlarges steadily over the subsequent decade. Its increasing development correlates with DHEAS levels.

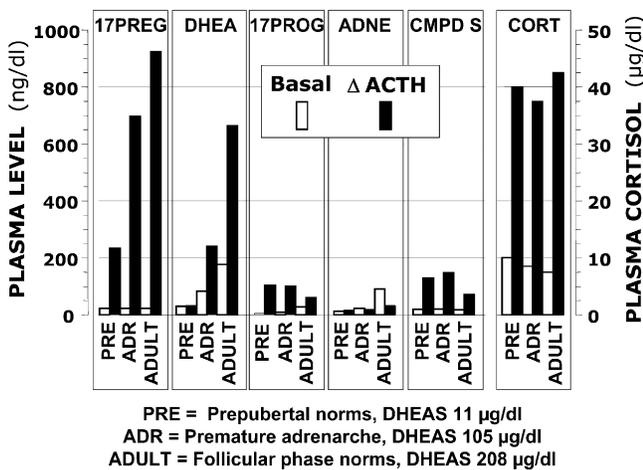


Fig. 16.20 Changing pattern of adrenal steroidogenic response to adrenocorticotrophic hormone with maturation. Shown are plasma steroid levels before (basal, 8:00 a.m. after dexamethasone 1 mg/m²) and the rise (Δ) 30 minutes after cosyntropin (ACTH) administration (10 μ g/m²) in healthy prepubertal children, children with premature adrenarche as an isolated phenomenon, and follicular phase adult women. Note that 17-hydroxypregnenolone (17PREG) and dehydroepiandrosterone (DHEA) responses of children with premature adrenarche are intermediate between prepubertal and adult responses. 17PROG, 17-hydroxyprogesterone; ADNE, androstenedione; CMPD S, 11-deoxycortisol; CORT, cortisol; DHEAS, dehydroepiandrosterone sulfate. (Data from Rich, B.H., Rosenfield, R.L., Lucky, A.W., Helke, J.C., Otto, P. (1981). Adrenarche: Changing adrenal response to adrenocorticotropin. *J Clin Endocrinol Metab*, 52, 1129.)

This zone's secretion pattern results from a unique enzyme expression profile: it expresses low 3 β -hydroxysteroid dehydrogenase type 2 (HSD3B2), but high cytochrome b5 (an enhancer of the 17,20-lyase activity of cytochrome P450c17) and steroid sulfotransferase (SULT2A1) activities.⁴⁰ The high secretion of DHEA and DHEAS is primarily attributable to these activities.⁴⁰ Enhanced expression of 17 β -hydroxysteroid dehydrogenase type 5 (HSD17B5) by this zone accounts for the small but significant adrenal contribution to testosterone secretion. Both testosterone and androstenedione are further metabolized by zona reticularis 11 β -hydroxylase type 1 (CYP11B1), which underlies adrenal 11 β -hydroxyandrostenedione and 11 β -hydroxytestosterone secretion.^{373,374} These are further metabolized, primarily in the periphery, by 11 β -hydroxysteroid dehydrogenases to their 11-keto congeners. These 11-oxy metabolites of testosterone are one-fifth as potent as testosterone at half-maximal dosage (ED₅₀).³⁷³

The factors causing and regulating zona reticularis development are unclear. Body growth is related to adrenarche.^{375–378} Insulin, IGF-1, and leptin have been suggested as determinants of this relationship. Insulin and IGF-1 stimulate expression of adrenal P450c17 and 3 β HSD2 activities^{379,380} and may be involved in progenitor cell proliferation.³⁸¹ Leptin, an adipocyte hormone, stimulates the 17,20-lyase activity of adrenocortical cells, which shunts adrenal steroidogenesis toward DHEAS production.³⁸²

Nutritional status, in particular, seems to play a role in the development of adrenarche, particularly in girls.^{376,383} Infants born small for gestational age have higher DHEAS levels at 5 to 8 years of age, and children born large for gestational age have lower levels than those with normal birth weight.³⁸⁴ Obesity is related to DHEAS levels, and rapid weight gain during early childhood is associated with adrenal androgen levels independently of birth weight.³⁸³

A pituitary hormone ("adrenarche factor") may well be required to bring about their adrenarchal development.⁴ It has been postulated to be an ACTH-related hormone distinct from ACTH because adrenal androgen production is more sensitive to glucocorticoid suppression than is cortisol production,³⁸⁵ falls more slowly than cortisol after dexamethasone administration,¹²⁵ and rises more sluggishly after its withdrawal.³⁸⁶ Candidates for a dexamethasone-suppressible adrenarchal factor include pro-ACTH-related peptides and CRH, but the data have not been convincing.³⁸⁷ Prolactin seems to be required.^{40,388} Currently the only established adrenal androgen-stimulating hormone in postnatal life is ACTH. Because the adrenarchal secretion pattern represents a change in the pattern of steroidogenic response to ACTH, an adrenarche factor need only control the growth and differentiation of zona reticularis cells or regulate their unique pattern of steroidogenic enzyme expression.

ACTH effects on adrenal androgen production are modulated by diverse signaling networks.^{40,389} Modulators of the androgenic response to ACTH include a stimulatory isoform of DENND1A (DENN/MADD domain-containing protein 1A; DENND1A.V2) that is known to be overexpressed in polycystic ovary syndrome (PCOS) theca cells, and BMP4, which is inhibitory. Intraadrenal cortisol may participate in the regulation of adrenal DHEA secretion through inhibition of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) activity.³⁷⁸ In addition, interleukin-6 is strongly expressed in the zona reticularis, where it directly stimulates production of all classes of adrenal steroids independently of ACTH.^{390,391} Although gonadal dysgenesis is associated with earlier adrenarche,³⁹² paradoxically, ovariectomy precipitates an early decline in DHEAS levels that is not reversed by estrogen replacement.³⁹³

Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development, apocrine gland development, and the growth of pubic hair. Sulfation of DHEA within the adrenal cortex prevents adrenal hyperandrogenism,³⁹⁴ and circulating DHEAS is a precursor for ovarian testosterone formation.³⁹⁵ It has been proposed that DHEAS elevation in response to obesity exerts a protective effect on plasma lipids.³⁹⁶ Whether adrenarche plays a more fundamental role in normal puberty is not established.³⁶⁸

There has been considerable interest in the possibility that adrenarchal steroids play a role in human neurobiological development. DHEA and testosterone serum levels differentially correlate with specific structural developmental changes in the cerebral corticolimbic system.³⁹⁷ DHEAS and its precursor, pregnenolone sulfate, as well as the progesterone metabolite allopregnanolone, have direct nongenomic neuroactive effects, which include modulation of neurotransmitter signaling and neuroplasticity.^{398–400} These steroid sulfates are actively transported across the blood-brain barrier.⁴⁰¹ The association of adrenarchal changes with the emergence of sexually dimorphic sexual attraction, stress-adaptive, and social maturational behavior during middle childhood, before true puberty, has led to the suggestion that adrenarchal steroids play a role in activating these behaviors.^{402–404} Many functions attributed to DHEAS have been inconsistent, and whether they differ from those of low-dose testosterone remains to be established.³⁶⁸

Hormonal Secretion, Transport, Metabolism, and Action

Peptide Hormones

Peptide hormones act after binding to specific receptors located in the plasma membranes of target cells. GnRH receptors and

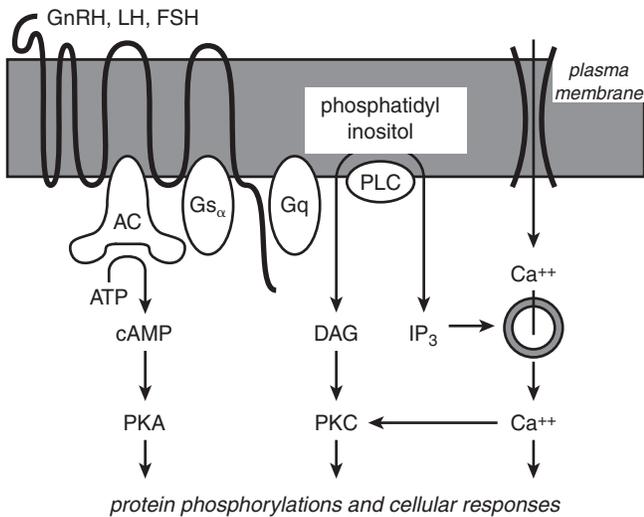


Fig. 16.21 Overview of the pathways established to mediate gonadotropin-releasing hormone (*GnRH*) and gonadotropin action. The receptors for these hormones are members of the seven-transmembrane family of receptors. Hormone-receptor binding alters receptor configuration. One consequence is to couple the receptor to adenylate cyclase (*AC*) via the stimulatory α -subunit of G-protein ($G_{s\alpha}$). This permits the efficient generation of cyclic adenosine monophosphate (*cAMP*) from adenosine triphosphate (*ATP*). Another consequence is to couple phospholipase C (*PLC*) to the receptor through *Gq*. *PLC* is a phosphodiesterase that hydrolyzes phosphatidylinositol to diacylglycerol (*DAG*) and inositol-1,4,5-triphosphate (*IP3*). *DAG* stimulates the calcium-sensitive protein kinase C (*PKC*). *IP3* mobilizes ionic calcium (Ca^{2+}) from intracellular organelles and stimulates Ca^{2+} influx through calcium ion channels. Protein kinase A (*PKA*), *PKC*, and Ca^{2+} then bring about cellular responses through protein phosphorylations. *FSH*, Follicle-stimulating hormone; *LH*, luteinizing hormone.

gonadotropin receptors are members of the 7-transmembrane receptor family. These receptors are necessary for the actions of their cognate hormones. Receptors expressed in nonclassical sites are not necessarily functionally mature.⁴⁰⁵ Mature receptors signal after coupling to a guanine nucleotide (G-protein) subunit (Fig. 16.21).^{5,326,330,406–408} G_s signaling activates adenylate cyclase and acts via phosphodiesterase-regulated cAMP to activate protein kinase A. G_q signaling activates phospholipase C, which acts via protein kinase C and Ca^{2+} ; Ca^{2+} may also be mobilized by other factors that influence ion channels. Phosphorylation of various cytoplasmic and nuclear proteins ultimately mediates the action of the peptide hormones, and secondarily involves the *RAS* and *EGF* signaling cascades in the case of gonadotropins.²⁶ The diversity among target cells in their responses to the action of protein kinases in part relates to diversity and type of kinase, intracellular compartmentalization, substrate availability, and other differences in gene expression that are specific to each type of target cell.

GnRH is a decapeptide [pyro]Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂.³²⁶ One gene encodes the single precursor protein for both *GnRH* and prolactin release-inhibiting factor.⁴⁰⁹ *GnRH* not only effects prompt release of preformed gonadotropins (the “readily releasable pool”), but also stimulates the synthesis of gonadotropins (the “reserve pool”).⁴¹⁰ Repeated administration of *GnRH* augments the pituitary responsiveness to subsequent *GnRH* pulses (“self-priming”).¹⁰⁷ This has been ascribed partly to upregulation of *GnRH* receptors. *GnRH* has an important paradoxical effect. As discussed earlier, it acutely stimulates gonadotropin secretion, but, upon protracted, continuous administration it downregulates pituitary gonadotropin secretion. The significance of the

expression of *GnRH* and its receptor in nonhypothalamic reproductive tissues⁴¹¹ is unclear.

An evolutionarily conserved form of *GnRH* (*GnRH-II*) acts primarily through the type 2 *GnRH* receptor;⁴¹² *GnRH-II* and the type 2 *GnRH* receptor are products of unique genes, rather than being modified products of the *GnRH* or type 1 *GnRH* receptor genes.⁴¹³ The cell bodies of the *GnRH-II* neurons lie predominantly in the midbrain and only a minority project to the hypothalamic-pituitary area. *GnRH-II* function in humans is unknown; there is speculation that it is a neurotransmitter involved in sexual behavior. A recent study using functional neuroimaging demonstrated that kisspeptin administration enhanced limbic brain activity in response to sexual and couple-bonding stimuli and attenuated negative mood. Whether kisspeptin may become a therapeutic agent for patients with reproductive function dysfunction is currently unclear.⁴¹⁴

LH and *FSH* are synthesized in a single type of cell, and both are sometimes identified within the same cell.⁴¹⁵ A vestigial population of hCG-secreting pituitary cells has been described.⁴¹⁶ *LH*, *FSH*, and hCG are glycoprotein hormones that consist of two chains.⁴¹⁷ After synthesis of these hormones on the ribosomes, the carbohydrate moieties, which constitute about 16% of the weight, are added in the rough endoplasmic reticulum and Golgi apparatus. The α chains of *LH*, *FSH*, hCG, and thyroid-stimulating hormone (*TSH*) are identical in amino acid sequence (92 amino acids). Although the β chain of each hormone is different in both primary amino acid sequence and length, these β chains nevertheless share 30% to 80% amino acid homology. Biological activity is conferred when an α and β chain are glycosylated and assemble within the cell. The α/β dimer is stabilized by a β -subunit derived “seat belt” that wraps around the α -subunit. Neither the isolated α nor the β glycosylated protein subunit exhibits biological activity unless noncovalently bound to one another.

The gonadotropins exhibit considerable molecular heterogeneity.^{100,418–420} The major basis for this is variation in the relative degree of glycosyl sialylation or sulfonation, steps which occur reciprocally in the pituitary gland.⁴²¹ These differences affect in vitro and in vivo bioactivity. Polymorphisms in amino acid sequence of the *LH- β* and hCG- β gene also may affect the expression or bioactivity of *LH* or hCG.⁴²² Reproductive status affects isoform distribution, with sialylated forms predominating in the hypogonadal state.⁴²³ Androgens increase and estrogens decrease in vitro *LH* biopotency by altering *LH* sialylation.^{424,425} Thus the pituitary gland contains multiple isoforms of *LH* and *FSH* that vary in bioactivity. Consequently, different pituitary *LH* and *FSH* standards, as well as serum, contain variable proportions of immunoreactive material of varying bioactivity.

One corollary of the molecular heterogeneity is that the antibodies generated from these gonadotropin moieties detect heterogeneous epitopes that are not necessarily bioactive; indeed some may even act as gonadotropin antagonists.⁴²⁶ These factors combine to cause the gonadotropin B/I to vary in a wide variety of circumstances.^{100,418} The purest of standards, even recombinant ones, interact very differently in the diverse immunoassay systems. Likewise, the putative level of *LH* or *FSH* in a serum sample differs substantially among immunoassays. Furthermore, bioactivity assessments vary with the bioassay model system.^{100,427} Monoclonal antibody-based immunometric assays yield results that correlate with, but are not necessarily equivalent to, those by bioassay.^{100,428} The “third-generation” immunometric assays have the advantage of being more sensitive and specific for low levels of gonadotropins in serum than polyclonal antiserum-based RIA, but B/I discrepancies remain.

The major determinant of in vivo gonadotropin bioactivity is the serum half-life. Terminal sialic acid residues retard clearance by the liver, the primary site of metabolism, whereas

TABLE 16.2 Average Hormone Blood Production Rates in Midfollicular Phase Women.^a

Hormone	Production Rate
Luteinizing hormone	615 IU/day ^b
Follicle-stimulating hormone	215 IU/day ^b
Androstenedione	3.4 mg/day
Dehydroepiandrosterone	7.0 mg/day
Dehydroepiandrosterone sulfate	7.0 mg/day ^c
Dihydrotestosterone	0.06 mg/day
Estradiol	0.1 mg/day
Estrone	0.1 mg/day
Progesterone	1.1 mg/day
17-Hydroxyprogesterone	1.2 mg/day
Testosterone	0.2 mg/day

^aThese production rates are roughly equivalent to those in midpuberty.

The average daily production of those hormones that fluctuate cyclically is substantially greater. For example, estradiol production transiently peaks to about 0.5 mg/day, and thus the average production over the monthly cycle is about 0.2 mg/day or 6 mg/mo.^{434–440}

^bIn terms of second International Reference Preparation, human menopausal gonadotropin.

^cApproximate urinary production rate, expressed as unconjugated steroid.

sulfonated ones facilitate clearance.⁴²¹ About 10% to 15% of gonadotropins are excreted in urine according to RIA⁴²⁹; only about one-third of this is in a biologically active form.⁴³⁰

LH is cleared more rapidly from the blood than FSH or hCG.^{431,432} LH disappears from blood in an exponential pattern: RIA indicates that the half-life of the first component is about 20 minutes and that of the second component is about 4 hours. The bioactive LH half-life is about 25% to 50% shorter.⁴³³ These respective components for immunoreactive FSH are 4 and 70 hours; those for hCG are 11 and 23 hours. Hormone production rates in follicular phase women, which approximate midpubertal values, are given in Table 16.2.^{434–440}

Prolactin has structural and functional similarities to GH and placental lactogen. Prolactin has a considerable degree of structural heterogeneity; this results from genetic and posttranslational events within pituitary cells, as well as modifications, such as glycosylation in the periphery.⁴⁴¹ Lactotrope growth and prolactin secretion are stimulated by estrogens. Prolactin release from the anterior pituitary is primarily under the control of hypothalamic inhibition, probably primarily mediated by dopamine.⁴⁴² A prolactin release-inhibiting factor has been described within the same precursor protein as GnRH,⁴⁰⁹ thus providing a potential mechanism for reciprocal control of these two peptides. Prolactin secretion also is inhibited by thyroxine and is directly responsive to thyrotropin-releasing hormone (TRH). Estrogen and suckling are stimulatory. These signals may be positively mediated by α -MSH.

Inhibins and activins are members of the TGF- β superfamily and signal accordingly.^{343,344} Inhibin was discovered as the result of the search for the nonsteroidal gonadal hormone capable of specifically suppressing FSH. Activin was serendipitously discovered as the FSH-stimulating activity in the side-fractions in these studies. These hormones are formed by the differential disulfide-linked dimerization of two of three subunits (α , β_A , and β_B), each encoded by a distinct gene. The combination of an α - and β -subunit yields the inhibins, inhibin-A ($\alpha\beta_A$) and inhibin-B ($\alpha\beta_B$). Activins are dimers of β -subunits, $\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$ (activin-A, B, and AB). Inhibin antagonizes all known actions of activin. The genes for all three subunits are differentially expressed in a wide variety of tissues. Furthermore, these factors, particularly activin, have proven to exert effects not only on gonadotropes, but within other pituitary cells, the gonads, and in nonsexual target tissues.

Steroid Hormones

The ovary and adrenocortical zona reticularis share the core of the steroid biosynthesis pathway (Fig. 16.22).^{40,444,445} Gonadal cholesterol seems to be derived mostly from the cholesterol esters of low-density lipoprotein in man.⁴⁴⁶ Most steroidogenic steps are mediated by cytochrome P450 family members. These are the terminal enzymes in electron transfer chains, which include P450 oxidoreductase (POR) as the clinically relevant electron donor for all in the endoplasmic reticulum. The initial step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. This is a two-stage process. The rapidity of the process depends upon the transport of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). The conversion itself is carried out by the cholesterol side chain cleavage activity (scc) of cytochrome P450scc. The next steps are either the 3β -HSD step or 17 α -hydroxylation. 3β -HSD converts Δ^5 - 3β -hydroxysteroids to steroids with the Δ^4 -3-keto configuration (e.g., pregnenolone to progesterone, 17-hydroxypregnenolone to 17-OHP, and DHEA to androstenedione). This step is obligatory for the synthesis of all potent steroid hormones. The type 2 3β -HSD isozyme accounts for the vast majority of the 3β activity in the human ovary and adrenal; the type 1 isozyme accounts for 3β -HSD activity in liver and skin. Pregnenolone alternatively undergoes a two-step conversion to the 17-KS DHEA along the Δ^5 -steroid pathway: this conversion is accomplished via cytochrome P450c17. P450c17 is a single enzyme with 17 α -hydroxylase and 17, 20-lyase activities, the latter being less efficient and critically dependent on electron transfer from cytochrome *b*. Progesterone undergoes a parallel transformation to androstenedione in the Δ^4 -steroid path: 17 α -hydroxylation of progesterone by P450c17 forms 17-hydroxyprogesterone, but in humans P450c17 does not efficiently utilize 17-hydroxyprogesterone as a substrate for 17,20-lyase activity, so P450c17 seems to form little if any androstenedione. There is some evidence for the existence of a P450c17-independent Δ^4 -pathway to androstenedione, but most seems to be formed from DHEA by the action of 3β -HSD.⁴⁴⁷ Sulfotransferase 2A1 is uniquely expressed in the adrenal zona reticularis and requires the cofactor 3'-phosphoadenosine-5'-phosphosulfate synthase type 2.³⁹⁴ Other sulfotransferases (e.g., for formation of estrone sulfate) and steroid sulfatase (for the reverse reaction) are widely expressed.⁴⁴⁸

17 β -Hydroxysteroid dehydrogenase (17 β -HSD) and aromatase activities are required for the formation of potent sex steroids. In the ovary, androstenedione is the major precursor for sex steroids. The conversion of 17-KS to 17 β -hydroxysteroids by 17 β -HSDs is essential for the formation of both androgen and estrogen: testosterone is formed in the ovary by 17 β -HSD type 5 (also termed *aldoketoreductase*, AKR1C3), whereas estradiol formation requires 17 β -HSD type 1.⁴⁴⁹ Aromatase activity, effected by P450arom, is essential for estradiol formation. Alternate promoters are used by the P450arom gene in the gonads, placenta, and adipose tissue, which yields alternatively spliced forms of aromatase. The organization and regulation of steroidogenesis in the developing follicle is depicted in Fig. 16.19.

The ovary normally accounts for about 25% of testosterone secretion in the mature female (0.06 mg daily), but it secretes about 30 times as much androstenedione (1.6 mg daily).⁴³⁶ These amounts are similar to those secreted by the adrenal. However, the ovary secretes less than 1/10 as much DHEA as the adrenal.

The "production rate" of a hormone equals its secretion rate plus (in the case of hormones formed outside of endocrine glands) the rate of formation of the hormone by peripheral conversion of secreted precursors. The "blood production rate" is calculated as metabolic clearance rate X serum concentration;

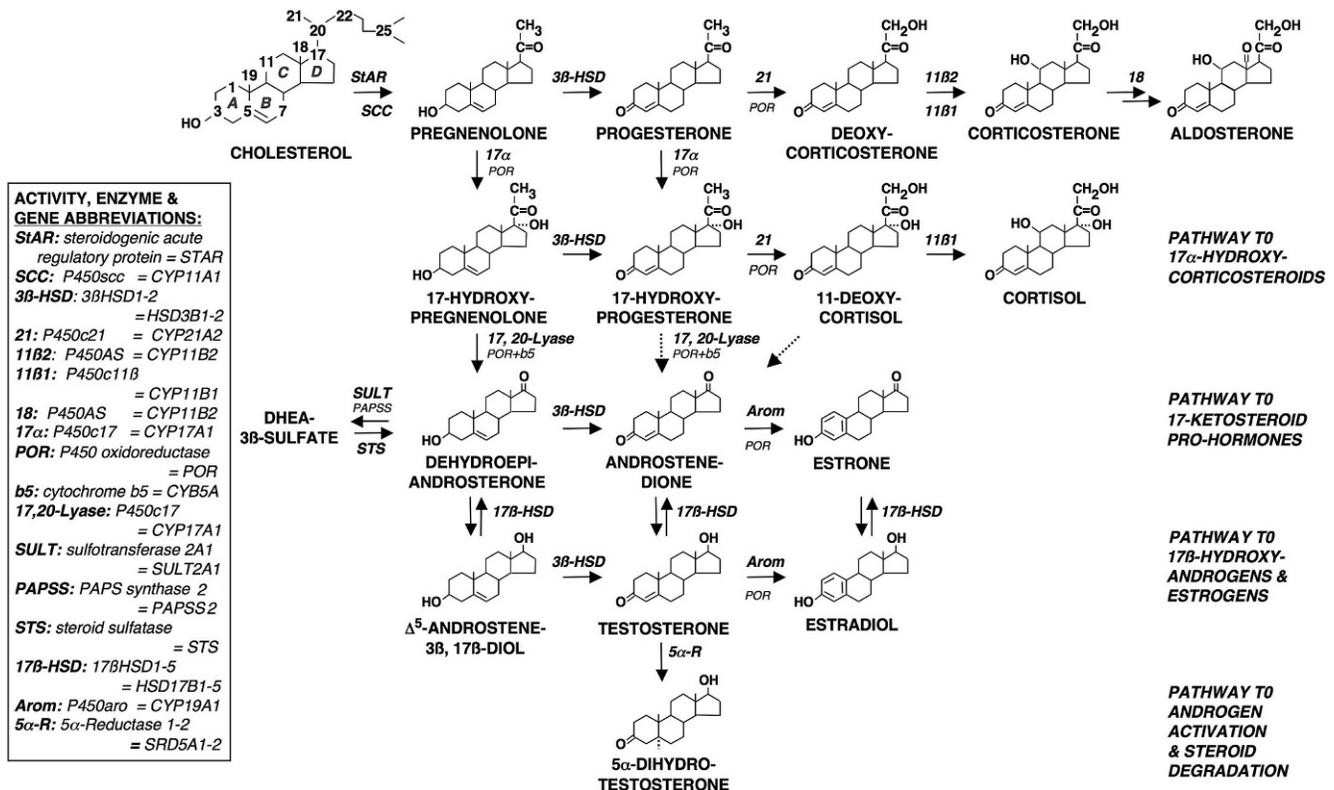


Fig. 16.22 Major pathways of steroid hormone biosynthesis from cholesterol. Carbon atoms of cholesterol are designated by conventional numbers and rings by conventional letters. The flow of hormonogenesis is generally downward and to the right. The top row shows the pathway to progesterone and mineralocorticoids, the second row the pathway to glucocorticoids, the third row the 17- β -hydroxysteroids, and the bottom row the activation of androgen. The steroidogenic enzymes are italicized. Abbreviations for enzymes include the following cytochrome P450 enzyme activities: cholesterol side chain cleavage (*scc*); 17 α -hydroxylase (17 α); 21-hydroxylase (21); 11 β -hydroxylase (11 β 1); aldosterone synthase (11 β 2, 18-hydroxylase/oxidase); aromatase (*Arom*). Non-P450 enzyme activity abbreviations include Δ^5 -isomerase- β -hydroxysteroid dehydrogenase (3 β) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD). Clinically relevant electron transfer enzymes include P450 oxidoreductase (*POR*), cytochrome b5 (*b5*), and 3'-phosphoadenosine-5'-phosphosulfate synthase type 2 (*PAPSS*). (Modified from Rosenfield, R.L., Lucky, A.W., Allen, T.D. (1980). The diagnosis and management of intersex. *Curr Prob in Pediatr*, 10, 1.)

in the steady state the amount of hormone irreversibly leaving the plasma compartment equals the amount entering it. Because of extensive steroid interconversions, the quantity of these hormones excreted in urine is **not** necessarily indicative of the amount reaching target tissues.⁴³⁶ For example, so large a fraction of urinary testosterone glucuronide is formed directly from androstenedione by compartmentalized metabolism within the liver that the range of urinary excretion of testosterone in women overlaps that in men (Fig. 16.23).⁴⁵⁰ Estrone sulfate, like DHEAS in the androgen pathway, forms a circulating reservoir of inactive estrogen that can be returned to the active pool by hepatic sulfatase activity.⁴⁵¹ The blood production rates of representative steroid hormones are given in Table 16.2 and are shown for estrogens in Fig. 16.24. During the luteal phase of the menstrual cycle, estradiol production doubles⁴³⁹ and progesterone production rises 16-fold or more.⁴⁵²

Sex hormones also have environmental origins. Structurally distinct biological estrogens include equine estrogens and plant-derived phytoestrogens.⁴⁵¹ Synthetic estrogens include pharmacological compounds, such as ethinyl estradiol, diethylstilbestrol, selective ER modulators (SERMs), and some industrial chemicals, such as organochlorines (*p,p'*-dichlorodiphenyltrichloroethane [DDT] and others) and plasticizers (such as bisphenol A and phthalates). Endocrine-disrupting chemicals (EDCs) interfere with any aspect of hormone action, with mechanisms including mimicking or blocking hormone signaling through its receptor, and modulating

the synthesis, release, transport, metabolism, binding or elimination of natural hormones. These compounds therefore may impact development of the reproductive tract and function of the reproductive axis.^{192,266} Animal studies have indicated that EDCs can impair ovarian development, inhibit ovarian follicle growth, increase follicular atresia, and disrupt steroid hormone levels.²⁶⁶

Peripheral conversion of secreted prehormones by non-endocrine organs accounts for a major portion of sex hormone production. The ovary and the adrenal cortex are sources of prehormones, as well as secreted hormones. About 50% of serum testosterone (0.1 mg daily) normally is formed indirectly by peripheral conversion. Although 85% of normal estrogen production in women arises by secretion in midcycle, 50% of estrogen production can arise from extraglandular sources during the low-estrogen phases of the menstrual cycle.⁴⁵³ Peripheral formation of active steroids occurs in a wide number of sites, including liver, fat, and target organs.^{40,454} For example, the liver has high levels of 3 α - 3 β -, and 17 β -hydroxysteroid dehydrogenase and 5 α -reductase activities (see Fig. 16.22).

Peripheral androgen metabolism is not tightly regulated by the neuroendocrine system. It seems determined to some extent by the perinatal androgenic milieu,⁴⁵⁵ the effect of which is possibly mediated by GH.⁴⁵⁶ Postnatally, it is influenced by the sex hormone binding globulin (SHBG) level and the state of nutrition. Adipose tissue becomes a major site of conversion of androstenedione to both estrone and testosterone in the obese.^{308,457}

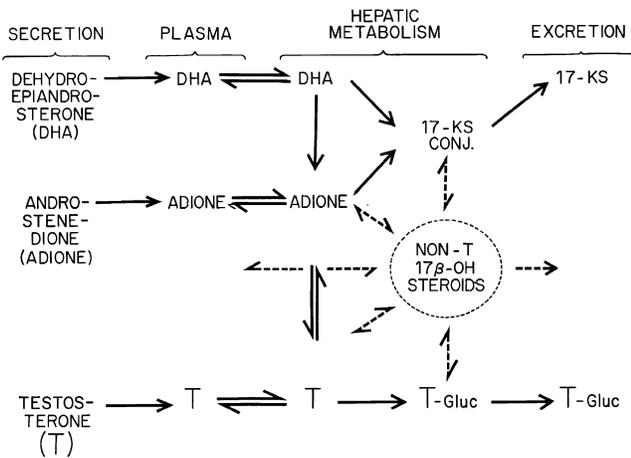


Fig. 16.23 Diagram illustrating the relationship among secreted, plasma, and urinary steroids. 17-Ketosteroid (17-KS) excretion does not reflect accurately the excretion of the most important plasma androgens. Only 25% or less of testosterone is excreted as 17-KS metabolites. Therefore important changes in testosterone production may not appreciably affect urinary 7-KS excretion. Furthermore, even the major 17-KS (*DHA-sulfate*) is excreted poorly until its production rate becomes quite high. On the other hand, about half of 17-KSs are not identified by the standard colorimetric test and 2 mg daily of 17-KS in adults results from hydrocortisone metabolism. In addition, testosterone glucuronide excretion does not accurately reflect the plasma testosterone level: less than 2% of testosterone appears in the urine as such. Furthermore, the plasma 17-KS androstenedione may be converted to testosterone glucuronide without ever circulating as unconjugated testosterone. (From Rosenfield, R.L. (1973). Relationship of androgens to female hirsutism and infertility. *J Reprod Med*, 11, 87.)

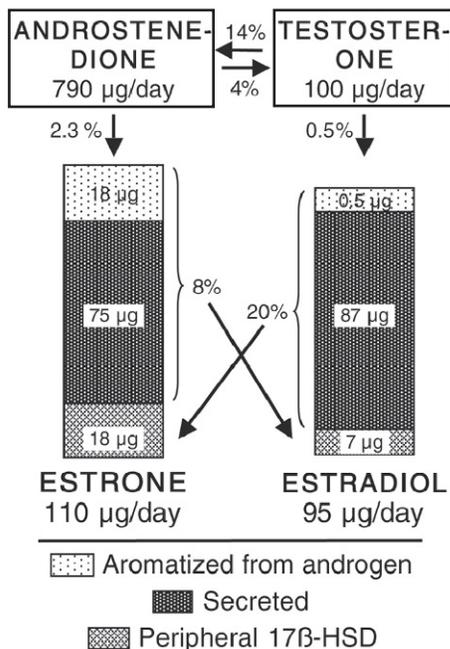


Fig. 16.24 Sources of estrone and estradiol in blood of follicular phase premenopausal women. Estrogen is derived from direct secretion by the gonad, aromatization of androgen, or conversion of an estrogen precursor by 17β-hydroxysteroid dehydrogenase (17β-HSD) activities. The percentage of substrate converted per day and total approximate production in micrograms per day are noted for each source. (Modified from Alonso, L.C., and Rosenfield, R.L. (2002). Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab*, 16, 13.)

Cytochrome P450 mixed function oxidases, the most important of which is CYP3A4, affect steroid efficacy by forming hydroxylated steroid metabolites of varying potency.^{458,459} They are subject to induction or inhibition by numerous drugs. Phytoestrogens increase estradiol bioavailability by inhibiting hepatic sulfotransferase.⁴⁶⁰

Plasma steroids appear to reach their sites of action and metabolism by simple diffusion from the vascular compartment.⁴⁶¹ The bioactive portion of serum testosterone seems to be the free testosterone and a portion of the albumin-bound testosterone that differs among tissues according to the diffusion characteristics of the vascular bed.⁴⁶² About 98% of serum testosterone and estradiol are bound to albumin and SHBG. The SHBG concentration determines the fraction of serum testosterone and other ligands (e.g., estradiol, DHT) that are free or bound to albumin. It is also a major determinant of ligand egress from plasma (Fig. 16.25).⁴⁶³ Some sex steroid effects may be mediated by SHBG binding to membrane receptors and activation of adenylate cyclase.^{464,465} A number of physiological and pathological states affect the SHBG level. It is increased by estrogen and thyroid hormone excess; it is decreased by androgen, insulin-resistant obesity, glucocorticoid, GH, and inflammatory cytokines.⁴⁶⁶⁻⁴⁶⁸

Target cell metabolism influences the cell's response to the steroid hormones that reach it (Fig. 16.26).⁴⁶⁹ The intracellular conversion of testosterone to DHT by one of the two isozymes of 5α-reductase is important for many but not all effects of testosterone,⁴⁷⁰ dependent upon the tissue-specific pattern of

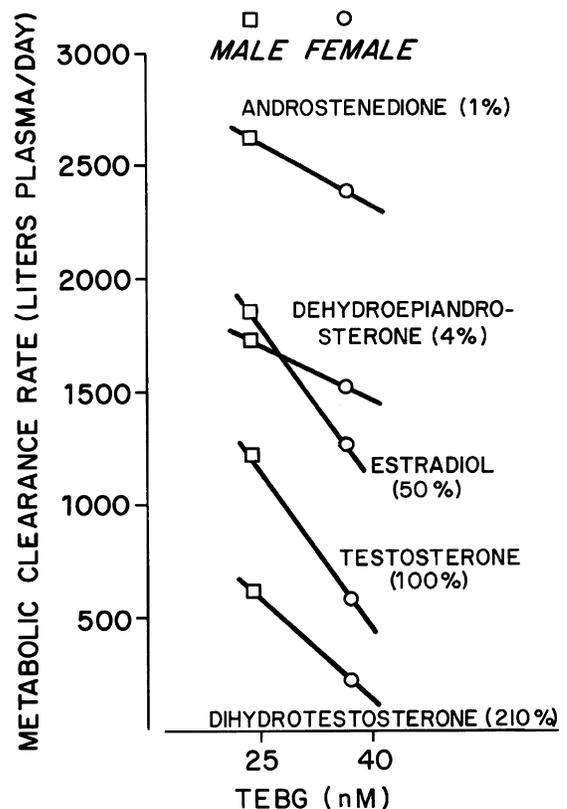


Fig. 16.25 The relationship between the metabolic clearance rate (MCR) and binding of sex hormones to sex hormone binding globulin (SHBG = testosterone-estradiol binding globulin [TEBG]). The MCR of each steroid has been related to the mean SHBG levels of men and women. The approximate affinity of each steroid for SHBG relative to testosterone is indicated in parentheses. (From Rosenfield, R.L. (1975). Studies of the relation of plasma androgen levels to androgen action in women. *J Steroid Biochem*, 6, 695.)

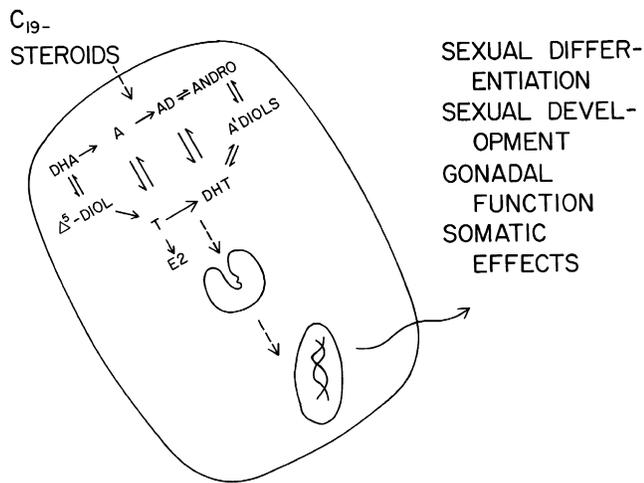


Fig. 16.26 Model of the mechanism of androgen action emphasizing the effect of steroid metabolism within a target cell on the mode of action. Solid arrows indicate pathways of steroid metabolism from 17-ketosteroid precursors as laid out in Fig. 14.23. Broken arrow indicates transport. The cell-specific intracellular pattern of C19-steroid metabolism determines the relative availability of testosterone or dihydrotestosterone (DHT) to the cytosol receptor for translocation to the nucleus. In cells, such as the rat granulosa cell in which $\Delta^5,3\beta$ -hydroxysteroid dehydrogenase activity is high, androstenediol (Δ^5 -diol) is as potent as testosterone. The human sebaceous gland has a similar pattern of steroid metabolism. A, androstenedione; AD, androstenedione; A'DIOLS, androstenediols; ANDRO, androsterone; DHA, dehydroepiandrosterone. E2, estradiol; T, testosterone. (From Nimrod, A., Rosenfield, R.L., Otto, P. (1980). Relationship of androgen action to androgen metabolism in isolated rat granulosa cells. *J Steroid Biochem*, 13, 1015. With permission from Elsevier Science.)

steroid metabolism. An important mode of testosterone action is via estradiol, notably within the brain. Although transformation is not fundamental to the mode of action of estradiol, estradiol effectiveness is influenced by target cell metabolism: the induction of 17β -hydroxysteroid oxidation in target tissues by progesterone, resulting in conversion of estradiol to the less potent estrogen estrone, counterbalances estrogenization.⁴⁷¹ There is also evidence that novel steroid metabolites exert tissue-specific effects.^{472,473}

Within target cells, all steroid hormones regulate the genome similarly, starting with binding to high-affinity intracellular receptors (Fig. 16.27).^{474–476} The steroid hormone receptors belong to the superfamily of nuclear hormone receptors. The estrogen, progesterone, and androgen receptors are, thus, homologous. Classic sex hormone effects are exerted by the interaction of steroid with receptor, not by either alone. Steroid binding triggers the dissociation of inhibitory chaperone heat shock proteins from the receptor.⁴⁷⁷ The active receptor-ligand complex then undergoes noncovalent dimerization and binding to its specific hormone response element on the gene. The deoxyribonucleic acid (DNA) bound steroid-receptor complex acts as a transcriptional regulator of the target gene promoter. Sensitivity to steroids is also modulated by molecular chaperone proteins that influence receptor configuration, intracellular trafficking, and receptor turnover, all which are determinants of steroid action.^{478,479}

The binding properties of steroids to their cognate receptors are the initial determinants of classical steroid action.^{474,475} Ligand-based selectivity is one element of this interaction. Estradiol is a more potent estrogen than estrone and estriol partly because it binds best to the steroid binding domain of the ER.⁴⁸⁰ DHT is an inherently more potent androgen than

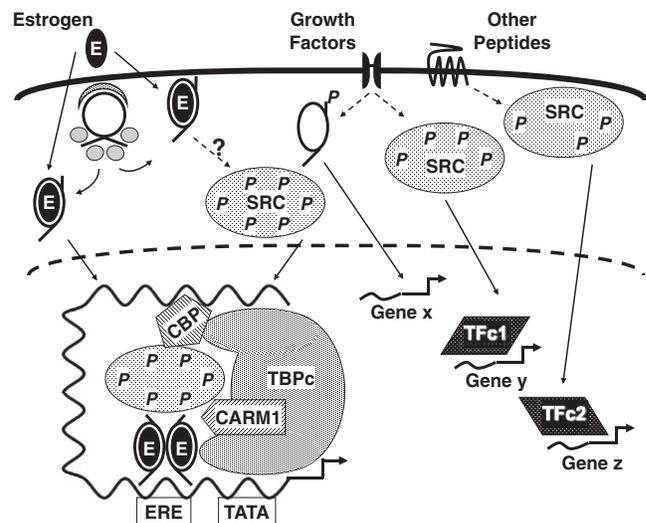


Fig. 16.27 A model for the mechanism of estrogen (E) action that emphasizes the role of interactions of the estrogen receptor with steroid receptor coregulator (SRC) and phosphorylation signaling. Estrogen causes the 4S subunit and heat-shock proteins to dissociate from the unliganded estrogen receptor. Then estrogen entry into the binding pocket causes a conformational change in the receptor. Estrogen also stimulates phosphorylation of SRC in a specific pattern (Ps), possibly via liganded membrane-bound estrogen receptor (ER) as it does some transcription factors, and recruits it to the nuclear deoxyribonucleic acid (DNA) steroid-receptor complex with the estrogen response element (ERE). SRC in turn recruits other cofactors, such as the cyclic adenosine monophosphate (cAMP) response element binding protein-binding protein (CBP) and coactivator-associated methyltransferase (CARM1) to the hormone binding complex. This aggregate then interacts with the TATA binding protein initiation complex (TBPC) to initiate estrogen-specific gene transcription. The genomic estrogen effect is modulated by the effects of environmental signals on other cell-specific transcription factors (TFs), some of which involve differentially phosphorylated SRC complexes (TFc) in gene activation, others of which involve ligand-independent ER. Dotted lines indicate diverse kinase pathways. ER recycling is not shown. (From Katzenellenbogen, B.S., Montano, M.M., Ediger, T.R., et al. (2000). Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*, 55, 163–193; O'Malley, B.W. (2005). A life-long search for the molecular pathways of steroid hormone action. *Mol Endocrinol*, 19, 1402–1411; McDevitt, M.A., Glidewell-Kenney, C., Jimenez, M.A., et al. (2008). New insights into the classical and nonclassical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol*, 290, 24–30.)

testosterone mainly because of its higher association rate constant and its lower dissociation rate constant.⁴⁸¹

The antiestrogens tamoxifen and clomiphene and the antiandrogens cyproterone acetate and spironolactone competitively inhibit the active ligands from binding to their specific receptor sites by weakly and transiently occupying receptor sites. These differences result from potent agonists snugly fitting into the binding pocket, which induces a receptor conformation different than that of antagonist-bound receptor. One such change is the C-terminal tail of the receptor flipping over to close the “door” when a potent agonist enters; this simultaneously provides a different outer surface for interaction with coregulator proteins.

Thus ligand-based selectivity arises not only because of tighter ligand binding, but because alternative ligands produce both intermediate and unique conformational changes in the receptor, which in turn induce altered receptor interactions

with coregulator proteins that result in a spectrum of activities.⁴⁸² Thus steroids do not simply switch receptors on; they induce selective functions that depend on the nature of the coregulators that are recruited to the complex.^{483–486} In part, this selectivity arises because different domains of these receptors mediate these different functions. For example, the activation function (AF)-1 domain of the ER mediates interactions with mitogen-activated protein (MAP) kinase and TGF- β 3, whereas the AF-2 domain mediates interactions with coregulator proteins.⁴⁸⁷ Coactivators, in turn, regulate alternative splicing, gene activation and repression (in some cases via their dual enzymatic functions), ubiquitin-proteasome-mediated turnover of the receptor-coregulator complex,⁴⁸⁸ and also determine cell-specific, site-based actions,⁴⁷⁵ as discussed later.

Receptor-based selectivity is a second element in steroid action. There are now known to be two isoforms of each of the sex steroid receptors. The α and β forms of the ER, although homologous, are coded by separate genes.¹⁷¹ A and B forms of the progesterone and androgen receptors exist.^{489,490} These forms of the PR arise by transcription from alternate promoters within the same gene, whereas those of the androgen receptor arise from posttranslational modification of a single messenger (m)RNA. These isoforms have variously been shown to manifest a differential tissue expression pattern and respond differentially to antagonists. Interactions of a steroid with different forms of receptors can regulate some target genes differentially. One role of ER β is apparently to modulate ER α activity: ER α and ER β can have opposite actions at AP-1 and SP-1 sites, and studies of transcriptional activity in bone and breast tissue of mice indicate a restraining effect of ER β on responses to estradiol.^{171,491} Thus different target tissues exposed to the same hormone may respond selectively because of a distinct repertoire of receptor isoform expression. Some examples are notable. Although both forms of ER are expressed in most target tissues, the classic form of the ER, ER α , plays the key role in regulation of LH and estrogen actions on the uterus, breasts, sex-specific behavior, and bone.^{171,492,493} In the mouse ovary, knockout experiments show that ER α is expressed in thecal cells where it prevents androgen excess in response to LH. In contrast, ER β is expressed only in granulosa cells, where its inhibition of androgen receptor expression is critical to prevent premature follicular atresia.¹⁷² Both are necessary for oocyte survival and the ability of preovulatory follicles to rupture. Furthermore, loss of both causes transdifferentiation of granulosa cells to Sertoli-like cells and massive oocyte death.¹⁵⁶ Liganded PR $_A$ is essential for ovulation⁴⁹⁴ and is the more effective antagonist of ER action.⁴⁷⁴ In addition, sequence variation in hormone response elements contributes to differential gene regulation.⁴⁹⁵

Effector site-based selectivity is a third variable in classical sex hormone action. In other words, the potency and character of a response to a ligand-receptor complex are not simply inherent properties of the complex. Rather, they depend on the array of effector molecules present in the site of action. Thus the array of genes expressed locally and the relative expression level of coregulators (coactivators and corepressors) are extremely important in the determination of appropriate and graded responses to a ligand by a target cell.^{474,496} Heterodimerization of the ER with other nuclear receptors can modulate its action.⁴⁹⁷ Androgens appear to exert some of their genomic effects by directly complexing with transcription factors other than the androgen receptor.⁴⁹⁸ Both estrogen and androgen appear to exert antiapoptotic effects in osteoblasts and osteocytes by activating a ligand-dependent, but nongenomic, kinase-mediated signaling pathway.⁴⁹⁹

Nuclear receptor coactivators are critical in sensing cell-specific environmental signals and to coordinate signals emanating from membrane receptors with nuclear receptor

action.⁴⁷⁵ Surface receptors send signals through kinase pathways that result in specific serine/threonine phosphorylation patterns of coactivators. These phosphorylation patterns serve as a code for the coactivator to preferentially bind and activate distinct sets of downstream transcription factors (see Fig. 16.27). Overexpression of steroid receptor coactivator-3 (SRC-3) is as important in the pathogenesis of some breast cancers as is ER positivity.

The effects of a given ligand-ER complex often differ from those of the estradiol (E2)-ER complex among cell types. This is the basis for the development of SERMs: these compounds exert effects in a tissue-specific manner depending on the cell context.^{474,482} The chemical structure of a SERM—or any ER ligand, for that matter—determines the configuration of the ER, resulting in a spectrum of activities from agonist to antagonist, depending on which coregulators are available for recruitment in the target cell. Raloxifene is an estradiol agonist in bone and epiphyseal cartilage but is antiestrogenic in uterus and breast; tamoxifen is estrogenic in uterus but antiestrogenic in breast and bone. Both appear to retain neural and endothelial estrogenic activity.^{500,501}

Nonclassical mechanisms play a role in sex steroid action.⁴⁷⁵ The nonclassical mechanisms are of two general types: (1) genotropic estrogen response element (ERE)-independent signaling, in which liganded ER acts as a coregulator of other transcription factors that act through their specific DNA response elements and (2) nongenotropic signaling, in which E2 binding to membrane-associated receptors, including ER α , rapidly stimulates phosphorylation pathways.^{502,503}

The nongenomic effects via membrane signaling occur rapidly (within minutes) and can mediate cell proliferation, apoptosis, and migration in cell-specific ways.^{499,504} Nongenomic E2 actions account for most of the LH-inhibitory and energy balance effects of E2.^{503,505} These effects can be mediated by binding to nuclear ER in plasma membrane domains provided by scaffolding proteins, such as caveolin. On such platforms, the E2-ER complex acts like a membrane receptor, coupling with G-proteins and activating cytoplasmic pathways involving SRC and MAP kinase. Androgens appear to act similarly. Nongenomic actions of nuclear PR have also been reported. Some nongenomic effects seem to involve the activation of novel G-protein-coupled transmembrane receptors for E2 and progesterone that interact either with steroids or their metabolites.

Genomic ER signaling may also be ligand independent. For example, cell membrane signaling by growth factors or other peptides stimulate ER phosphorylation. EGF activates phosphorylation of the ER and simulates diverse estrogen effects.⁵⁰⁶ Activation of unliganded ER α seems to be involved in repressing expression of the androgenic 17 β -HSD testicular isoform in the ovary.³³⁵

Steroids that act by binding to membrane-bound receptors in the brain are termed *neuroactive*.³⁹⁷ Neuroactive steroids synthesized in the brain are termed *neurosteroids*.^{507–509} The best documented of these effects are on neurotransmitters, which control ion channels. Allopregnanolone (3 α -hydroxy-5 α -tetrahydroprogesterone) and 3 α -androstane-20-one are GABA $_A$ receptor agonists and so have sedative and antiepileptic properties.⁵¹⁰ Pregnenolone sulfate and DHEAS have the opposite effect, the former also stimulating the glutamate receptors. Receptors for 5-hydroxytryptamine have been implicated in mediating some of the effects of sex steroids and certain of their metabolites.^{511,512} Some estrogen effects in brain are membrane-mediated.⁵¹³

The tissue-specific posttranscriptional events involved in sex steroid signaling are poorly understood. Estradiol and progesterone modulate the actions of each other through effects on their specific receptors: increased estrogens in the preovulatory phase of the cycle upregulate target organ receptors for both estradiol and progesterone; luteal phase levels of progesterone

then suppress the production of both receptors.^{514,515} Estrogen prevents bone loss by blocking the production of proinflammatory cytokines.⁵¹⁶ Androgen action has been reported to be mediated by prostaglandins in genitalia,⁵¹⁷ and testosterone stimulates the IGF-1 system in epiphyseal cartilage.⁵¹⁸

Maturation of Sex Hormone Target Organs

Genital Tract

The Müllerian system of the embryo gives rise to the uterus, cervix, upper vagina, and fallopian tubes in the absence of AMH secretion by fetal testes during the first trimester of gestation.⁴⁴⁴ Genital swelling develops to engulf the base of the penis-like clitoris between 11 and 20 weeks' gestation in parallel with the development of the ovarian follicular system.⁵¹⁹ ERs are expressed in the labia minora, prepuce, and glans in females, but not in the homologous structures of males.⁵²⁰ An association between antiestrogen and genital ambiguity has been reported.⁵²¹ Diethylstilbestrol induces dysplasia of the genital tracts.⁵²² These data suggest that estrogen may play a direct role in female genital tract differentiation. However, knock-out of ERs has no obvious effect on genital tract differentiation.¹⁵⁶

The infantile uterus and cervix enlarge under the influence of estrogen during puberty. The endometrium and cervical glands then undergo cyclical changes in concert with cyclic ovarian function. In response to rising estrogen during the follicular phase of the cycle, the endometrial epithelium and stroma proliferate. The uterine glands increase in number and lengthen. Endometrial hyperplasia is prevented by progesterin⁵²³ and androgen excess.⁵²⁴ In response to progesterone secretion after ovulation, the endometrium increases in thickness: stromal edema occurs, and the uterine glands enlarge, become sacculated, and secrete a glycogen-rich mucoid fluid. The coiled arteries lengthen further during this time and become increasingly spiral. These changes are critical to permit implantation. High-dose progesterin is an effective postcoital contraceptive because it prevents implantation when taken within 3 days of unprotected intercourse.⁵²⁵

Endocervical gland secretions lubricate the vaginal vault. The endocervical mucus is scanty and relatively thin during the low-estrogen phase of the cycle. The increase in mucus flow with advancing follicular development seems to require tissue-specific stimulation of the cystic fibrosis transmembrane regulator by estrogen.⁵²⁶ Cervical mucus becomes more viscous and elastic as estrogens rise in the later follicular phase of the cycle—the extent to which it can be stretched into a long spindle, *spinnbarkeit*, is a function of the estrogen level.

The mucosa of the vagina and the urogenital tract is comprised of hormone-responsive stratified squamous epithelium (Fig. 16.28).² The basal layer is the regenerative area. In the absence of estrogen, there is only a parabasal layer of cells over this, and the vagina is thin, with a tendency to alkalinity, which predisposes it to local infection (nonspecific vaginitis).⁵²⁷ In response to estrogen, epithelial proliferation occurs, with formation of successive intermediate and superficial layers. With this maturation, the cytoplasm of each cell first expands, leading to formation of small intermediate cells. With further estrogenization, the nuclei become pyknotic and large intermediate cells form. Greater estrogenization brings about their transformation to cornified squamous superficial cells: the cytoplasm changes from basophilic to acidophilic with the accumulation of glycogen. Resistance to infection of the fully developed vaginal mucosa results from its thickness and from its acid pH, which occurs from the fermentation of the glycogen of the superficial cells. In response to luteal phase progesterone, degenerative changes appear in vaginal mucosal cells:

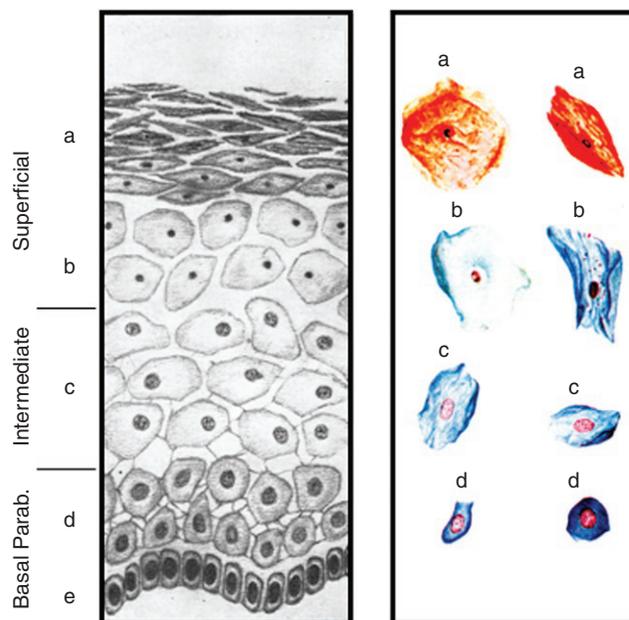


Fig. 16.28 The layers of vaginal epithelium of the well-estrogenized adult. The superficial layer contains surface cells that are cornified (squamous) with eosinophilic cytoplasm and pyknotic nuclei (a) as well as large intraepithelial cells that are also karyopyknotic but basophilic (b). The intermediate zone contains basophilic cells that have less cytoplasm and intermediate-size nuclei (c). The basal and parabasal cells have a relatively small amount of basophilic cytoplasm and relatively vesicular nuclei (d, e). (Modified from Wilkins, L. (1968). *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. Springfield, IL, Charles C Thomas.)

superficial cells decrease, the cytoplasm assumes a “crinkled” appearance, cells degenerate, and bacterial proliferation increases.

Vaginal smears show the characteristic cyclic changes in the cell types comprising the vaginal epithelium (see Fig. 16.28).⁵²⁸ In the prepubertal years, parabasal cells predominate, and characteristically 10% or less are small intermediate cells. A pattern consisting entirely of intermediate cells is typical of early puberty. The early follicular phase of the menstrual cycle is characterized by the predominance of large intermediate cells with few, if any, superficial cells. Peak maturation is reached at midcycle, at which time 35% to 85% of the cells seen on vaginal smear are superficial; the remainder are large intermediate cells. This cornification develops over a 1-week period in response to estradiol levels of about 70 pg/mL and persists 1 to 2 weeks after estrogen withdrawal (see Fig. 16.11).⁵²⁹

Progesterone antagonizes estrogen effects on the vaginal epithelium and cervix. Inhibition of cervical ripening by progesterins is used to prevent recurrent spontaneous preterm delivery.^{530,531}

Many normal variations have been recognized in the appearance of the hymen. The transverse diameter increases with age.^{532,533}

Mammary Glands

Multiple rudimentary branching mammary ducts are found beneath the nipple in infancy; they grow and branch very slowly during the prepubertal years.⁵³⁴ Estrogen stimulates the nipples to grow, mammary terminal duct branching to progress to the stage at which ductules are formed, and fatty stromal growth to increase until it constitutes about 85% of the

mass of the breast. GH (via IGF-1) and glucocorticoids play a permissive role.^{535,536} These hormones interact with breast stroma and local growth factors to stimulate the development of breast epithelium. Lobulation appears around menarche, when multiple blind saccular buds form by branching of the terminal ducts. These effects are caused by the presence of progesterone. The breast stroma swells cyclically during each luteal phase. Full alveolar development normally only occurs during pregnancy under the influence of additional progesterone and prolactin. Prolactin does not play a role in breast growth without priming by female hormones.⁵³⁷

Estrogen and progesterone also play a role in breast cancer susceptibility.⁵³⁸ Earlier than average age at menarche is a modest risk factor for breast cancer, regardless of BRCA status;^{539,540} notably, however, breast cancer risk has not been shown to be increased in precocious puberty. The *BRCA1* gene normally restrains mammary growth, at least in part, by inhibiting expression of ER α and PRs, and cancer-related mutations reverse these processes.⁵⁴¹

Pilosebaceous Unit

The pilosebaceous unit (PSU), with but few exceptions, consists of both a piliary and a sebaceous component.⁵⁴² Androgens are a prerequisite for the growth and development of PSUs in their characteristic pattern. Androgens exert their effects both on the dermal papilla, which regulates the hair growth cycle, and on PSU epithelium. Before puberty, the androgen-dependent PSU consists of a prepubertal vellus follicle in which the hair and sebaceous gland components are virtually invisible to the naked eye (Fig. 16.29). Under the influence of androgens, in the sexual hair areas, the PSU switches to producing a medullated terminal hair follicle that expresses a unique type of keratin that is androgen responsive.⁵⁴³ The difference in the apparent density of sexual hair between men and women is caused by differences in the density of terminal hairs that develop in response to androgen. In the balding-prone area of scalp in individuals genetically predisposed to male-pattern alopecia, androgens weakly attenuate the hair growth cycle, so that the PSU gradually generates only vellus follicles.⁵⁴⁴ In acne-prone areas, androgen causes the prepubertal vellus

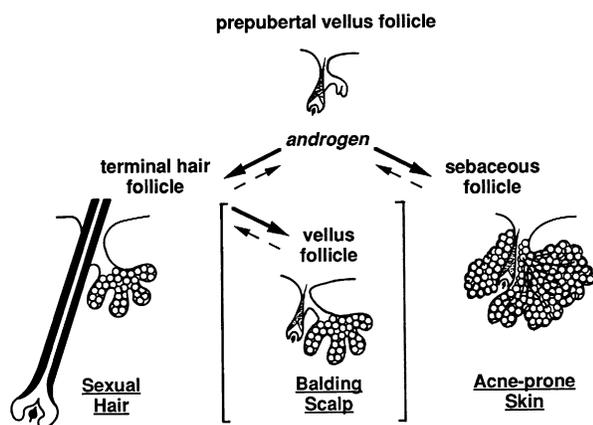


Fig. 16.29 Role of androgen in the development of the pilosebaceous unit. Androgens (solid lines) are responsible for the patterned differentiation of the pilosebaceous unit at puberty. Dotted lines indicate effects of antiandrogens. Hairs are depicted only in the anagen (growing) phase of the growth cycle. In balding scalp (bracketed area), terminal hairs not previously dependent on androgen regress to vellus hairs under the influence of androgen. (From Rosenfield, R.L., Deplewski, D. (1994). Role of androgens in the developmental biology of the pilosebaceous unit. *Am J Med*, 97(5A), 80.)

follicle to develop into a sebaceous follicle, in which the sebaceous epithelium develops and the hair remains vellus. Adrenarchal levels of androgens suffice to successively initiate sebaceous gland development and the growth of pubic hair. Progressively, greater amounts of androgen are in general required to stimulate terminal hair development along a pubic to cranial gradient. All these effects of androgen are to some extent reversible by antiandrogens.

Estrogens modestly stimulate hair growth, probably by inhibiting the catagen (resting) phase of the hair cycle;⁵⁴⁵ this may well be caused by induction of androgen receptors by estrogen. Estrogens also directly inhibit sebum secretion. GH synergizes with androgen action on the PSU, in part via IGF-1 signaling. Retinoic acid receptor agonists antagonize the effects of androgen on the sebaceous gland by inhibiting sebocyte proliferation and differentiation. Insulin, prolactin, glucocorticoids, thyroxine, and catecholamines also play roles in PSU growth, development, and function.

Bone

Increased secretion of sex hormones clearly initiates the pubertal growth spurt. About half of this effect of sex hormones is caused by their stimulation of the GH-IGF axis.⁵⁴⁶ The remainder of the effects of sex steroids on skeletal growth is direct.^{547,548}

Differences between the actions of sex hormones contribute to women's bones being shorter and narrower than men's.^{549,550} The basis for these differences are diverse and involve interactions with IGF-1 and effects on cortical, cancellous, and periosteal bone formation.

Estrogen and androgen both stimulate epiphyseal growth. Estradiol is the critical hormone that brings about epiphyseal closure.⁵⁴⁸ Estrogen also is particularly effective in reducing bone turnover. To some extent these effects may be prenatally programmed.⁵⁵¹ Bone accrual during puberty is a major determinant of adult fracture risk. Menarche after 15 years carries a 1.5-fold increase in fracture risk, and the risk rises with age of menarche.⁵⁵²

Adipose Tissue

Women have a greater percentage of body fat than men.⁵⁵³ During puberty, they develop both more and larger fat cells than men in the lower body, which favors a lower body (gluteofemoral) fat distribution, in contrast to men's upper-body (visceral) fat accumulation. The critical periods for establishment of the adipocyte population are fetal life and adolescence, after which lipid accumulation occurs primarily by cell hypertrophy.⁴⁰ Serum levels of leptin rise throughout puberty to reach higher levels in females than males,²⁸² whereas levels of the antilipolytic adipocytokine adiponectin remain stable in females but fall in males⁵⁵⁴ in response to androgen.⁵⁵⁵

Insulin signaling is of major importance to the size and function of adipose tissue—stimulating adipogenesis (development of preadipocytes into adipocytes) and lipogenesis, while inhibiting lipolysis.⁴⁰ Beta-adrenergic catecholamines stimulate lipolysis, countering inhibition by insulin.^{556,557} Visceral white adipose tissue (VWAT) lipolysis is less sensitive to insulin and more sensitive to catecholamines than subcutaneous SCWAT.⁵⁵⁷

Androgens cause a masculine physique at puberty primarily by inhibiting adipogenesis reciprocally to stimulating myogenesis.⁵⁵⁸ They act by inhibiting adipogenic differentiation of human mesenchymal pluripotent stem cells reciprocally to their stimulation of the myogenic lineage, in a dose-dependent fashion.⁵⁵⁹ Local androgen generation by adipose tissue as it differentiates in response to insulin⁵⁶⁰ likely serves to limit insulin-generated adipogenesis.⁵⁶¹ In adipocytes, androgen has been reported to inhibit lipogenesis.^{562,563} Androgen also

inhibits catechol-stimulated lipolysis in female SCWAT, opposing the effect of insulin, but not in omental WAT.^{564,565}

Estrogen has been reported to suppress lipogenesis through inhibition of adipocyte lipoprotein lipase activity, according to most in vitro studies.⁵⁶⁶ Estradiol attenuates the lipolytic response to catecholamines, specifically in SCWAT adipocytes,⁵⁶⁷ but promotes lipolysis by stimulating hormone-sensitive lipase.⁵⁶⁶ The development of obesity in postmenopausal women and oophorectomized animal models has led to the concept that estrogen deficiency causes obesity,⁵⁶⁸ but the role of FSH elevation in mediating this obesity by inhibiting the formation of brown adipose tissue⁵⁶⁹ confounds the interpretation of all such studies. Progesterone generally counters estrogen and androgen effects on white and brown adipose tissue in experimental models.^{568,570}

Female sex steroid effects on serum lipids are modest.^{571,572} Physiological (transdermal) estradiol replacement slightly raises high-density lipoprotein cholesterol (HDL-C)⁵⁷² and lowers very low-density lipoprotein (VLDL) triglycerides.⁵⁷³ The lower LDL-C during the normal luteal phase seems primarily because of its consumption by the corpus luteum as steroidogenic substrate.⁵⁷² Oral estrogen replacement therapy raises HDL-C more than does transdermal replacement, but differs from it in raising VLDL-triglycerides and decreasing LDL-C, whereas androgenic progestins lower HDL-C.⁵⁷⁴

Progesterone deficiency is responsible for the increased postprandial chylomicron triglyceride concentrations that occur as the result of low peripheral lipoprotein lipase activity when the pituitary-gonadal axis is acutely suppressed.⁵⁷⁵ In humans, use of the progesterone analogue megestrol acetate is approved to stimulate appetite and weight gain; use of progestins is associated with insulin resistance.⁵⁷⁶

Muscle

Testosterone administration increases muscle mass and decreases fat mass reciprocally. Androgen does so by promoting the commitment of mesenchymal pluripotent stem cells to the myogenic lineage while inhibiting adipogenesis.^{559,577} Testosterone effects are exerted via androgen-receptor-mediated and nonclassical pathways. Human skeletal muscle formation of DHT is mediated primarily by type 3 5- α -reductase.⁵⁷⁸ Androgens then exert dose-related stimulation of muscle cell hypertrophy, as well as hyperplasia along with associated tissues, such as motor neurons. Hyperandrogenic women have increased muscle mass and strength, which seems to give them an advantage in athletic competition.⁵⁷⁹ Consequently, there is active debate about regulation of women's androgen levels in elite athletic competition.

Central Nervous System

Concordance of gender identity (self-identification as male or female) and gender orientation (sexual preference) with gender assignment on the basis of genital anatomy is the norm, which is consistent with an important role of androgen in programming these aspects of neuropsychosocial development. However, nonhormonal genetic and epigenetic factors influence sexually dimorphic aspects of human development.⁵⁸⁰⁻⁵⁸²

Before sex hormone differences are detectable, several genes are differentially expressed in the brains of male and female mice.⁵⁸³ Sex chromosomes directly program sexually dimorphic neuronal differentiation⁵⁸⁴ and behaviors, such as aggression, parenting, and social interaction.⁵⁸⁵ The maternally inherited X chromosome is preferentially expressed in glutamatergic neurons of the cerebral cortex, and sex-specific imprinting of autosomal genes of the hypothalamus is common and appears to be the default state in females.^{586,587}

Testosterone exposure during both the period of transient activation of the HPG axis in the fetal-perinatal period and again during puberty plays a role in organizing neural gene expression and development in a sexually dimorphic manner according to extensive studies in animal models, which are consistent with observations in humans.⁵⁸⁸⁻⁵⁹⁰ The critical period for this hormonal programming on behavioral patterns closes after puberty. This has consequences both for sex-typical neuroendocrine function and sex-typical and nonsexual behavior that is activated by the pubertal hormonal milieu.^{404,588,591,592}

The critical period for hormonal sensitivity of sexually dimorphic areas of the brain occurs during the early newborn period of rodents, which is thought to be comparable to the early second trimester of humans.⁵⁹³ In rats, the preoptic nucleus of the hypothalamus is larger in males, and treatment of newborn females with testosterone (or estradiol) permanently increases neuronal development to duplicate this effect and causes subsequent masculinized sexual behavior and anovulation.^{13,594} The anovulation results from masculinization of the LH secretory pattern (that is, both increased LH secretion and suppressed capacity to mount LH surges in response to estrogen priming) that appear to be the consequence of permanent suppression of hypothalamic PR expression.¹⁶

Testosterone administration to experimental animals stimulates the growth of sexually dimorphic brain areas to adult male size.⁵⁹⁵ Maintenance of differences in adult nuclear size and androgen receptor expression of sexually dimorphic areas of the brain is dependent upon the ambient androgen level.^{596,597} Peripubertal testosterone and female hormone administration have different effects on behavior.^{595,598}

Several human brain structures are sexually dimorphic, some becoming so at puberty.⁵⁹⁹⁻⁶⁰¹ Characteristic cortical and subcortical sex differences are discernable at puberty by structural magnetic resonance imaging (MRI),^{589,602,603} although most human brains have a mosaic of "male" and "female" features.⁶⁰⁴ Fetal amniotic testosterone reportedly correlates positively with many of the regional male sexual dimorphisms in the gray matter, including the amygdala, as with diverse differences in gender behavior.^{603,605} Functional MRI has shown endogenous testosterone levels to correlate with, and exogenous testosterone administration to females to activate, amygdala and parahippocampal regions and other brain areas in response to social-affective stimuli.⁶⁰³ Studies in transsexuals have shown that virilizing doses of testosterone affects the size of specific cortical and subcortical areas of the brain, and antiandrogen/estrogen treatment robustly inhibited hippocampal size.⁶⁰⁶

Some testosterone effects are androgen specific.^{607,608} However, many testosterone behavioral effects appear to be mediated by intraneuronal aromatization of testosterone to estradiol in a manner that is regulated in site-specific fashion by androgen and estrogen.⁶⁰⁹⁻⁶¹¹ Thus it has been postulated that low levels of estradiol promote the development of the brain and greater amounts masculinize it. These higher levels of estradiol are generated in the male brain by neuronal aromatization of circulating testosterone. ER α knock-out in female mice reduces sexual behavior and parenting behavior, while increasing aggressiveness.⁶¹²

The mechanism by which estrogens mediate androgenic masculinization of rodent sexual behavior involves prostaglandin E₂ mediation.^{593,613} Estradiol acts through ER α to induce microglia, the resident immune cells of the brain, to secrete prostaglandin E₂, which reduces DNA methyl transferase activity so as to release epigenetic repression of the default female behavioral state;⁵⁸⁷ this initiates enhanced neurite dendritic spine formation and masculinized behavior. Release of epigenetic repression appears to be an important postreceptor mechanism of testosterone action in the brain, also affecting

embryonic neural stem cells.⁶¹⁴ The involvement of prostaglandin E₂ in mediating the androgen effect on neural synapsing lends credence to a role for androgen excess contributing to autism spectrum disorders.^{593,615}

This hormonal organization of the brain involves hormone-specific effects on cell proliferation versus survival and synapse formation versus pruning.^{588,589} Androgens have a trophic effect on the dendritic spine cells of sexually dimorphic nuclei of rodents that promotes increased synaptic density.⁵⁹³ Estrogen alters the pattern of synaptic connections in spatially-specific and precise patterns that appear to fine-tune the sensitivity of certain regions of the brain to excitatory and inhibitory amino acids.^{616,617} Hypothalamic changes in synaptic remodeling have been correlated with the preovulatory surge of GnRH. There is also sexual dimorphism in cerebral progesterin receptors, and progesterone attenuates testosterone effects on the brain.^{618,619} Progesterin-estrogen administration is neuroprotective in animal studies.⁶²⁰ These hormones may counteract brain and spinal cord injury in adult women,^{620,621} but not in the sexually immature state.⁶²²

On average, women tend to perform better than men on tasks that involve object memory, verbal skills, processing speed and accuracy, and fine-motor skills, whereas men tend to excel in visual-spatial memory, while the sexes do not differ in vocabulary or math skills.^{590,592,623–625} These differences are quantitatively modest, of the order of 0.4 to 1.0 standard deviation (SD), leading, therefore, to large overlaps in these skills among the sexes. The male advantage in visual-spatial skills is established by 4.5 years of age. Because both boys and girls who are congenitally sex hormone deficient are relatively poor in visual-spatial abilities, and sex hormone treatment at puberty does not ameliorate these deficits, this difference seems to be the result of estrogen-mediated patterning in both sexes. The extent to which this difference is innate or because of socio-cultural factors is a subject of considerable debate.

A wide variety of gender dimorphic behaviors are found in young children, but normally they have a different character than in adults.⁶²⁶ Gender identity is established in midchildhood,⁵⁸¹ probably by 3 years of age.⁶⁰⁰ Sexual orientation is established by 10 years of age; it has been postulated that this is dependent upon adrenarche rather than true puberty.⁴⁰⁴ Early pubertal amounts of androgen or estrogen have little effect on sexual behaviors, but increase some aspects of aggressive behavior.^{627,628} Only in later puberty is there activation of the sex drive, which has been programmed in earlier development.

Discordant gender identity (transsexualism/transgenderism) and sexual orientation (homosexuality, bisexuality) occur in a small proportion of the population.^{580,581} Their prevalence seems to be increased in disorders of sex development (DSD). Studies of DSD indicate that a male level of androgen acting through the androgen receptor pre- or perinatally is an important determinant of male gender behavior (role) and mildly disruptive to female gender identity.^{600,629} However, DSD is uncommon among homosexuals and transsexuals, whereas heritability estimates approximate 20% to 60%.^{580,581} Neuroimaging studies suggest that these disorders have a biological basis. Homosexuality is associated with loss of sex differences in brain structures^{630,631} and transsexuality with less differentiation of brain areas dealing with body and self-perception.⁶³⁰ Neuroimaging indicates that male homosexuals have a pattern of nuclear activation in response to pheromone-like chemosignals resembling heterosexual women rather than that of heterosexual men, and homosexual women have an intermediate type of activation.⁶³²

Androgen and estrogen metabolites in sweat and urine, which contain unusual steroids, such as androst-4,14-diene-3-one,⁶³³ have been found to exert sexually dimorphic activation of the anterior hypothalamus that is independent of their

odor.⁶³² Therefore they appear to act as pheromone-equivalent chemosignals. Human pheromones appear to modulate the timing of ovulation⁶³⁴ and mood.⁶³⁵ It is likely that a dedicated population of olfactory receptors that project to GnRH neurons act as pheromone receptors.⁶³⁶

Other Targets of Sex Hormone Action

Sex steroid hormones affect a wide variety of tissues in ways that are often unrecognized. An estrogen effect on stabilizing muscle integrity has been noted in muscular dystrophy.⁶³⁷

Autoimmune disorders are in general more common in females, particularly after puberty.⁶³⁸ Estrogen downregulates blood levels of the inflammatory cytokine interleukin-6⁶³⁹ and thymic autoimmune regulator (*AIRE*) gene expression.⁶⁴⁰ Progesterone has a similar effect and androgen the opposite effect on *AIRE*. Sex dimorphism in predisposition to autoimmune disorders is partly explicable by sex hormone action on *AIRE* network genes during the neonatal minipuberty.⁶⁴¹ However, sex genotype influences the autoimmune system independently of sex steroids.^{585,638}

The cardiovascular effects of estrogen include upregulation of estrogen and PRs in vascular tissue and nongenomic effects on endothelial nitric oxide synthase.⁶⁴² Estrogen improves the disturbed endothelial dysfunction of young hypogonadal women and is necessary for the cardioprotective effect of exercise.⁶⁴³ Estradiol replacement therapy, oral or transdermal, lowers blood pressure, although estradiol causes salt and water retention.⁵⁷¹ This contrasts with contraceptives containing the more potent estrogen ethinyl estradiol, which raise blood pressure significantly, unless containing an antimineralocorticoid progesterin.

Estrogens and progestins also exert hemostatic effects that are associated with increased resistance to the anticoagulant action of activated protein C.⁶⁴⁴ Combined oral contraceptives containing estrogen carry about a fourfold increased risk of venous thromboembolism in first-time users.^{645,646} The risk falls with decreasing dose of estrogen and duration of use and rises about 50% in those containing third-generation (e.g., desogestrel) and antiandrogenic progestins.⁶⁴⁷ Nevertheless, the risk is less than that of pregnancy. Progesterin-only contraceptives are not associated with any increased risk of venous or arterial thrombosis.^{645,648}

The differences between the sexes in lipid levels are not explained by physiological differences in estrogen levels.⁶⁴⁹ Although oral estrogens raise triglycerides, this is caused by a first-pass hepatic effect. Differences in androgen (lowers HDL-cholesterol) and progesterone (lowers triglycerides and HDL-cholesterol) levels only explain part of the difference.

NORMAL HORMONAL AND SEXUAL DEVELOPMENTAL STAGES

The Fetus and Neonate

The fetus grows in a richer steroidal milieu than the pubertal female owing to the function of the fetoplacental unit. Concentrations of estrogens in fetal serum are extremely high. Umbilical cord plasma free testosterone levels are modestly greater than those of normal adult females.⁴³⁶ Dehydroepiandrosterone sulfate is at an adrenarchal level. The newborn shows some signs of the pubertal degree of hormonal stimulation from the intrauterine environment. Hypertrophic labia minora and superficial cell transformation of the urogenital epithelium are consistently observed estrogen effects, and a palpable breast bud is present at term in one-third of babies.⁶⁵⁰ The mean (SD) uterine length at birth is 4.15 ± 0.56 cm.⁶⁵¹ Sebaceous gland hypertrophy results from the androgenic state,⁶⁵² and the

clitoral shaft sometimes is prominent, particularly in small premature babies.⁵¹⁹

Steroid hormone levels from birth through puberty are shown in Table 16.1.^{15,66,69,93,650,653–656} Upon birth, withdrawal from the intrauterine environment occurs. Pituitary-gonadal axis hormone levels fall to a prepubertal-like nadir within days of withdrawal from the intrauterine environment.¹⁷ Menstrual bleeding and colostrum production sometimes occur as the newborn is withdrawn from the estrogenic environment. The mini-puberty of the newborn then begins.

This neonatal mini-puberty evolves according to a developmental program determined by gestational age. At term gestational age, it commences with a gradual but transient rise to pubertal hormone levels. In girls these reach maximal values in the early pubertal range at 3 to 4 months of age, about 2 months later than in boys, before they regress as seen in Figs. 16.5 and 16.8. The activation of the HPG axis of the newborn stimulates breast and genital tract development that commonly persists for several months.^{657,658}

Premature neonates, in contrast to term newborns, develop high gonadotropin levels, of the magnitude seen in ovarian insufficiency, that persist until antral follicle development begins near 40 weeks, gestational age.^{17,18,659} As antral follicles develop, ovarian estrogen and AMH secretion commence, and the compensatorily high gonadotropins gradually fall to the low levels normal for term infants.¹⁷ Coincidentally, adrenal contributions to steroid intermediate levels are higher in premature infants because of the persistence of the fetal adrenocortical zone and immaturity in size and apparent 11β -hydroxylase activity of the definitive adrenocortical zones.^{660–664}

Transient ovarian hyperstimulation has been reported in preterm babies as a consequence of high gonadotropin levels persisting until or beyond late-term corrected gestational age.^{665–667} It manifests at several months of age as ovarian cysts with hyperestrogenism, causing genital swelling, persistent breast development, and/or delayed menstrual bleeding.

These phenomena then regress progressively through later infancy as the inhibitory tone of the neuroendocrine-gonadal axis undergoes juvenile maturation. Nevertheless, according to an ultrasensitive recombinant cell bioassay, girls' estrogen levels in late infancy are several fold greater than those of boys, averaging 1 pg/mL and ranging up to 3 pg/mL.⁶⁶⁸ On occasion, there may be subclinical but detectable estrogen effects on urogenital cytology.⁹⁵ Whether the transient "mini-puberty" activity of the neuroendocrine-gonadal axis in the newborn has a programming influence on subsequent behaviors remains unclear.⁶⁰⁰

The developmental pattern of serum AMH differs from that of other reproductive hormones because it reflects follicular growth and development rather than neuroendocrine activity. AMH rises from undetectable to low in cord blood to 0.6 to 4.1 ng/mL (4.3–29 pM) at 3 months; it then continues to slowly rise about 1.5-fold more to reach an adult level by the postmenarcheal period.⁶⁶⁹

Childhood

As the neuroendocrine-gonadal axis becomes quiescent and the fetal zone of the adrenal cortex regresses, steroid hormone levels fall through infancy to reach a nadir in midchildhood (see Table 16.1). The earliest hormonal change during childhood is the adrenarchal rise in serum DHEAS that is discernable at about 6 years (see Table 16.1). Although childhood gonadotropin levels are low and there is seldom obvious sexual development as a consequence of prepubertal gonadotropin production, there is a low level of bioactive gonadotropin production and ovarian follicular development^{31,86,670} and occasional evidence of transient estrogen secretion.⁶⁷¹ In

midchildhood, GnRH agonist stimulation of gonadotropin secretion elicits a prompt small rise in estradiol secretion.^{93,672} AMH levels of girls rise minimally in midchildhood, to levels about 3% those of boys.⁶⁶⁹

In late prepuberty, girls begin to experience increasing diurnal production of gonadotropins, and estradiol levels rise in diurnal fashion to approximate 10 pg/mL in midmorning.^{79,97}

Adolescence

Hormonal

The earliest hormonal changes of true puberty occur gradually during late preadolescence. Clinically prepubertal 10-year-olds develop greater average gonadotropin and sex hormone levels than do prepubertal 7-year-olds.⁷⁹

In the average girl, serum gonadotropins achieve pubertal levels after 8 years of age. However, the chronological age at which puberty begins varies considerably among children. Therefore the pubertal rise in gonadotropins is best appreciated by relating gonadotropin levels to pubertal stage. Daytime serum LH rises 25-fold from prepuberty to late puberty according to bioassay, but this rise is underestimated by polyclonal RIA.⁹⁹ "Third-generation" immunometric assays, using monoclonal antibodies and a more purified standard than earlier RIAs, show a rise similar to that found by bioassay.^{77,79}

The hallmark of early puberty is an increase in the sleep-related rise in LH (see Fig. 16.12).^{79,98} Daytime sampling underestimates the rise in gonadotropins in early puberty because it does not detect most of this sleep-related increase. In early puberty, current assays show that LH rises during sleep to reach peaks in the lower adult range, generally above 1.0 U/L and then typically falls during the day to 0.6 U/L or less.^{79,93,673,674} A single daytime sample also does not necessarily truly represent a child's pubertal status because it does not account for episodic and cyclic changes in gonadotropin secretion.^{94,675} The serum LH response to GnRH is slightly more indicative of the pubertal status than a morning basal sample (see Fig. 16.9) (Table 16.1).^{87,673,674} An LH level 1-h post-GnRH agonist of 3.2 U/L or more is 90% sensitive and 5.5 U/L or more is 95% specific for the onset of puberty in girls.⁹³ The response of LH to GnRH or GnRH agonist administration increases more than that of FSH during puberty, with a resultant increase in the LH:FSH ratio.^{77,676} GnRH agonists add a dimension to GnRH testing: they provide a sufficiently potent and prolonged stimulus to LH and FSH release to bring about an increase in ovarian estradiol secretion in pubertal girls.⁶⁷⁷ These responses likewise increase characteristically with sexual maturation (see Fig. 16.6).⁶⁸

Sex hormone levels rise further as the consequence of ovarian and adrenal maturation. Pubertal levels are intermediate between those of prepubertal and sexually mature individuals. Table 16.1 shows typical normal ranges for serum levels of the major steroid hormones. Once pubertal levels of estrogens and androgens are achieved, their effects ordinarily become obvious within 6 months.

Serum AMH levels stabilize at an adult level of 0.5 to 6.2 ng/mL (3.5–45 pM) in postmenarcheal females with normal ovarian morphology.^{40,669} They do not fluctuate during the normal menstrual cycle,⁶⁷⁸ reflecting a balance between recruitment of growing follicles and growth of antral follicles.⁶⁶⁹ The AMH serum level is an indicator of the number of growing follicles, and thus indexes the size of the oocyte pool ("ovarian reserve"); AMH begins to fall with the oocyte pool in the premenopausal adult⁶⁶⁹ and becomes undetectable after menopause.⁶⁷⁹ AMH levels also reflect the intrafollicular androgenic status of fertile females, probably because androgens stimulate the early phases of follicular growth.⁴⁰ Serum prolactin rises moderately

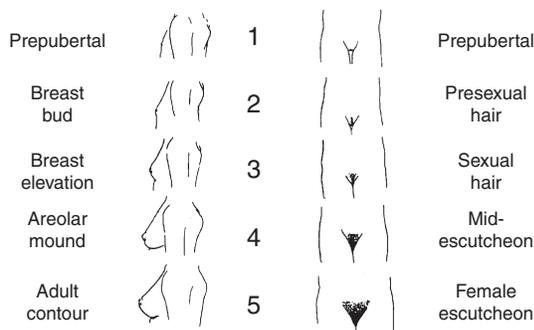


Fig. 16.30 The stages of breast and pubic hair development. (Modified from Ross, G.T., Vande Wiele, R. (1974). *The ovary*. In: Williams, R.H. (ed.), *Textbook of Endocrinology*, 5th ed. Philadelphia, WB Saunders.)

in females at about 14 years of age.⁶⁸⁰ This may be a response to estrogen secretion because it does not occur in boys.

Clinical

The first physical sign of puberty is breast development (thelarche). In a minority of girls, pubic hair development (pubarche) occurs before thelarche. Thelarche represents a response to estrogen, and pubarche a response to androgen. When pubarche precedes thelarche, it is usually a reflection of adrenal androgen production (adrenarche) rather than a sign of true puberty. The stages of breast and pubic hair development are shown in Fig. 16.30.^{681,682} Tanner stage 1 is prepubertal. The initial stage of breast development (stage 2, B2) is appreciated as a palpable subareolar bud before it can be seen as an elevation. Stage B3 is obvious enlargement and elevation of the whole breast. Stage B4, the phase of areolar mounding, is very transient and may not necessarily appear. Stage B5 is the stage of attainment of mature breast contour. Pubic hair first starts as presexual pubic hair development (PH2)—hairs which are shorter, lighter, and straighter than sexual pubic hairs, but longer than vellus body hair. Hypertrichosis is sometimes mistaken for stage 2 pubic hair; these can be differentiated by comparing genital hair to that on the forearm. Sexual pubic hair development (PH3)—curly terminal (long, dark) hair—usually commences on the labia majora before spreading to the pubis. Pubic hair then gradually progresses to the mature female escutcheon (inverted triangle pattern, stage 5). Axillary hair usually appears about a year later than pubic hair and passes through similar stages.

The age at which pubertal milestones are normally attained is not known with certainty. There has been considerable debate about the normalcy of pubertal changes between 6.0 and 8.0 years of age.^{190,203,683} The debate stems from office practice observations that breast and pubic hair development were found more frequently than expected in black girls in this age range. The prevalence of pubertal milestones has subsequently been estimated for the general US population by modeling cross-sectional data collected 1988 to 1994 on children 8.0 years of age and older by the National Health and Nutrition Examination Survey III (NHANES III).^{198,199,276} NHANES data had the advantage of nationally representative sampling, but the breast stage data was based on observation and so data quality was questionable, and modeling assumptions that permit extrapolation to younger ages may not be valid.^{276,684,685} The median ages at which the major pubertal milestones were attained in normal-weight US girls according to this database are given in Table 16.3.²⁷⁶ These data indicated that puberty begins before 8.0 years in less than 5% of the

TABLE 16.3 Pubertal Milestone Attainment in Normal Body Mass Index Girls in the General US Population, NHANES III, 1988–1994.

Stage	5%	50%	95%
Breast Stage 2	8.25	10.2	12.1
Pubic Hair Stage 3	9.25	11.6	13.9
Menarche	11.0	12.6	14.1

Before age 8.0 years breasts appeared in 12% to 19% and sexual pubic hair (stage 3) in $\leq 3\%$ of normal-body mass index in non-Hispanic black and Mexican American girls. Menarcheal milestones are attained at similar ages in these ethnic groups except for the 5th percentile being significantly earlier in blacks (10.5 y) than non-Hispanic whites (11.3 y). (Modified from Rosenfield, R.L., Lipton, R.B., Drum, M.L. (2009). Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics*, 123, 84–88.)

normal general female population, although breasts may normally appear during the seventh year in blacks and Mexican Americans.

A more contemporary study of female puberty was conducted from 2004 to 2011. The design was improved: data collection was longitudinal and breast development was ascertained by palpation. However, although the design included broad racial/ethnic and socioeconomic representation, it was not nationally representative, the study population enriched for Asians, for example.^{686,687} The overall distribution of pubertal timing was shifted to earlier ages by approximately 0.75 year for breast development and 0.35 year for menarche compared with NHANES III. The data confirmed that normal-weight non-Hispanic black girls achieved pubertal milestones earlier than white girls, by about 0.75 to 1.0 year for thelarche and 0.5 to 0.75 year for menarche; thus thelarche normally may occur in the seventh year of life in blacks. Pubertal milestones in Asian girls were similar to those in whites, and in Hispanics to blacks.

Obesity and ethnic factors now appear to be independent factors that advance the onset of puberty and menarche, with obesity having the greater influence.^{276,686} Longitudinal study indicates that obesity is associated with advancement of the age of thelarche (0.7 year) more closely and twice as much as advancement of the age of menarche (0.3 year).

Pubertal tempo, the span of time between the onset of breast development (B2) and menarche, is normally 2.3 ± 1.0 years. However, obesity is paradoxically associated with significant advancement of the age of thelarche while significantly slowing pubertal tempo and preserving height potential.^{687,688} It is possible that the advancement of the age of thelarche by obesity is not entirely because of an advancement of neuroendocrine puberty but rather arises in part from extragonadal formation of estrogen from adrenal precursors in excess adipose tissue; this may explain, in part,⁶⁸⁹ the apparent blunting of early neuroendocrine puberty in obese girls with early thelarche.^{93,687,690} On the other hand, a subgroup of early maturers with a history of intrauterine growth retardation seem to have an unusually rapid tempo of puberty and lose height potential.⁶⁹¹

The onset of puberty is more closely related to an individual's bone age than to chronological age. This is particularly important in the case of subjects who are later than average in entering puberty, as discussed previously. The great majority of girls can be expected to begin puberty by the time their skeletal age reaches 12.5 years and to experience menarche by the time skeletal age reaches 14 years.

The pubertal growth spurt in girls occurs during early adolescence. The peak of linear growth velocity corresponds most closely with stage B2⁶⁹² and the increase in serum alkaline phosphatase levels with B3.⁶⁹³ Fat accumulation increases

and fat distribution changes as well.⁶⁹⁴ As a consequence of these pubertal changes occurring out of phase with chronological age, girls begin to differ considerably in size and habitus during the ninth year of life.

Adult Menstrual Cycle

The menstrual cycle of young adults averages 28 days in length (normal adult 24–38 days).⁶⁹⁵ The variation in cycle length is almost entirely because of differences in the duration of the follicular phase. The luteal phase, the time between ovulation and the onset of menses, invariably lasts 14 ± 1 (SD) days.¹²⁶

The cyclic changes of LH, FSH, estradiol, and progesterone serum levels during the menstrual cycle are shown in Fig. 16.13. Diurnal and episodic fluctuations are superimposed upon these cyclic changes. Since testosterone and androstenedione have both adrenal and ovarian origins, their levels fluctuate to some extent in cyclic, diurnal, and episodic patterns. For example, testosterone levels tend to be 20% greater in the morning than in the evening and to double in midcycle.^{125,696} The normal range for most of the important ovarian sex hormone levels of women during the early follicular phase of the menstrual cycle is given in Table 16.1. Progesterone levels are below 100 ng/dL until the periovulatory phase of the cycle and then peak to over 500 ng/dL in the midluteal phase. Hormonal production rates for the midfollicular phase in women are given in Table 16.2. Serum prolactin increases transiently in midcycle with maximum ovarian estradiol secretion.⁶⁹⁷ Prolactin levels also transiently rise in response to mammary stimulation and psychological factors.⁶⁹⁸

Normal Variations in Pubertal Development

Although the onset of breast development (stage B2) characteristically precedes the appearance of sexual pubic hair (PH3) and the onset of menses substantially (see Table 16.3), there is considerable variation in the sequence of these events. Pubic hair may appear before breasts begin to develop, a situation arising from lack of direct linkage between adrenarche and gonadarche. Menarche may occur within months after the appearance of breasts; however, this is so unusual that its occurrence demands exclusion of an abnormal hyperestrogenic state.

A common normal variant is the unilateral onset of breast development. Unilateral breast development may exist up to 2 years before the other breast becomes palpable. This phenomenon seems related to an asymmetry that normally persists into adulthood. Excisional biopsy of a normal unilateral breast papilla in search of a nonexistent tumor should be avoided, because such a procedure excises the entire breast anlage.

Two extreme variations of normal are the most common causes of premature sexual development.⁶⁹⁹ These are the isolated appearance of breast development (premature thelarche) and the isolated appearance of sexual hair (premature pubarche).

Premature Thelarche

Breast development before 8.0 years is traditionally considered premature. Premature thelarche is a very mild, non- or slowly progressive incomplete form of premature puberty that is a variant of normal. In the 6- to 8-year age group, it is usually because of obesity, which may either accelerate the onset of puberty, account for increased peripheral estrogen production, or cause artefactual adipomastia.^{93,686,687,690,700} Otherwise, it usually seems to be caused by idiopathic subtle overfunction of the pituitary-ovarian axis, occurring in those girls whose FSH levels tend to be sustained about the upper end of the

prepubertal normal range.⁶⁷¹ Average serum levels of FSH at baseline and in response to GnRH are significantly increased, whereas those of LH are not. Estradiol levels are generally below the level of detection in most standard assays, but are significantly elevated according to ultrasensitive assay,⁷⁰¹ and intermittent low-grade estrogenization of the urogenital mucosa is sometimes found (see Fig. 16.11). Ovarian ultrasound examination shows an increased prevalence of antral follicles (“microcysts”) and uterine enlargement.⁷⁰² Nevertheless, a growth spurt does not occur, the bone age advancement rate is not abnormal, and menses do not appear until the usual age.

In infants, the syndrome seems to be caused by a lag in inhibition of the transient activation of the HPG axis of the newborn and is usually unsustained. In older children, the breast development is more likely to persist. A subgroup with “exaggerated thelarche” has an increased growth rate with relatively proportionate bone age advancement. Their unsustained or intermittent neuroendocrine activation seems to lie on a spectrum between ordinary premature thelarche and true sexual precocity (Fig. 16.31).⁷⁰³ However, the McCune-Albright syndrome mutation is found in the peripheral blood of about 25% of such patients.⁷⁰⁴ Premature thelarche may be the first sign of feminizing disorders (see Precocious Puberty). Therefore follow-up of these patients is indicated.

Premature Pubarche

The isolated appearance of sexual pubic hair before 8.0 years of age in girls (premature pubarche) is usually caused by premature adrenarche, as discussed next. However, it may occur at androgen levels that are normal for preschool children (idiopathic premature pubarche). This likely reflects increased sensitivity of the PSU to low preadrenarchal androgen concentrations, analogous to idiopathic hirsutism in adults, in which increased sexual hair growth occurs in the absence of other evidence of hyperandrogenism. The mechanism may be caused by increased androgen receptor gene activity.⁷⁰⁵

Premature adrenarche is a very mild, slowly progressive incomplete form of premature puberty that is usually a variant

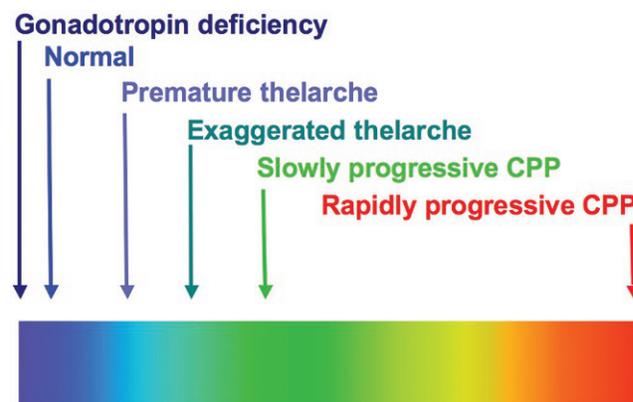


Fig. 16.31 The spectrum of gonadotropin secretion in girls with normal and abnormal puberty. Normal girls are conceptualized as having a small amount of pituitary-ovarian axis activation, which is more than that of congenitally hypogonadotropic girls. Premature thelarche, exaggerated thelarche, slowly progressive precocity, and rapidly progressive precocity fall along a spectrum of increasing activation of the axis—with deterioration of height potential occurring only in those near the most activated end. *CPP*, central precocious puberty. (Modified from Kreiter, M.L., Cara, J.F., Rosenfield, R.L. (1993). Modifying the outcome of complete precocious puberty: To treat or not to treat. In: Grave, G.D., Cutler, G.B. (eds.), *Sexual Precocity: Etiology, Diagnosis, and Management*. New York, Raven Press, p. 109–120.)

of normal. It usually appears to be caused by advanced development of the zona reticularis as indicated by increase of adrenal androgen levels above those of preschool children.^{15,706} The condition is ordinarily detected when a child presents with premature pubarche or other clinical manifestation of androgen action, such as adult-type body odor or acne.⁷⁰⁷ The androgen excess is ordinarily so subtle that there is no obvious growth spurt, the bone age typically does not advance abnormally, and there are no other signs of sexual maturation. The diagnosis requires biochemical demonstration of a serum steroid pattern indicative of adrenarche before 8 years of age in girls, for example, DHEAS 40 to 130 mcg/dL and other androgen levels only slightly elevated for age (see Table 16.1). The term is sometimes used to describe biochemical evidence of adrenarche irrespective of clinical manifestations.

Exaggerated adrenarche is a clinically extreme type of premature adrenarche.^{15,706} These girls have clinical features that suggest subtle androgen excess (e.g., significant bone age advancement, but not clitoromegaly) or insulin resistance (e.g., central adiposity or acanthosis nigricans). Such children generally have a slightly advanced onset of true puberty, but height potential is not compromised. The term exaggerated adrenarche has been variously applied but is here when the atypical clinical picture is associated with serum androgen levels above normal for early puberty (see Table 16.1), for example, DHEAS above 130 mcg/dL or testosterone over 35 ng/dL, or bone age above height age by 20%. Virilizing disorders should be excluded in girls with exaggerated adrenarche so defined.

The cause of premature adrenarche usually seems simply to be caused by advanced onset of normal zona reticularis development, but it sometimes seems to be an early manifestation of the steroidogenic dysregulation of polycystic ovary syndrome.^{15,706} Premature adrenarche appears to carry about a 10% to 20% risk of developing polycystic ovary syndrome; it is unclear whether those with exaggerated adrenarche are primarily at risk. Premature adrenarche with insulin resistance is independently associated with obesity, rapid weight gain during early childhood, and low birth weight.^{15,383,708} The IGF-1 excess of GH treatment may be a risk factor.⁷⁰⁹ Associations also exist between premature adrenarche and temporal lobe lesions⁷¹⁰ and poliomyelitic scoliosis.⁷¹¹ Heritable factors are also important. The heritability of DHEAS sulfate blood levels is estimated at 26% to 66%.⁷¹² Carriers for CAH may be over-represented in this group.^{713,714} Epigenetic programming seems to be a factor: prenatal virilization of nonhuman primates causes functional adrenal hyperandrogenism as part of the PCOS spectrum,⁷¹⁵ and fetal undernutrition has been postulated to program for insulin resistance by activating the limbic-hypothalamic-pituitary-adrenal system to cause fetal corticoid excess.⁷¹⁶

The differential diagnosis of premature pubarche and adrenarche includes virilizing disorders, of which nonclassic CAH is the most common. Girls with premature adrenarche should be followed through puberty for the possible development of hyperandrogenism. A suggested approach to rule out serious hyperandrogenic disorders in childhood is suggested in the accompanying algorithm (Fig. 16.32).⁷¹⁷

Constitutional Delay of Growth and Pubertal Development

By statistical definition, delayed puberty occurs in 3% of girls. Most of these girls are otherwise normal, in which case this is termed *constitutional delay of growth and pubertal development* (CDGP). It is familial;^{200,201} 80% in a large series had a parent with objectively delayed puberty.⁷¹⁸ A family history of delayed puberty is found in 50% to 75% of patients with CDGP.²⁰¹ Although its inheritance is likely complex, some predisposing

genetic factors seem to have a dominant effect. It has long been recognized that delayed puberty was overrepresented in families with idiopathic hypogonadotropic hypogonadism or hypothalamic amenorrhea cases, and rare variants in genes underlying these conditions appear to contribute to the etiology.^{719,720} Recent molecular investigation revealed that CDGP indeed segregates within families with complex patterns of inheritance that include X-linked, autosomal dominant and recessive and bilinear,²⁰⁰ although sporadic cases also occur. Autosomal dominant is the most prevalent pattern of inheritance (with or without complete penetrance).^{200,721} Initially, candidate genes associated with CDGP have been identified using GWAS, linkage analysis, and targeted sequencing strategies.^{718,722} However, recently whole exome and genome sequencing are increasingly being used to identify novel candidate genes. Despite these advances, the genetic basis and neuroendocrine pathophysiology remains unknown in the majority of patients with CDGP.

Mutations in several genes have been associated with CDGP. Six unrelated families from a Finnish cohort with delayed puberty were found to have two mutations in immunoglobulin superfamily member 10 (IGSF10)⁷²³ and four other families were found to have two rare variants with unknown significance. Mutations in IGSF10 appear to cause decreased levels of IGSF10 expression during embryogenesis resulting in delayed migration of GnRH neurons from the olfactory bulbs to the hypothalamus. The dysregulation of GnRH neuronal migration results in an abnormal configuration of the GnRH neuronal network and a functional defect in GnRH secretion that becomes crucial when a threshold level of GnRH secretion must be achieved for pubertal onset. IGSF10 mutations were also found in women with a hypothalamic amenorrhea and in patients with congenital hypogonadotropic hypogonadism (CHH), although these mutations did not alone appear sufficient to cause the phenotype. Loss-of-function mutations in *IGSF1* have been identified in patients with X-linked central hypothyroidism,⁷²⁴ delayed pubertal growth, and delayed increase in testosterone levels.⁷²⁵

Mutations in genes found in patients with CHH are also found in CDGP. Mutations in heparan sulfate 6-O-sulfotransferase 1 (HS6ST1), fibroblast growth factor receptor 1 (FGFR1), and Klotho β (KLB) have been found in several kindreds with CHH and their relatives with CDGP.⁷²⁶⁻⁷²⁸ Variants in the genes encoding gonadotropin-releasing hormone receptor (*GNRHR*), tachykinin 3 (TAC3) and its receptor (TACR3), interleukin-17 receptor D (IL17RD), and semaphorin 3A (SEMA3A) known to cause CHH have been found in patients with CDGP.⁷²⁹ Mutations in 24 genes associated with GnRH deficiency were found in probands with CHH with greater frequency than in CDGP, leading to the conclusion that CHH and CDGP are likely to have different genetic backgrounds.⁷³⁰ Mutations in Kallmann syndrome genes such as anosmin 1 (*ANOS1*) and N-methyl-D-aspartic acid receptor synaptonuclear signaling and neuronal migration factor (*NSMF*) have not to date been identified in pedigrees with CDGP. Loss-of-function mutations within the GnRH receptor are the most frequent cause of autosomal recessive CHH, accounting for 16% to 40% of patients. Mutations have been found within the extracellular, transmembrane, and intracellular domains of the receptor leading to impaired GnRH action.⁷³¹ A homozygous partial loss-of-function mutation in *GNRHR* was found in two brothers, one with CDGP and one with idiopathic HH,⁷³² and a further heterozygous mutation was found in one male with self-limited CDGP.⁷³³ Thus the genetic background of CHH and CDGP may be different, or shared by as yet undiscovered genes.⁷³³

Girls with constitutional delay are generally more slight in habitus and have lower bone mineral density upon entering

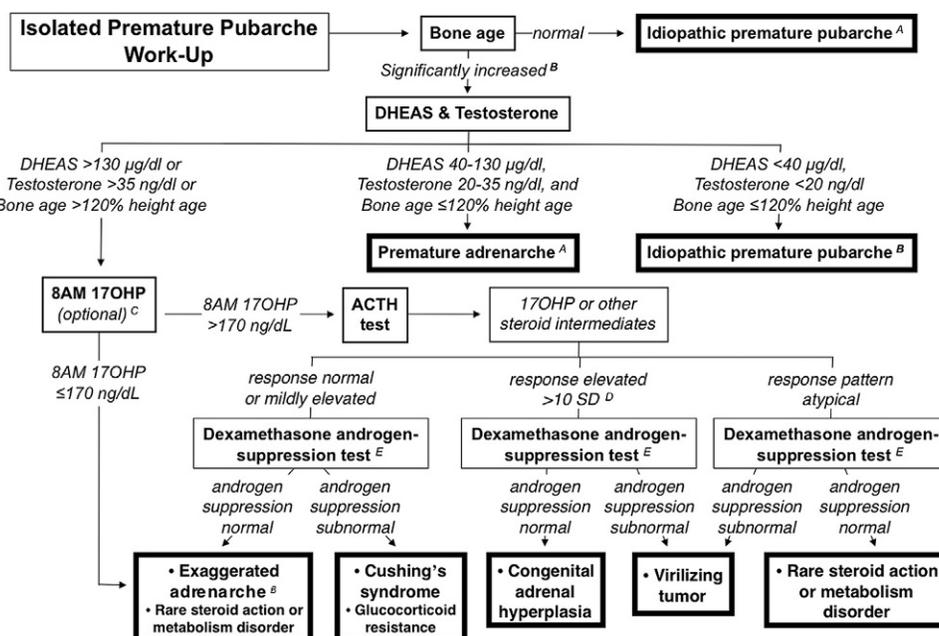


Fig. 16.32 A workup to screen for hyperandrogenic-virilizing disorders as a cause of premature pubarche. Premature or exaggerated adrenarche accounts for the great majority of premature pubarche; nonclassical congenital adrenal hyperplasia caused by 21-hydroxylase deficiency, the most common hyperandrogenic cause, accounts for less than 10% in most populations/ethnic groups. *SD*, Standard deviations. (Modified with permission from Rosenfield, R.L. (2018). Premature adrenarche. UpToDate.)

Footnotes:

- Diagnoses of idiopathic premature pubarche, premature adrenarche, or exaggerated adrenarche, or based on bone age and/or hormonal measurements are provisional diagnoses. Even if these tests are normal or only mildly elevated, clinical follow-up is indicated to rule out inordinate progression of pubarche or evidence of virilization. This is because bone age interpretation and the precision of testosterone assays at low levels are often problematic, and because this algorithm will not detect some very mild hyperandrogenism cases.
- A bone age that is significantly advanced for chronological age is an indication for a screening laboratory workup for childhood hyperandrogenism, consisting of dehydroepiandrosterone sulfate (*DHEAS*) and testosterone levels. A bone age that exceeds height age indicates less than average height potential. A compromised height potential (indicated by a predicted adult height less than expected for the family or a bone age >120% of height age in a young child) or *DHEAS* or testosterone level above the normal adrenarchal range (130 mcg/dL and 35 ng/dL, respectively) suggests that the premature pubarche may be caused by a virilizing disorder rather than ordinary premature adrenarche.
- An 8:00 AM baseline measurement of 17-hydroxyprogesterone (*17-OHP*) is an option to an adrenocorticotropic hormone (*ACTH*) test in a family, population, or ethnic group in which the risk is relatively low for nonclassical congenital adrenal hyperplasia caused by 21-hydroxylase deficiency. A level over 170 ng/mL (5.1 nmol/L) has a 95% or greater sensitivity and specificity for this disorder among premature pubarche patients when obtained at 8:00 AM, before the level wanes with diurnal *ACTH* secretion. A normal baseline level does not necessarily exclude this or more rare forms of nonclassical congenital adrenal hyperplasia.
- For *ACTH* testing, the typical response in congenital adrenal hyperplasia (*CAH*) is that the steroid immediately before the enzyme block is extremely elevated, and steroids earlier in the biosynthetic pathway are successively less elevated the further removed they are from the block. For example, in 21-hydroxylase deficiency, 17-OHP is extremely elevated and androstenedione and testosterone are successively less elevated; in 3β-hydroxysteroid dehydrogenase type 2 deficiency, 17-hydroxypregnenolone and *DHEA* responses are extremely elevated and 17-hydroxyprogesterone, androstenedione, and testosterone are mild to moderately elevated. An elevated baseline level of 17-OHP may preclude a clear response to the *ACTH* test. For *ACTH* testing, an atypical response pattern is one that is not typical for any type of *CAH*. An example of an atypical response would be baseline or post-*ACTH* androstenedione or testosterone elevations greater than those of 17-OHP.
- The dexamethasone androgen-suppression test consists of administering dexamethasone, 1 mg/m²/day in three to four divided doses daily for 4 days, and then measuring the serum cortisol, *DHEAS*, and androgens on the morning of the fifth day after a final dexamethasone dose. Normally, serum cortisol falls below 1 mcg/dL (28 nmol/L), testosterone to less than 10 ng/dL, and *DHEAS* to less than 40 mcg/dL. Serum androgen intermediates also fall to prepubertal concentrations.

puberty than earlier maturing girls.⁷³⁴ Girls do not usually become concerned about this until they enter high school at 14 years of age and realize that not only has pubertal development not begun, but also most of their friends are menstruating. When puberty does ensue in such subjects it is perfectly normal in tempo. Endocrinological status is normal for the stage of puberty. The differential diagnosis includes chronic endocrine, metabolic, and systemic disease of almost any kind, as well as gonadotropin deficiency, which it closely resembles and from which it is distinguished with difficulty (see Gonadotropin Deficiency and Functional Hypothalamic Anovulation).

Physiological Adolescent Anovulation

Immaturity of the hypothalamic-pituitary-ovarian axis causes menstrual cycles to be longer and more irregular during the early postmenarcheal years (Fig. 16.33).^{124,695,735} About half of menstrual cycles during the first 2 years after menarche are anovulatory by standard criteria; half of these actually have evidence of the attenuated ovulation that results in luteal insufficiency (see Luteal Phase Defects).^{124,736} Although about half of these cycles with ovulatory abnormalities are irregular, half are of normal length by adult standard, so normal adolescent

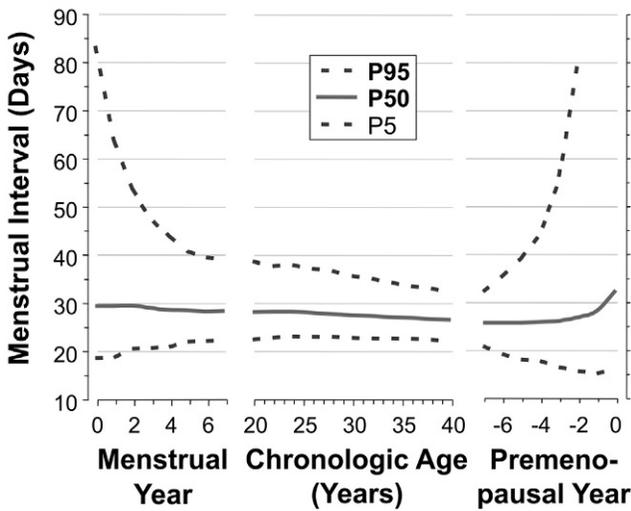


Fig. 16.33 Normal range for interval between menstrual cycles. Note that cycle intervals averaging more than 90 days or fewer than 21 (19 days in first year after menarche) days are abnormal at any age. P5 and P95 = 5th and 95th percentiles, respectively. (Modified from Treloar, A., Boynton, R., Benn, B., Brown, B. (1967). Variation of human menstrual cycle through reproductive life. *Int J Fertil*, 12, 77–84.)

menstrual cyclicity differs only slightly from that of reproductive-age adults. Thus most adolescent ovulatory abnormalities are asymptomatic, with cyclic menstrual bleeding occurring at 21- to 45-day intervals even in the first postmenarcheal year (Fig. 16.34); this paradox arises because immature cyclic ovarian function is usually occurring during these intervals.⁷³⁷ Serum hormonal changes during normal adolescent menstrual cycles confirm that substantial but immature cyclic follicular development occurs in such girls and some aluteal adolescents (Fig. 16.35).^{735,736,738}

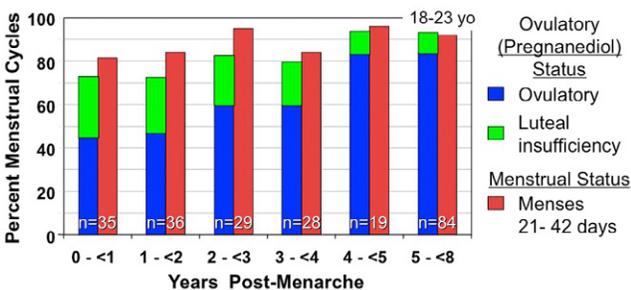


Fig. 16.34 Comparison of the percent of menstrual cycles that are 21 to 42 days duration (red) and percentage of menstrual cycles that are ovulatory (blue) by postmenarcheal age through young adulthood. Many more menstrual cycles are regular (21–42 days) than are normal, mature ovulatory cycles. Ovulation normalcy was determined with reference to adult standards of the major urinary progesterone metabolite, pregnanediol glucuronide, in weekly samples collected during the last 12 days of each menstrual cycle. Clearly detectable but subnormal pregnanediol is here designated as having luteal insufficiency (green). It can be seen that most of the cycles that are not mature ovulatory had sufficient cyclic follicular activity to generate an immature corpus luteum, which indicates antecedent ovulation, rather than being truly anovulatory as the investigators had labeled them. (Data from Metcalf, M.G., Skidmore, D.S., Lowry, G.F., Mackenzie, J.A. (1983). Incidence of ovulation in the years after the menarche. *J Endocrinol*, 97, 213–219; Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. With permission.)

Although there is considerable variation in the time it takes for menstrual cycles to mature, menstrual regularity approximates adult standards in most girls within a year of menarche: three-quarters have a cycle length between 21 and 45 days, and 5% more fall within these bounds each of the next 3 years.^{739,740} By 5 gynecological years, 90% of menstrual cycles last 22 to 40 days, and about 80% of cycles are normal ovulatory ones. Menstrual cycle length narrows to 24 to 38 days in midadulthood⁶⁹⁵ as ovulatory rates approach 90%.¹⁸⁷

Menstrual abnormalities in adolescence can be defined similarly to abnormal uterine bleeding in adults.^{736,741} Primary amenorrhea is failure to begin menses at a normal age (by 15 years of age or within 3 years of thelarche when it has been delayed). Secondary amenorrhea is the absence of menstrual periods for 90 days or more after initially menstruating. Oligomenorrhea is defined as subnormal menstrual frequency, the normal limits for which gradually change during the 6 years after menarche (Box 16.2). Anovulatory cycles may also cause excessive uterine bleeding, as discussed in the later section Ovulatory Dysfunction: Dysfunctional Uterine Bleeding.

Symptomatic menstrual abnormalities in adolescents are increasingly unlikely to represent “physiological” adolescent anovulation with time. By 1 year postmenarche, failure to establish and sustain a normal adult menstrual pattern carries approximately a 50% risk of persistent oligoovulation, and failure to do so by 2 years after menarche carries approximately a two-thirds risk (Fig. 16.36).⁵⁷⁵ Thus persistence of menstrual irregularity for 1 to 2 years or more is a strong indication for investigation (see section Abnormal Puberty).

Serum LH, testosterone, and androstenedione levels are significantly higher in adolescents with anovulatory than those with ovulatory cycles.^{742,743} It is unclear whether this is the cause or the result of the anovulation; however, if hyperandrogenemia is found, it seldom regresses.¹²³ A polycystic ovary is common in adolescents and is usually a variant of normal, unless associated with menstrual abnormality or hyperandrogenism.^{83,123,656,744–746}

Other Normal Adolescent Variations

Three-quarters of adolescent girls experience mild to severe comedonal acne, and one-quarter mild inflammatory acne.⁷⁴⁷ Mild hirsutism also arises commonly among perimenarcheal girls. However, inflammatory acne that is moderate or severe (i.e., >10 lesions of face or other region) is uncommon during the perimenarcheal years, and hyperandrogenism should be considered in such girls as it should in those with mild hirsutism and menstrual irregularity (see Hyperandrogenism in Adolescence). The initiation of acne is more closely related to blood levels of DHEAS than of other androgens, as is cystic acne.⁵⁴²

Profound psychological changes occur during adolescence. Sexually immature girls tend to be socially immature, and the onset of puberty is associated with increased independence and profound changes in outlook on life and intellectual capacities. The extent to which these developments occur in reaction to the physical changes of puberty and the extent to which they are direct effects of sex hormones are unknown. Masculine tomboyish traits usually have no clear hormonal basis, although there is some evidence that they may have prenatal hormonal determinants. Social interactions have effects on these aspects of development. They affect even the synchrony of the menstrual cycle.⁷⁴⁸

Despite the popular notion that adolescence is inherently a period of turmoil, the majority of teenagers do not develop significant social, emotional, or behavioral difficulties.⁷⁴⁹ Occasional experimentation and risk-taking are normal, as are withdrawal from and conflict with parents. Adolescent behavior must be understood in the context of individual

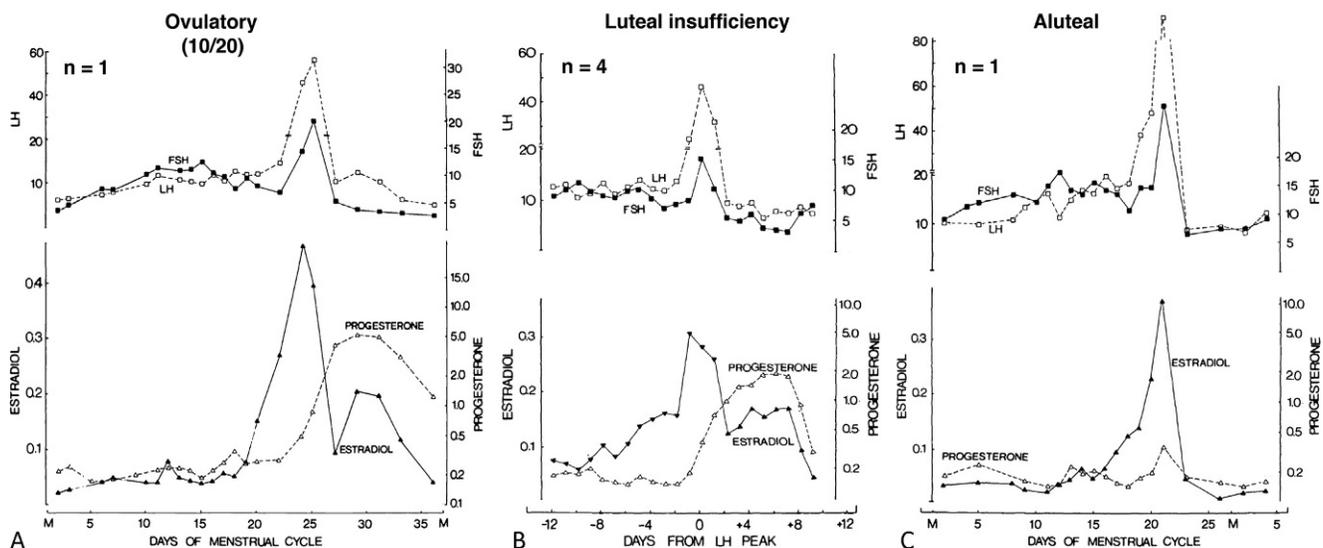


Fig. 16.35 Serum hormonal patterns of normal adolescent menstrual cycles among 20 healthy schoolgirls. **Panel A.** A normal ovulatory cycle in a 16-year-old female, representative of 10/20 of the study group who were ovulatory. Note normal preovulatory estradiol and progesterone rises, the subsequent midcycle luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surges, followed by progesterone levels that reach 5.0 ng/mL or higher during the luteal phase of the menstrual cycle. Although this is a standard criterion for an ovulatory cycle, the illustrated cycle would be considered luteally insufficient in an adult. **Panel B.** Mean hormone levels of the group defined as having luteal insufficiency: their short luteal phases lasted 4 to 8 days, but cycle lengths were normal (23–30 days) caused by prolonged follicular phase. Note the slightly low preovulatory estradiol and absent progesterone rises, followed by blunted midcycle LH and FSH surges that presage the low progesterone levels of the short luteal phases. **Panel C.** An aluteal anovulatory cycle in a 14-year-old female. Note normal preovulatory estradiol and LH surges, but absent preovulatory progesterone rise that presaged an absent luteal phase progesterone increase. The remainder of the five anovulatory study subjects did not attain clear preovulatory development and progesterone levels were consistently under 1.0 ng/mL, yet the menstrual cycles were abnormally long in only 2/5. M, menses. Scales differ slightly, and progesterone scales are logarithmic. Steroid levels in ng/mL LH and FSH levels in mIU/mL. (Data from Apter, D., Viinikka, L., Vihko, R. (1978). Hormonal pattern of adolescent menstrual cycles. *J Clin Endocrinol Metab*, 47, 944–954; Modified from Rosenfield, R.L. (2013). Clinical review: adolescent anovulation: maturational mechanisms and implications. *J Clin Endocrinol Metab*, 98, 3572–3583. With permission.)

susceptibility, family upbringing and interactions, peer group interactions, changes in brain maturation, and adolescents' reaction to their perception of the bodily changes and to the sexual urges that are the direct consequences of puberty. Simply because a problem is displayed during adolescence does not mean that it is a direct consequence of puberty.

Many behavioral problems that emerge during adolescence have earlier roots. Although the prevalence of depression increases during puberty, many children who develop depression during adolescence have had preexisting symptoms of psychological distress. Likewise, most delinquent teenagers have had antecedent problems at home and school.

Early-maturing girls in Western cultures are more popular, but they have more emotional problems; lower self-image; and higher rates of depression, anxiety, and disordered eating than their peers. Early maturation appears particularly to be a risk factor for problem behavior among girls who have had a history of difficulties before adolescence, when they have more opposite sex friendships and relationships, and when they attend coeducational schools.

Short-term administration of testosterone or estrogen has minimal effects on behavior or mood in adolescents.^{627,750} Thus variation in hormone levels accounts for only a small fraction of adolescents' affective issues, and social influences account for considerably more. Although there is little evidence that psychological difficulties stem directly from hormonal changes during normal puberty, it is likely that the bodily changes of adolescence play a role in the development of a negative body image when they occur out of synchrony with sociocultural norms.

Problems with initiating and maintaining sleep are common in adolescents and contribute a small amount to poor

school performance.⁷⁵¹ Although insufficient sleep might be caused by environmental factors (e.g., social and academic pressures), intrinsic factors clearly play an important role. A 50% decline in the intensity of deep (slow wave, delta) sleep occurs during adolescence, and one-half of this change occurs between 12 and 14 years of age.^{752,753} Recent evidence indicates that this change is related to age and sex, beginning earlier in girls, but not to pubertal stage. It has been proposed that this shift is a manifestation of the widespread synaptic pruning that is related to the emergence of adult cognitive capacity.

The causal direction of the link between pubertal development and the quality of family relationships has come into question. Several studies have indicated that family dynamics may affect the timing and course of puberty, with earlier and faster maturation observed among adolescents raised in homes characterized by more conflict and among girls from homes in which the biological father is not present.⁷⁴⁹

ABNORMAL PUBERTY

Abnormal Development

Disorders of Sex Development

Patients with DSD (formerly termed *intersex*)—those whose genitalia are ambiguous or inappropriate for their gonadal sex as a result of endocrinopathy—may come to a physician's attention for the first time at puberty. These syndromes have been categorized as 46, XX DSD, which encompasses cases formerly termed *female pseudohermaphroditism* and including 46, XX testicular DSD (formerly termed *XX sex reversal*); 46, XY DSD, which encompasses cases

BOX 16.2 Definition of Types Of Abnormal Uterine Bleeding in Adolescents

Descriptor	Definition
Primary amenorrhea	Lack of menarche by 15 years of age or by 3 years after the onset of breast development ^a
Secondary amenorrhea	Over 90 days without a menstrual period after initially menstruating (in consecutive periods during first year post-menarche)
Oligomenorrhea (infrequent abnormal uterine bleeding)	Postmenarcheal year 1: average cycle length >90 day (fewer than four periods in the year) Postmenarcheal year 2: average cycle length >60 days (fewer than six periods in the year) Postmenarcheal years 3: average cycle length >45 days (fewer than eight periods per year) Postmenarcheal years ≥4: cycle length >38 days (<9 periods per year)
Excessive uterine bleeding ^b	Menstrual bleeding that occurs more frequently than every 21 days (19 days in year 1) or is prolonged (lasts >7 days) or heavy (soaks more than one pad or tampon every 1–2 h large clots, or gushing)

(Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. With permission; Modified according to Teede, H.J., Misso, M.L., Costello, M.F., et al. (2018). Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril*, 110, 364–79).

^aBone age of 15 years may be substituted for chronological age in girls with earlier than average age at puberty onset.

^bEncompasses frequent, intermenstrual, heavy, and/or prolonged abnormal uterine bleeding. Alternatively termed *dysfunctional uterine bleeding* or *excessive abnormal uterine bleeding caused by ovulatory dysfunction*.

formerly termed *male pseudohermaphroditism* and including 46, XY complete gonadal dysgenesis (XY sex reversal, Swyer syndrome); and sex chromosome DSD, which includes Turner syndrome, Klinefelter syndrome, mixed gonadal dysgenesis, and chimeric ovotesticular DSD.⁶⁰⁰ In the absence of chromosomal mosaicism, ovotesticular DSD, formerly termed *true hermaphroditism*, is categorized as either 46, XX DSD or 46, XY DSD. Patients with any of these disorders may undergo inappropriate puberty. They may present with clitoromegaly and be found upon examination to have a degree of genital ambiguity which was previously overlooked. Virilization beginning at puberty is sometimes the presenting complaint. Ovotesticular DSD or 46, XX DSD because of CAH are compatible with fertility.⁴⁴⁴ Androgen insensitivity syndrome in a genetic male may present as primary amenorrhea in an otherwise phenotypically normal adolescent girl. The disorders of sexual differentiation are reviewed in detail in Chapter 6.

Congenital virilization of the female developmentally programs the emergence of PCOS at puberty. These observations are consistent with studies of fetal androgenization of the female in several species, including primates.^{15,16,754} There is a persistent increase in LH pulse frequency and impairment of the negative feedback effect of progesterone on LH release that appear to be related to suppression of hypothalamic PR

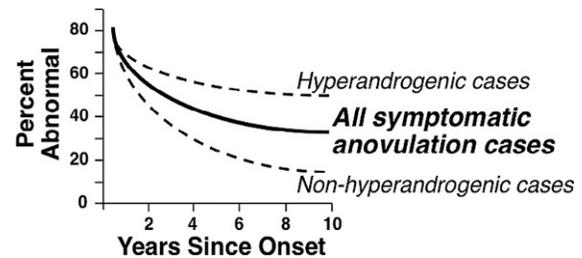


Fig. 16.36 Probability that an adolescent with a symptomatic menstrual abnormality severe enough to result in gynecological consultation will have continued menstrual abnormality. The lines show the cumulative rates at which subjects with menstrual abnormalities converted to normal patterns. The heavy curve shows the average incidence of continued menstrual abnormality in adolescents when considering all symptomatic cases of abnormal uterine bleeding presenting to a reproductive endocrine clinic regardless of time of symptom onset. (Data from Southam, A.L., Richart, E.M. (1966). The prognosis for adolescents with menstrual abnormalities. *Am J Obstet Gynecol*, 94, 637.) Dysfunctional uterine bleeding of onset within 1 year of menarche carries the furthest from average poor prognosis for continuing menstrual abnormality, and oligomenorrhea of relatively short duration occurring after a normal menstrual pattern has been established carries the furthest from average best prognosis. Note that if the menstrual abnormality persists for 1 year there is about a 50% probability, and if for 2 years over a 60% probability, that the patient will not spontaneously evolve to normal cycles. Similarly, if the problem persists for 5 years there is an 80% likelihood of persistence of the abnormality. “Hyperandrogenic” and “Nonhyperandrogenic” curves are hypothetical, based on data reviewed in Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165. Hyperandrogenic cases are predominantly a mix of physiological anovulation and polycystic ovary syndrome (PCOS), with PCOS persisting. Nonhyperandrogenic cases are a mix of physiological anovulation and hypogonadal cases, ranging from primary hypogonadism through hypothalamic amenorrhea to hypogonadotropic hypogonadism, with hypogonadal cases persisting. (Modified from Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165.)

in response to estradiol. Experimental animals exposed to androgen excess early in gestation develop classic PCOS features: these animals have elevated LH levels, ovarian and adrenal hyperandrogenism, oligomenorrhea, and polyfollicular ovaries. They also have abdominal obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia, which likewise appear to result from developmental programming.

Genetic males with complete androgen insensitivity have low LH levels and poor LH responsiveness to GnRH in the neonatal period.⁶⁴ However, gonadotropin levels are normal-to-high at puberty, and, paradoxically, androgen signaling via the androgen receptor enhances the capacity of females to mount an LH surge in response to estrogen positive feedback.^{337,338,755}

Other Dysgenetic Disorders

Failure of the onset of menses can result from structural abnormalities of the genital tract that do not have an endocrinological basis. The hymen may be imperforate, which results in hydrocolpos if the vagina is intact. The vagina may be aplastic, which will result in hydrometrocolpos if the uterus is intact.⁷⁵⁶ The uterus may be congenitally aplastic. Uterine synechiae develop as the consequence of endometritis, which may result from infection or irradiation (Asherman syndrome). Congenital absence of the vagina may be associated with varying degrees of uterine aplasia; this is the Mayer-Rokitansky-Kuster-Hauser syndrome.⁷⁵⁷ This syndrome seems to occur as a

single gene defect or as an acquired teratogenic event involving mesodermal development and the mesonephric kidney, the latter resulting in abnormalities of the genital tract and sometimes the urinary tract. A subtype caused by *Wnt4* gene defects is associated with hyperandrogenism.⁷⁵⁸

Precocious Puberty

Causes

When breast or sexual pubic hair development begins before the age of 8.0 years or menses begin before the age of 9.5 years, puberty is traditionally considered precocious, or premature. It should be kept in mind that breast development during the seventh year is within normal limits in ethnic minority girls. In addition, presexual pubic hair (stage 2) may be normal in 6- and 7-year-old ethnic minority girls.

Puberty can occur prematurely as an extreme variation of normal, because of a disturbance in the HPG axis normally involved in sexual maturation, or because of a disturbance outside the HPG axis. Depending on which part of this hormonal axis is involved, different forms of precocious puberty are distinguished. A classification of the causes of premature puberty together with typical findings is given in Table 16.4.

It is important to distinguish between true precocious puberty and pseudoprecocious puberty. True precocious puberty is gonadotropin dependent; thus *central* is another term applied to this type of precocity. Maturation is complete: both breasts and pubic hair develop as the result of CNS activating pituitary secretion of the respective gonadotropins FSH and LH (although breast development may be the sole manifestation of early complete precocity for as long as 6–12 months). Patients with true precocious puberty have “isosexual” precocity because the secondary sexual characteristics are appropriate for the sex of the child. Pseudoprecocious puberty is gonadotropin independent; it is not mediated by pubertal pituitary gonadotropin secretion and is sometimes

termed *peripheral*. Maturation is incomplete, with only one type of secondary sexual characteristic developing early. Peripheral precocity has diverse causes. In some patients with pseudoprecocity, pubertal development is isosexual, in others it is “contrasexual,” meaning that characteristics of the opposite sex are manifested.

Complete Precocious Puberty. True isosexual precocity results from pubertal function of the HPG axis. About 95% of true precocity in girls is idiopathic. Idiopathic true sexual precocity appears to be caused by premature triggering of the normal pubertal mechanism. Pubertal development usually is qualitatively and quantitatively normal except for its early occurrence. The predominance of the idiopathic cases in females and its benign nature are compatible with the likelihood that this disorder is an extreme exaggeration of the normal tendency of girls to have higher gonadotropin levels than boys. Most cases are sporadic, a few familial. The majority of these patients seem to go on to have normal menstrual cycles and fertility.⁷⁵⁹ Indeed, pregnancy has been documented to occur as early as 4 years of age.

Rapidly progressive puberty with a growth spurt ensues when activation of the pituitary-ovarian axis is sustained. However, precocious puberty is not necessarily sufficiently intense or sustained to cause inexorable progression or bring about deterioration of height potential.⁷⁶⁰ Precocity in the 6- to 8-year age range usually is not rapidly progressive and most commonly seems to be caused by excessive adiposity.²⁷⁶

Any type of intracranial disturbance can cause true isosexual precocity. These neurogenic disturbances are presumed to cause true sexual precocity by increasing the prevalence of excitatory inputs or by interfering with CNS inhibition of hypothalamic GnRH secretion.⁷⁶¹ These include congenital brain dysfunction, such as cerebral palsy or hydrocephalus, or acquired disorders, such as irradiation,⁷⁶² trauma, chronic inflammatory disorders, or masses in the region of the hypothalamus. The activation of GnRH release by hypothalamic injury may be

TABLE 16.4 Typical Findings in Female Sexual Precocity

Locus	Type	HA ^a	BA ^a	Estrogens ^U	Androgens ^U	LH/FSH ^U	Pathology	Characteristics
COMPLETE PRECOCITY (CENTRAL, GONADOTROPIN-DEPENDENT)								
Hypothalamic	Isosexual	+	++	+	+	+	Idiopathic Neurogenic Advanced somatic maturation	95% of female cases
INCOMPLETE PRECOCITY (PERIPHERAL, GONADOTROPIN-INDEPENDENT)								
Normal variant	Isosexual	-	-	±	-	±	None	Thelarche
	Isosexual	-	-	-	±	-	None	Pubarche/adrenarche
Neuroendocrine	Contrasexual	+	++	-	+	+ /+++	LH/hCG excess	Familial or tumor
	Isosexual	Low	Low	-	-	±	Hypothyroid	Growth arrest
Ovary	Isosexual	+	++	+ /+++	-	-	McCune-Albright	Bone lesions ± nevi ± ovarian cysts
	Isosexual/ contrasexual	+	++	+ /+++	+ /+++	-	Tumor	
Adrenal	Contrasexual	+	++	±	+++	-	Congenital adrenal hyperplasia	Dexamethasone suppressible
	Contrasexual/ isosexual	+	++	+ /+++	+ /+++	-	Tumor	
Ectopic	Isosexual	+	++	+ /+++	-	-	Aromatase excess	
	Contrasexual/ isosexual	±	±	-	-	-	Sex steroid exposure	
End organ	Isosexual/ contrasexual	-	-	-	-	=	Vaginal foreign body, abuse, tumor	

Hormone levels; - normal prepubertal; + pubertal level; ++ adult level; +++ abnormally high.

FSH, Follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

^aHA (height for age) and BA (bone age); - normal; + advanced; ++ markedly advanced.

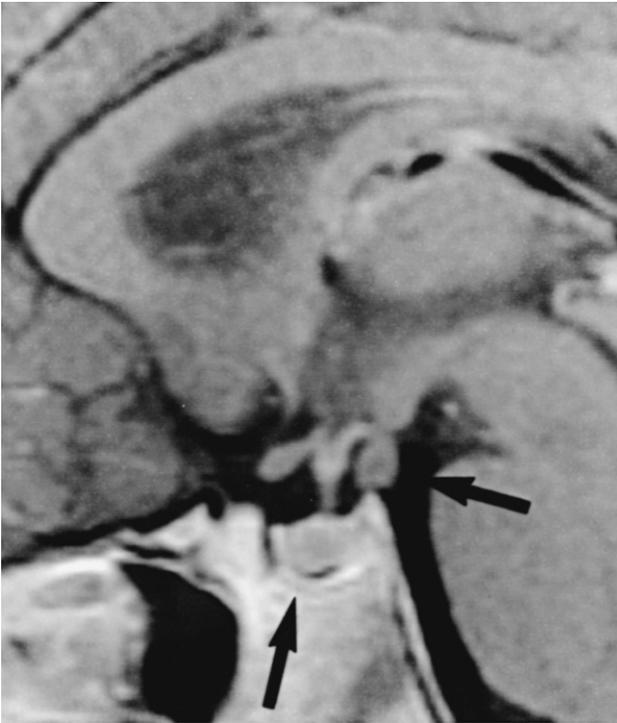


Fig. 16.37 Magnetic resonance image showing a hypothalamic hamartoma (right-hand arrow) as the cause of true sexual precocity in a 2.5-year-old girl. The hamartoma is hanging from the floor of the hypothalamus just posterior to the pituitary infundibulum. The sella turcica (bottom arrow) contains a normal pituitary gland with pituitary stalk hanging from the infundibulum.

mediated by TGF- β , trauma, chronic inflammatory disorders, or masses in the region of the hypothalamus. An empty sella is occasionally found.⁷⁶³ The precocity of neurofibromatosis type I (von Recklinghausen disease) usually results from an optic glioma, which is often of low-grade malignancy, or from a hamartoma,⁷⁶⁴ although occasionally with neither.⁷⁶⁵ Hamartoma of the hypothalamus may cause central sexual precocity; this effect is most likely caused by an anatomic effect on hypothalamic structures rather than by acting as an “accessory hypothalamus” that releases pulses of GnRH into the pituitary portal circulation.^{766,767} Fig. 16.37 shows a hypothalamic hamartoma.

A small proportion of pineal tumors cause true sexual precocity.^{768,769} The incidence of sexual precocity is about 3.5 times as great in nonparenchymatous neoplasms (such as gliomas and teratomas) as in parenchymatous pineal tumors. This suggests that these tumors cause sexual precocity via absence of a normal pineal inhibitory factor rather than by destructive effects on inhibitory tracts. Although pineal masses may cause paralysis of upward gaze by pressure on the corpora quadrigemina, this sign is present in only a minority of cases.

Pineal or hypothalamic hCG-secreting germ cell tumors occasionally cause true sexual precocity.^{770,771} Because hCG is an LH receptor agonist, possible explanations for this unusual situation are disinhibition of hypothalamic GnRH release attributed to a mass effect of the neoplasm, the weak FSH effect of massive elevation of hCG, or the capacity of some dysgerminomas to secrete estradiol, as well as hCG.⁷⁷²

Advancement of somatic maturation caused by peripheral endocrine disorders that advance the bone age to a pubertal level sometimes cause true sexual precocity. Thus true puberty may begin after correction of virilizing or feminizing disorders that have advanced the bone age to 10 to 12 years.^{264,265}

The hypergonadotropinism of premature ovarian insufficiency (POI) has been reported to cause sexual precocity or rapid progression of puberty before premature ovarian failure.^{773,774} Although PCOS has occasionally been reported to follow true sexual precocity,⁷⁷⁵ there is currently no clear evidence of a significant association.^{759,776}

A family history of complete precocious puberty (CPP) is common in patients with early puberty; however, a clear genetic etiology is usually unknown. Monogenic causes of CPP have recently been identified in four genes. Gain-of-function mutations have been found in kisspeptin and its receptor, KISS1R.^{777,778} The patients were heterozygous for the mutations, with an autosomal dominant inheritance.⁷⁷⁹ The first mutation to be described was in an adopted girl who had progressive thelarche from birth, with accelerated growth velocity and skeletal maturation noted at age 7 years. A heterozygous activating mutation of KISS1R (p. Arg386Pro) was identified and in vitro studies showed that the mutation led to prolonged activation of intracellular signaling pathways resulting in higher sustained inositol phosphate accumulation.⁷⁷⁷ A mutation in kisspeptin, p. Pro74Ser, was identified in a boy with CPP at age 1 year, with high concentrations of serum LH and testosterone. Interestingly, his mother and maternal grandmother had normal pubertal development, carried the p. Pro74Ser mutation in the heterozygous state, suggesting incomplete penetrance. In vitro, the mutated protein was able to stimulate signal transduction to a greater extent than the wild type, suggesting it may be more resistant to degradation, resulting in greater kisspeptin bioavailability.⁷⁷⁸ However, large-scale studies in children with CPP have not been able to identify additional patients and families with CPP with these mutations, hence kisspeptin and KISS1R activating mutations are relatively rare causes of CPP.⁷⁷⁹

Another genetic abnormality linked to CPP involves loss-of-function mutations in the Makorin RING-finger protein 3 (*MKRN3*) gene, a maternally imprinted gene located on the long arm of chromosome 15 (Prader-Willi region) encoding a protein that is involved in gene transcription and ubiquitination. Expression of *MKRN3* was found to be high in the arcuate nucleus in prepubertal mice, decreases before puberty, and is low after puberty, thus *MKRN3* appears to be acting as an inhibitor of puberty. Interestingly, *MKRN3* polyubiquitylates Nptx1, another protein of unclear function that is also expressed at low levels prepubertally, and highly during puberty.⁷⁸⁰ Fifteen families with a history of CPP were studied, and mutations in *MKRN3* that resulted in truncated proteins or missense mutations predicted to disrupt protein function were found in one-third of them. At present, 10 different mutations have been found, and include frameshift, missense, or nonsense mutations predicted to result in loss-of-function of the protein.^{246,781–787} *MKRN3* is maternally methylated, explaining the autosomal dominant paternal inheritance in all cases, with no de novo mutations described.⁷⁸¹ All patients described to date exhibit a typical pattern of early pubertal development. A few patients with *MKRN3* mutations were described as having syndromic features that included esotropia, a high-arched palate, dental abnormalities, clinodactyly, and hyperlordosis.⁷⁸⁵ In a genetic study of 20 boys with idiopathic CPP, eight were found to have *MKRN3* mutations and one had a *KISS1*-activating mutation,⁷⁸⁸ perhaps indicating that *MKRN3* mutations are a relatively frequent cause of CPP.

Delta-like homolog 1 (*DLK1*), a paternally expressed imprinted gene, encodes a protein expressed in kisspeptin-expressing neurons.⁷⁸⁹ *DLK1* is a part of the delta-notch pathway, which is an evolutionarily conserved signaling pathway with roles in proliferation and differentiation during development.⁷⁹⁰ In the pituitary, *DLK1* and notch signaling appear to be important in pituitary cell type differentiation.⁷⁹⁰

A mutation in *DLK1* was found in a family with five girls with CPP. The mutation was a 14-kb deletion along with a 269-bp duplication. Serum levels of *DLK1* were undetectable in these girls.⁷⁸⁹ In another study, the *DLK1* gene was sequenced in 60 girls with idiopathic CPP, and no mutations were found.⁷⁹¹

Distinct chromosomal abnormalities associated with specific syndromes may include CPP. These include: 1p36 deletion, 7q11.23 microdeletion (Williams-Beuren syndrome),⁷⁹² 9p deletion, maternal uniparental disomy of chromosomes 7 (Silver-Russell syndrome) and 14 (Temple syndrome),⁷⁹³ inversion duplication of chromosome 15,⁷⁹⁴ de novo interstitial deletion and maternal uniparental disomy of chromosome 15 (Prader-Willi syndrome),⁷⁹⁵ and a de novo deletion in the cyclin-dependent kinase-like 5 gene (*CDKL5*; located in the Xp22 region)⁷⁹⁶ (phenotype similar to Rett syndrome).

Incomplete Precocity. The most common causes of incomplete sexual precocity in girls are the extreme variants of normal mentioned previously, premature thelarche and premature pubarche. These are incomplete forms of sexual precocity in which either breast development (thelarche) or sexual hair development (pubarche) is of a degree appropriate for an early stage of puberty and isosexual. Isolated prepubertal menses is a rare disorder that has been attributed to transient ovarian activity.⁷⁹⁷

LH- or hCG-producing tumors have not been reported to virilize girls, perhaps because of their limited capacity for thecal androgen production over short periods of time. However, familial isolated elevation of LH has been reported to cause mild virilization of siblings;⁷⁹⁸ one was a girl who developed premature pubarche and clitoral hypertrophy at 4 years of age, with slight to moderate advances in height and bone age in association with an adrenarchal level of DHEAS and a moderately elevated testosterone level (91 ng/dL).

The van Wyk-Grumbach syndrome is one of the most puzzling pediatric complexes.⁷⁹⁹ This is an unusual syndrome of sexual precocity associated with juvenile hypothyroidism. A case is illustrated in Fig. 16.38. This syndrome is often characterized by galactorrhea, which is often not spontaneous; a few drops of milky fluid may become apparent only upon "milking" the subareolar ductal tissue. Multicystic ovaries are often demonstrable by ultrasonography.⁸⁰⁰ Modern assays show that levels of LH at baseline and post-GnRH are suppressed and those of FSH are early pubertal.⁸⁰¹ There is little, if any, sexual hair development. There is another clinically unique feature about the sexual precocity of hypothyroidism: it is the only form of sexual precocity in which growth is arrested rather than stimulated and is an exception to the general rule, indeed followed by most chronically hypothyroid children, that a delayed growth pattern is associated with delayed puberty.

Van Wyk and Grumbach postulated that this syndrome resulted from hormonal "overlap" in the negative feedback regulation of pituitary hormone secretion, with overproduction of gonadotropins, as well as TSH in response to the thyroid deficiency. The specific nature of hormonal overlap has been considerably clarified in recent years, but the pathogenesis of the precocity remains unclear. The increases in serum TSH and prolactin that characterize the syndrome could well be accounted for by common neurohumoral control systems, TRH stimulating and dopamine inhibiting both hormones. It has been suggested instead that the ovarian FSH receptor is activated by the weak intrinsic FSH activity of extreme TSH elevation,⁸⁰² analogous to the rare ovarian hyperstimulation syndrome in which pregnancy levels of hCG activate the FSH receptor.⁸⁰³

Prolactin excess has been postulated to underlie the FSH-predominant gonadotropin pattern by slowing GnRH pulsations, and it is this FSH stimulation of the TSH-sensitized ovary that seems to be the proximate cause of the sexual precocity.⁸⁰⁴



Fig. 16.38 Sexual precocity caused by hypothyroidism in a 9.1-year-old with breast development since 7 years and menarche at 9.0 years. Growth failure had occurred, and her height age was 6 years. In addition to breast enlargement and galactorrhea, the labia minora were noted to be enlarged and pigmented. There was no sexual hair or clitoromegaly. Rectal examination revealed an enlarged and palpable cervix without adnexal masses. There were typical physical findings of hypothyroidism. Bone age was 6.2 years. Thyroxine was less than 1 mcg/dL. Thyrotropin-stimulating hormone was 438 μ U/mL. Prolactin was 66 ng/mL. Serum estrogens were 72 to 182 pg/mL. Vaginal smear showed 45% superficial cells and 55% large intermediate cells. Immunoreactive luteinizing hormone (LH) and follicle-stimulating hormone were 300 and 174 ng LER-907/mL, respectively (see Fig. 16.5 for reference ranges). However, bioactive LH was undetectable. Immunoreactive gonadotropins failed to suppress upon estrogen administration. Their response to a 100-mcg gonadotropin-stimulating hormone bolus was minimal, and they seemed responsive to thyrotropin-releasing hormone. All of these hormonal findings were not obviously different from those of hypothyroid girls without sexual precocity except for the higher estrogens. She had withdrawal bleeding and evidence of regression of breast development within the first 3 months of thyroid hormone replacement treatment. After 6 months' treatment, normal puberty began. Menarche occurred at 12.5 years of age.

Hyperprolactinemia alone does not correspond with pubertal development in normal or hypothyroid children. However, hyperprolactinemia may itself also sensitize the ovaries to gonadotropins. Induced hyperprolactinemia causes sexual precocity in female rats.³⁶⁵ Ovarian estrogen and progesterone responsiveness to hCG is increased by prolactin, possibly by its induction of ovarian LH receptors. Conversely, suppression of hyperprolactinemia in experimental hypothyroidism blocks the ovarian cyst formation characteristic of hypothyroidism.⁸⁰⁵

McCune-Albright syndrome is another intriguing disorder causing incomplete isosexual feminization.^{806,807} This is a syndrome of precocious puberty, cafe-au-lait pigmentation

occurring in nevi that have an irregular (“coast of Maine”) border, and polyostotic fibrous dysplasia. The disorder is caused by a somatic activating mutation of the G_s -alpha subunit protein that couples transmembrane receptors to adenylate cyclase. The syndrome has been recognized predominantly in females and occurs in incomplete, as well as expanded forms. Precocious puberty or monoostotic bone lesions may occur in the absence of cutaneous pigmentation; not all patients have sexual precocity. The sexual precocity is of the gonadotropin-independent type. Luteinized follicular cysts within the ovaries function autonomously. Pituitary adenomas capable of secreting excess LH, FSH, GH, and/or prolactin have been reported. Patients may have Cushing syndrome and hyperthyroidism because of autonomous multinodular hyperplasia. These girls may be at increased risk of breast carcinoma.⁸⁰⁸ Nonendocrine abnormalities include cardiopulmonary disease, hypertension, and hepatobiliary disease (including severe neonatal cholestasis).⁸⁰⁹ Molecular studies have shown an R201H mutation in over 90% of cases where an affected tissue could be studied, but in only 50% of blood samples.⁸¹⁰ Because of the variation in the number and degree of tissue involvement in individual patients, caused in large part by the extent of mosaicism present, precocious puberty may be the only feature present in an individual who is mosaic for the activating mutation of G_s -alpha. Thus these mutations have been found in blood samples from 25% to 33% of subjects with isolated gonadotropin-independent precocity or exaggerated thelarche.^{704,810}

CAH is a well-known cause of premature pubarche. Either nonclassic CAH, a form of the disorder which is so mild that there is no genital defect in girls, or poor control of classic CAH may be responsible. Each form on occasion has been reported to mimic true sex precocity.^{811,812}

Tumor may cause isosexual or contrasexual development. The most common tumor is the feminizing benign ovarian follicular cyst.^{813,814} Most are isolated and large (>1.0 cm in diameter). The cells lining these cysts are often luteinized. Estrogen levels may be markedly elevated. Testosterone levels tend to be in the adult female range (about 40 ng/dL). Many function intermittently. They may be gonadotropin dependent and respond to GnRH agonist or progestin therapy. A case is illustrated in Fig. 16.39. The second most common hormonally active ovarian neoplasm in girls is the juvenile granulosa cell tumor.^{815,816} These have variable degrees of ovarian sex cord-stromal elements and are usually localized and benign in spite of having a malignant histological appearance. They are more commonly feminizing than masculinizing in young children. They may produce hCG, AMH, and inhibin. Elevation of hCG is found in many ovarian dysgerminomas (a primitive germ cell tumor), with hypercalcemia in some, although less frequently than in small cell carcinoma.^{815,817} Granulosa-theca cell tumor is occasionally associated with mesodermal dysplasia syndromes. It has been reported in the adrenal gland, presumably arising in an ovarian rest.⁸¹⁸ FOXL2 mutations are typical of adult-type granulosa cell tumors, but are found in only 10% of the juvenile type. A related feminizing ovarian sex cord-stromal tumor may be caused by loss of a tumor-suppressor gene, as in Peutz-Jeghers syndrome.⁸¹⁹ Adult-type ovarian carcinoma of epithelial cell origin is rare. Ovarian masculinizing tumors are discussed in the section Hyperandrogenism in Adolescence.

Adrenocortical tumors, as discussed in Chapter 14, typically cause rapid virilization characterized by very high DHEAS production; however, androstenedione is the predominant androgen in many cases. A case discussion is given with Fig. 16.40. Many are accompanied by cushingoid changes. On occasion, they cause feminization. When adrenocortical tumors secrete both androgen and estrogen, the clinical picture may resemble complete isosexual precocity.⁸²⁰ Structural abnormalities of

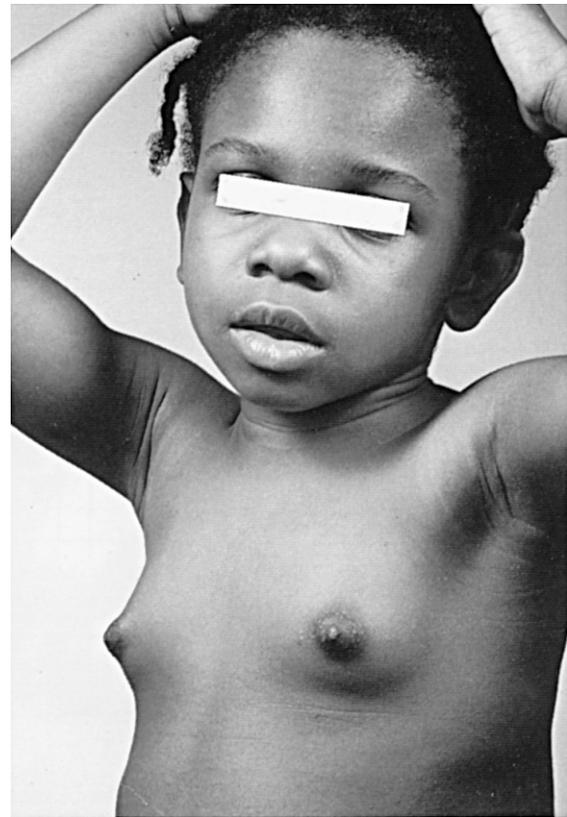


Fig. 16.39 The appearance of a 5.2-year-old child with complete isosexual precocity caused by a luteinized follicular cyst. Her breast development is no different from that of girls with idiopathic premature thelarche. She presented at 4.7 years with a 2-week history of breast development. Height and bone ages were 5 years. Over a 5-month follow-up period, breast development progressed, she developed sexual pubic hair and presexual axillary hair, and menstrual flow commenced at 3- to 5-week intervals. Four weekly determinations of plasma unconjugated estrogens (estradiol and estrone) showed them to consistently range between 158 and 215 pg/mL. Luteinizing hormone was pubertal and follicle-stimulating hormone was suppressed (averaging 50 and 13 ng LER-907/mL, respectively; see Fig. 16.5 for reference ranges). Testosterone was 37 ng/dL, and dehydroepiandrosterone sulfate (DHEAS) was 82 mcg/dL. Exploratory laparotomy was performed when she was 5.2-years-old. Her height age was 5.8 years, and her bone age was 7.5. The laparotomy revealed a right ovarian cyst about 5 cm in diameter, which was removed. Subsequently, there was a rapid fall in plasma estrogens and testosterone to prepubertal levels. However, DHEAS was unchanged. Menses ceased, but intermittent vaginal cornification (maturation index 90/10/0 to 0/90/10) was repeatedly found. Breast enlargement and sexual hair development resumed at 8.5 years, with normal pubertal sex hormone levels. Menarche occurred at 9.5 years.

chromosome 11p15 and mutations of the tumor suppressor p53 are fairly common in pediatric adrenocortical tumors.⁸²¹

Aromatase excess syndrome is a rare cause of ectopic feminization.⁸²² This is an autosomal dominant disorder with variable penetrance that results from constitutive aromatase gene overexpression.

Exogenous steroids can cause sexual precocity. Estrogen-containing contraceptive pills and creams are widely available. Some cases of precocious thelarche may be caused by ingesting food contaminated with artificial estrogens.⁸²³ Soy formulas are potential sources of phytoestrogens, as are many commonly consumed foods, herbs, and topicals (including products containing lavender oils and tea tree oils).⁸²⁴⁻⁸²⁷ It has been



Fig. 16.40 Abdominal ultrasound (decubitus view) showing a pedunculated encapsulated 5-cm adrenal adenoma (*large arrow*) near upper pole of kidney (*small arrow*). This 1.3-year-old girl was virilized. Pubic hair had appeared 2 months previously, height had changed from the 10th to the 30th percentile, and clitoromegaly had occurred. Bone age was 2.3 years. Dehydroepiandrosterone sulfate was 3000 to 4271 mcg/dL. Testosterone was 121 ng/dL. A, Anterior; P, posterior.

proposed that childhood exposure to estrogenic chemical contaminants in underdeveloped countries predisposes to sexual precocity when children emigrate to developed countries.¹⁹⁰ Premature pubarche or acne can result from anabolic steroid use. Topical nonprescription androgen use by a parent, such as for sexual dysfunction or anabolic effects, can cause premature pubarche without necessarily being detectable by standard tests.⁸²⁸

Vaginal bleeding in the absence of breast development suggests foreign body, sexual abuse, or tumors of the genital tract. A rare cause is "premature menarche," which may result from an extreme variation in the normal intermittent ovarian activation of young girls.⁹³ Malodorous discharge is highly suggestive of foreign body. A hymenal opening of greater than 5 mm or posterior notches is compatible with sexual abuse.^{532,533} Neurofibromas have been reported to simulate breast development and clitoral hypertrophy.

Differential Diagnosis

A physician need not be experienced in endocrinology to diagnose and manage most girls presenting with early breast or pubic hair development. For the most part, the isolated appearance of one of these signs is caused by the benign processes of either premature thelarche or premature pubarche, respectively. An otherwise normal history and physical examination, together with a normal bone age, constitutes sufficient workup.

In the history, the physician should inquire about the possibility of exposure or access to exogenous steroids in the form of pills, diet, or topical substances (such as estrogen creams or personal care products containing lavender oil or tea tree oil). The possibility of sexual abuse, vaginal infection, or foreign body must be kept in mind when evaluating the child with

isolated vaginal bleeding. In the examination, the physician should search for nevi, acanthosis nigricans, signs that might suggest intracranial or abdominal-pelvic disease, and inspect the external genitalia. The child's height and weight should be carefully recorded, the growth curve examined, and the BMI percentile plotted.

If the history and examination are unremarkable, only a bone age determination is indicated to screen for whether the symptom is indeed an isolated phenomenon or whether appreciable hormone excess exists. If the skeletal age is not abnormally advanced relative to height age, it is likely that the presenting symptom is an isolated and benign extreme variant of normal, which requires no treatment.^{671,829} To confirm the diagnosis of premature thelarche or premature adrenarche, the child must be similarly reevaluated after 3 to 6 months. If the results are still negative, the family can be reassured with a high degree of confidence that true puberty, including menses, will not occur until the usual age.

If more than one sign of precocious puberty is present or develops or if the growth is accelerated, a more extensive workup is indicated. For example, if a young girl with early breast development begins to grow pubic hair, or vice versa, then something more than premature thelarche or premature adrenarche is involved. The same is true if she develops a growth spurt or if she begins menstruating. These additional signs indicate the need for more extensive studies. Isolated vaginal bleeding, that is, bleeding in the absence of secondary sex characteristics, suggests sexual abuse, foreign body, or genital tract tumor, rarely isolated menarche. Cytology, anaerobic culture, pelvic ultrasound, and serum estradiol examinations are indicated.

Bone age advancement that is or becomes disproportionate to height (as indicated by compromised height potential or bone age $\geq 20\%$ greater than height age) suggests a sustained excess of sex hormone, and is an indication for a more extensive investigation to determine the cause of the precocity. An algorithmic approach to the differential diagnosis of premature pubarche is presented in Fig. 16.32. The importance of the recheck is illustrated by the case presented in Fig. 16.39.

One must be particularly aware that girls with neurogenic precocity, especially those who have had cranial irradiation, are at risk of paradoxically having concomitant GH deficiency.⁷⁰³ Coexistent GH deficiency masks the seriousness of the precocity: the growth rate is normal (not accelerated) and breast development is attenuated. However, the disparity between bone age and height age is extreme.

If the clinical picture and bone age are suggestive that premature breast development may be due to sex precocity, the laboratory investigation of premature pubertal development requires determinations of sex steroids, LH, and FSH by assays of high sensitivity: at least 10 pg/mL for estradiol, 10 ng/dL for testosterone, 5 mcg/dL for DHEAS, and 0.2 U/L for LH and FSH.⁷⁵⁹ The modern multichannel platform assays that are widely available generally meet these specifications for the assays of DHEAS, LH, and FSH, but they are totally inadequate for testosterone and estradiol. These require assays of high sensitivity and specificity, such as are provided by postchromatographic RIA or tandem mass spectrometry, in laboratories with well-established normal ranges for children.⁸³⁰ Prepubertal estradiol levels are normally less than 10 pg/mL, and prepubertal testosterone is less than 20 ng/dL. Measurement of serum thyroxine and prolactin is indicated if the sexual precocity is accompanied by growth arrest and/or galactorrhea.

In central precocity, daytime serum estradiol concentration is usually pubertal, 10 pg/mL (37 pmol/L) or more.⁸³¹ An estradiol level in the upper premenarcheal normal range (≥ 75 pg/mL) is atypical and necessitates a prompt workup to distinguish ovarian or adrenal tumor from true isosexual precocity.⁸³² If the estradiol level is atypically high, weekly determinations of estradiol may be helpful to determine

whether the level is fluctuating in the normal cyclic fashion of true precocious puberty. Because of the episodic and cyclic nature of sex hormone secretion, examination of the vaginal mucosa (see Fig. 16.28) for estrogen effect is a more sensitive indicator of the presence of early puberty than is an estradiol blood level, because it represents the integrated effect of estrogen over the preceding 2 to 3 weeks. Uterine size (e.g., uterine length >3.8 cm and endometrial thickness of ≥ 2 mm) has been used as an objective indicator of overall estrogenization.^{85,86} Androgen levels are appropriate for the stage of pubarche in true precocity.

Third-generation, monoclonal-antibody-based, "pediatric" immunoassays for gonadotropins are necessary for early detection and monitoring of therapy.⁷⁵⁹ Early morning basal LH greater than 0.3 to 0.6 U/L has been reported to be 62% to 95% sensitive and 92% to 100% specific for the diagnosis of central precocious puberty in girls.^{673,833,834} A post-GnRH peak LH greater than 6.9 U/L has been reported to be 92% sensitive and 100% specific,⁶⁷³ whereas a post-GnRH agonist peak LH over 4.0 to 5.0 U/L has been reported to be 90% or more accurate for the diagnosis of central precocious puberty.^{676,835,836}

A study of GnRH agonist-stimulated LH levels in healthy prepubertal girls found mean levels of 5.2 ± 4.0 and 2.9 ± 2.5 U/L in girls 0.8 to 3 years and 3 to 6 years, suggesting an LH cutoff level of 9 U/L and LH/FSH ratio of 0.4 in girls under 3 years.⁸³⁷ The diagnostic specificity of GnRH agonist testing is complicated by the overlap between the gonadotropin responses of prepubertal and pubertal girls. A study of healthy 6- to 13-year-old girls showed the 95th percentile for prepubertal girls to be 8.9 U/L and the 5th percentile for pubertal girls to be 2.8 U/L (Table 16.1).⁹³ In a subgroup of peripubertal 8- to 11-year-old girls, the most discriminating LH values were 3.2 U/L (85% specific) and 5.5 U/L (72% sensitive) 1 hour post-GnRH agonist. The GnRH agonist test also permits assessment of the ovarian gonadotropin-responsiveness: an estradiol peak of 34 pg/mL or more is approximately 90% sensitive and 60 pg/mL or more 95% specific for puberty (see Table 16.1).⁹³

FSH levels are not as helpful diagnostically because prepubertal values overlap considerably with pubertal ones and they may be elevated in premature thelarche. In response to GnRH or GnRH agonist testing, children with unsustained pseudopuberty as variants of normal will have a minimal gonadotropin response, whereas children with gonadotropin-independent precocity will have suppressed responses.^{838,839} Demonstration of a sleep-related rise of serum LH is an alternate diagnostic procedure. Box 16.3 summarizes laboratory criteria for the diagnosis of complete sexual precocity.⁸⁴⁰

Premature pubarche must be distinguished from hypertrichosis, the generalized excessive growth of vellus body hair that is prominent in nonsexual areas. The most common cause, by far, of premature pubarche is premature adrenarche. Premature adrenarche must be distinguished from virilizing disorders, the most common of which is virilizing nonclassic CAH and the most serious of which, although rare, is virilizing tumor.

Determination of the early morning baseline serum androgen pattern is useful in discriminating among the causes of premature pubarche and virilization. Premature adrenarche is characterized by a pubertal level of DHEAS, whereas serum testosterone and androstenedione are, at most, marginally elevated above the prepubertal range. A greatly elevated level of DHEAS suggests either adrenal tumor or the 3β -HSD deficiency form of CAH. Androstenedione and 17-OHP levels are disproportionately elevated compared with testosterone or DHEAS levels in other forms of virilizing CAH and many ovarian tumors.

An ACTH stimulation test is the definitive test to exclude CAH. We advise performing an ACTH test in children with premature pubarche who have unusually high serum

BOX 16.3 Laboratory Criteria for Diagnosis of Complete Precocious Puberty in Girls

BONE AGE ADVANCEMENT

Bone age > height age > chronological age
Compromised predicted adult height

LH LEVEL PUBERTAL^a

Sleep-associated LH peak > 1.0 U/L
LH (early morning) ≥ 0.6 U/L^b
Post-GnRH (1 h) agonist LH ≥ 3.2 –5.5 U/L^b
Suppressible by chronic GnRH agonist administration

SEX HORMONE LEVEL PUBERTAL

Estradiol (early morning, cyclically): >9 pg/mL^a
DHEAS normal for age or early puberty

DHEA, Dehydroepiandrosterone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

^aEssential criterion.

^bTypical values for sensitive assays. Exact values vary among laboratories.

androgens and bone age advancement and an early morning serum 17-OHP concentration above 170 ng/dL (5.1 nmol/L) (Fig. 16.32),⁸⁴¹ although levels above 1500 ng/dL do not require a confirmatory ACTH stimulation test. The diagnosis of CAH is discussed in detail in Chapter 14. The differential diagnosis of hyperandrogenic disorders is discussed further in the section Hyperandrogenism in Adolescence.

Ultrasonography is indicated to screen for abdominal or pelvic masses when feminizing or virilizing disorders are suspected.^{842,843} The ovaries of girls with true sexual precocity resemble those of normal pubertal girls.^{82,84,844} A "cyst" of 10 mm or more in diameter is usually caused by a transient preovulatory follicle. However, the differential diagnosis of a persistent cyst or multicystic ovaries includes McCune-Albright syndrome,⁸⁰⁷ tumor,⁸⁴⁵ and premature ovarian failure.⁸⁴⁶

The ability of ultrasonography to detect small adrenal neoplasms is highly dependent on the expertise of the ultrasonographer. On rare occasions, ultrasonography has been insensitive in detecting an ovarian tumor in adults.⁸⁴⁷ Computed tomography and MRI permit better visualization and more detailed assessment of tumors.

A systematic review that included 1853 subjects found that brain MRI identified a pathological finding in 9% of girls with central precocious puberty, with a much higher prevalence in those under 6 years of age (25%), compared with those that were 6 to 8 years of age (3%).⁸⁴⁸ The most common finding was a hypothalamic hamartoma in approximately 3.7%, whereas 1.3% of the subjects had another type of CNS tumor. Thus MRI of the hypothalamic-pituitary area is indicated in progressive central precocious puberty in those less than 6 years old or those at risk of organic causes by virtue of their underlying condition or neurological symptoms and signs,^{831,849} while the necessity of such imaging in girls presenting at 6 to 8 years of age without neurological indications is less certain. An argument for genetic testing of patients with CPP has been made, citing cost-effectiveness and safety in cases where MRIs with sedation are required.⁸⁵⁰

Management

The goals in management are to rule out an organic disorder that requires treatment in-and-of-itself and to ascertain whether sexual precocity is either compromising height potential or resulting in important secondary emotional disturbances in the child.

The situation of a girl presenting with the onset of breast development or pubic hair between 6.0 and 8.0 years of age warrants special consideration. Breast development between 7.0 and 8.0 years of age is normal in blacks and Hispanics. Although presexual pubic hair (stage 2) may be seen in 6- to 8-year-old black and Hispanic girls, sexual pubic hair (stage 3) is abnormal if present before 8 years of age (see Table 16.3). However, pubertal development in girls in the 6.0- to 8.0-year age range may be associated with pathology, with excessive adiposity being the major consideration in most.²⁷⁶

Many 6.0- to 8.0-year-old girls with central precocious puberty, including whites, have slowly progressive precocity, with a normal timing of menarche, and are at low risk of short adult stature. Most such girls do not require GnRH agonist therapy to preserve adult stature.^{759,760,851,852} Thus a less comprehensive investigation may be warranted in selected girls presenting with thelarche or stage 2 pubic hair between 6 and 8 years of age. For most such girls, a complete history and physical examination, including obesity evaluation and a bone age determination, may be all that is needed, along with careful longitudinal follow-up to rule-out a disorder that requires therapy.^{760,852} However, 6- to 8-year-old girls with a suggestion of rapidly progressive or excessive androgenization or feminization, neurological symptoms, linear growth acceleration, or significant bone age advancement should be more completely evaluated, as outlined earlier.

Intracranial lesions must be treated by appropriate measures, such as neurosurgery or irradiation. Shunting for hydrocephalus may stop the precocity. Granulosa cell tumors confined to the ovary have a good prognosis for cure by unilateral oophorectomy. Recurrence of tumor may occur up to 20 years after the initial operation, however. Biopsy of the opposite ovary is indicated in unilateral ovarian neoplasms. Compensatory ovarian hypertrophy can be expected at any age after removal of a single ovary.⁸⁵³

The only permanent physical complication of true isosexual precocity, all else being normal, is short adult height. There is no increase in the risk of obesity, metabolic derangements (diabetes, hypertension, dyslipidemia), or cancer in adults with a history of central precocious puberty, whether they were or were not treated with GnRH agonist.⁸⁵⁴ Women with a history of central precocious puberty, whether treated or not, do have a higher rate of hirsutism and irregular menses, and women who were not treated for the sexual precocity may have decreased fertility and a higher need for assisted fertilization.⁷⁷⁶ However, fertility is normal in women who received treatment with GnRH agonist or with cyproterone acetate for central precocious puberty, with a spontaneous pregnancy rate that is no different than in controls.⁷⁷⁶ Pregnancy outcome is normal in both treated and untreated women with a history of central precocious puberty.⁷⁷⁶ With regard to height, excessive sex hormone production in the first decade of life causes early maturation of the epiphyses, resulting in their premature closure. About half the girls with this disorder reach an adult height of 53 to 59 inches and the remainder are over 60 inches tall.^{760,852} The mismatch between physical, hormonal, and psychological development may cause behavior changes ranging from social withdrawal to aggression or sexuality. However, frank behavioral problems are unusual in girls and so are by themselves seldom indications for treatment.

When central precocity is accompanied by documented progression of pubertal development that accelerates growth and compromises normal height potential, GnRH agonist treatment is indicated.⁷⁵⁹ Documentation typically requires 3 to 6 months' observation, although this may be unnecessary if puberty is substantially advanced clinically and hormonally on presentation. The downregulating effect of GnRH agonists

BOX 16.4 Indications for Gonadotropin-Releasing Hormone Agonist Therapy of Precocious Puberty

1. Documentation of central precocious puberty
2. Documentation of pubertal progression
3. Plus presence of one of the following:
 - Progressive compromise of predicted adult height or
 - Emotional or behavioral disturbance or
 - Menses in the emotionally immature or disabled

(Modified from Rosenfield, R.L. (1994). Selection of children with precocious puberty for treatment with gonadotropin releasing hormone analogs. *J Pediatr*, 124, 989–991.)

on pituitary gonadotropin release inhibits gonadotropin secretion within 1 month. Suggested criteria for the use of these drugs are presented in Box 16.4.^{759,840} The commonly used agents in the United States are leuprolide acetate (ordinarily given as Lupron Depot-Ped[®] 7.5–15 mg/mo or 11.25–30 mg/3 mo intramuscular [IM]),^{855,856} nafarelin acetate (Synarel[®] 800 mcg bid intranasal), a histrelin implant (Supprelin[®] LA 50 mg implant yearly),⁸⁵⁷ and triptorelin (Triptodur[®] 22.5 mg/24wk IM).⁸⁵⁸ Dosage can be adjusted later as necessary.

Treatment is adequate if the estradiol and baseline LH levels become prepubertal⁸⁵⁹ or LH is below 4.0 (1 hour) to 6.6 U/L (2 hours) after GnRH agonist^{856,860} 1 month after institution of therapy. Withdrawal menses may occur at that time, but none should be expected thereafter. Arrest of breast development and the pubertal growth spurt become apparent by 3 to 6 months. Concomitantly, epiphyseal closure is delayed and adult height potential is improved, because a type of catch-up growth occurs in which height age catches up to bone age. Adult height is greatest when treatment is started soon after onset at an early age, yielding an average height gain above pretreatment height prediction of about 1.4 cm for each year of therapy.⁷⁵⁹ Adult height prediction at the end of treatment tends to be overestimated from bone age. Therefore prolonging treatment beyond a chronological or bone age of 12.0 to 12.5 years of age generally leads to little further increase in adult height potential, regardless of the prediction of residual height potential. Menses occurs an average of 12 to 20 months after discontinuation of GnRH agonist treatment.^{861,862}

Coincident GH deficiency must be treated for optimal growth.⁸⁶³ GH-sufficient patients with central precocity who are started on treatment relatively late and whose height velocity falls below 4 cm/y after 2 to 3 years, appear to gain an average of 2 cm/y when GH therapy is added.^{864,865}

Use of the depot form of GnRH agonist is complicated by sterile abscesses at injection sites in about 5% of cases. The histrelin implant breaks on its removal over 20% of the time,⁸⁶⁶ necessitating care that the complete implant is removed. Anaphylaxis is a rare complication.⁸⁶⁷ No other serious side effects have come to light. During treatment, girls with central precocious puberty do not differ in their cognitive or psychosocial functioning compared with controls.⁸⁶⁸ Long-term safety data remain incomplete, but current studies following subjects into young adulthood are reassuring, including no difference in menstrual cycle characteristics or pregnancy outcomes of treated compared with untreated subjects⁸⁶⁹ and some evidence of improved fertility with GnRH agonist treatment compared with that in women with a history of untreated central precocious puberty.⁷⁷⁶ GnRH agonist treatment does not seem to cause

or aggravate obesity, as judged from BMI.^{759,854} Bone mineral density dips with the onset of treatment, but becomes normal after discontinuation of GnRH agonist therapy of precocious puberty.⁷⁵⁹

Girls with idiopathic slowly progressive puberty of onset between 6 and 8 years of age or with early fast puberty between 8 and 9 years of age tend to be tall at the onset of puberty, follow an advanced growth pattern, and reach their target height without GnRH agonist therapy.^{760,852,870–872} Therefore this treatment is only indicated if height potential is compromised or there are other compelling reasons to slow the pace of puberty.

Medroxyprogesterone acetate (Depo-Provera) is useful for stopping menses and as a contraceptive in girls with intellectual disability in whom preservation of height potential is not important. It is begun in a dosage commencing at 50 mg/month IM. Doses as high as 400 mg/month have been used, although cushingoid side effects may be observed at this level.⁸⁷³ Although this treatment reverses some of the physical changes of premature puberty, it does not reverse the inordinately rapid maturation of the skeleton, possibly because of its inherently weak androgenicity. In addition, use of medroxyprogesterone acetate is associated with a loss of bone mineral density, which must be considered if long-term use is being considered.⁸⁷⁴

A variety of drugs have been used off-label to treat gonadotropin-independent precocity. Both antiestrogen and aromatase inhibitor treatments have demonstrated partial efficacy for McCune-Albright syndrome.^{856,875–877} However, there should be a period of observation before initiating treatment for patients with McCune-Albright syndrome, as there can be marked variability in the clinical course, with some patients having extended periods of disease inactivity in whom treatment may not be indicated.⁸⁷⁷ There have been reports of surgical intervention in children with McCune-Albright syndrome, including resection of an ovarian cyst or ovariectomy. In many of these cases, however, there has been recurrence of precocious puberty symptoms.⁸⁷⁸ Therefore such treatment should only be considered in very select situations, such as a girl with a large ovarian cyst at risk of ovarian torsion. Ketoconazole, an antifungal agent that inhibits 17,20-lyase activity and other steroidogenic enzymes, may be useful.⁸⁷⁹ GnRH agonist treatment may be necessary for those in whom true puberty becomes superimposed because the bone age has reached a pubertal level.^{264,265,880} Bisphosphonates are often effective at relieving the bone pain of fibrous dysplasia in McCune-Albright syndrome, although they do not appear to have an effect on the course of the lesions and are not a suitable long-term treatment.⁸⁸¹

Patients with premature thelarche or pubarche as variations of normal are counseled as follows. The child's early development seems to be a matter of a normal stage of puberty occurring early. It is caused either by an incomplete, slow kind of puberty or by increased sensitivity to the trace levels of hormones that are normally present in childhood. Feminization with breast development and eventual menstruation can be expected to occur at an appropriate age. No treatment is indicated. To exclude subtle sex hormone excess or eventual anovulatory syndromes, long-term follow-up is advisable.

Besides dealing with the physical consequences of true isosexual precocity, the physician must be ready to help the family and child cope with the psychological problems that come with early physical maturation. The doctor can help the family by explaining that even though their child looks older and more mature than other children of the same age, the child will not behave more maturely. The libido of young children with precocity is not increased. The family should be advised to take some precautions to downplay their child's development, for example, in the choice of clothing and swimsuits. Early on,

children with these disorders tend to be withdrawn because they feel that they are different from their peers. Later on, they tend to enter into romantic relationships early. It is important to remind the family and child that in a few years, the child will not be unique from the standpoint of sexual development.⁷⁰³ The following books may be helpful in explaining precocious puberty: for children, *What's Happening to Me?*, by Peter Mayle (Lyle Stuart, Inc., Secaucus, NJ, 1973); for parents, *Sex Errors of the Body*, by John Money (Paul H Brookes Publishing Co., Inc, Baltimore, MD, ed. 2, 1994).

Hypogonadism

Causes

If hypogonadism is complete and present prepubertally, it causes sexual infantilism. In genetic males, congenital primary hypogonadism may also cause a completely female or ambiguous phenotype (see Chapter 6). If hypogonadism in girls is partial or of onset in the early teenage years, feminization will be of too limited a degree to permit the onset of menses at a normal age (primary amenorrhea). Milder, partial, or incomplete forms of hypogonadism may present in adolescence with abnormal uterine bleeding (Box 16.2). Consequently, disorders causing hypogonadism appear in the differential diagnosis of disorders of sexual differentiation, sexual infantilism, failure of pubertal progression, and menstrual irregularity. The causes of hypogonadism are listed in the differential diagnosis of amenorrhea in Box 16.5.

Primary Ovarian Failure. Primary ovarian failure is characterized by high levels of gonadotropins, particularly FSH. Two exceptions exist to this rule. First, the gonadotropins may not be elevated until CNS maturation has reached a pubertal stage, as indicated by a bone age of approximately 10 to 11 years (Table 16.5).⁸⁸² Secondly, patients with early or partial ovarian failure (primary ovarian insufficiency), as normally occurs during the menopausal transition, do not have high baseline gonadotropin levels.^{883,884} FSH may hyperrespond to GnRH and estrogen hyporespond to GnRH agonist challenge. It seems as if relatively few ovarian follicles—too few to permit the cyclic emergence of preovulatory follicles—suffice to prevent the characteristic rise in basal FSH levels. Serum AMH levels are a less sensitive indicator of ovarian failure than FSH but may be useful in prognosticating the potential for fertility.^{885,886}

Primary ovarian failure may occur before or during puberty, causing primary amenorrhea, or after puberty has occurred, causing secondary amenorrhea. The latter is termed *premature ovarian insufficiency* (POI; in its complete form termed *premature ovarian failure*) and clinically resembles premature menopause, except that about 25% of the cases sometimes resume ovarian function and there is a 4.4% spontaneous pregnancy rate.⁸⁸⁷

Gonadal dysgenesis caused by deficiency of genes on the X-chromosome is the most common cause of primary ovarian failure and POI. It is usually caused by a relatively large-scale deletion of X-chromosomal material, which is associated with a characteristic, but variable, phenotype and is termed *Turner syndrome* (see Chapter 17). Fetuses with a 45,X karyotype have a normal number of oocytes in the ovary at midgestation, but a drastic reduction in the number of follicles,⁴¹ which appears to cause gonadal streaks via an accelerated rate of apoptosis. However, the gonadal dysgenesis, like other features of the syndrome, is often incompletely expressed.^{888,889} Thus Turner syndrome should be considered in all girls with primary hypogonadism or secondary amenorrhea whether or not they have the typical stigmata of Turner syndrome.

BOX 16.5 Differential Diagnosis of Amenorrhea: Structural and Anovulatory Disorders**ABNORMAL GENITAL STRUCTURE**

- Ambiguous genitalia
 - Disorders of sex development
 - Pseudointersex
- Aplasia^a
 - Hymenal
 - Müllerian
 - Disorders of sex development
- Endometrial adhesions

ANOVLATORY DISORDERS**Hypoestrogenism, FSH Elevated**

- Primary ovarian failure
 - Congenital
 - Gonadal dysgenesis
 - Chromosomal
 - Genetic
 - Other genetic disorders
 - Acquired
 - Oophorectomy
 - Radiotherapy or chemotherapy
 - Oophoritis
 - Idiopathic

Hypoestrogenism, FSH not Elevated

- Primary ovarian insufficiency
 - Complete if bone age <11 years^a
 - Incomplete if bone age >11 years

- Delayed puberty
 - Constitutional delay of growth and puberty^a
 - Growth-retarding disease
- Gonadotropin deficiency
 - Congenital
 - Acquired
 - Organic
 - Functional
 - Virilization

Estrogenized

- Hypothalamic anovulation
 - Functional hypothalamic amenorrhea
 - Athletic amenorrhea
 - Psychogenic amenorrhea
 - Epilepsy
- Nonhypothalamic nonovarian disorders
 - Pregnancy
 - Obesity or undernutrition
 - Chronic disease
 - Cushing syndrome
 - Hypothyroidism
 - Drug abuse
 - Hyperprolactinemia
 - Postpill amenorrhea
- Hyperandrogenism
 - Polycystic ovary syndrome
 - Other hyperandrogenic disorders

FSH, Follicle-stimulating hormone.

^aCause only primary amenorrhea.**TABLE 16.5** Bone Age in Workup of Sexually Infantile Girls With Normal Follicle-Stimulating Hormone Level

	Bone Age (Years)		
	<11	11–13	>13
Primary hypogonadism	Yes		
Delayed puberty	Yes	Yes	
Gonadotropin deficiency	Yes	Yes	Yes

(From Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491.)

Specific loci on the X-chromosome are associated with primary ovarian failure. Xp11.2 harbors BMP15, a specific ovarian differentiation factor, heterozygous mutation of which is a rare cause of gonadal dysgenesis. Xq harbors two independent loci, in addition to the fragile X premutation, that are associated with about 5% of sporadic and 14% of familial POI.⁸⁹⁰ The premutation is an expansion of CGG repeats in the fragile X mental retardation 1 (*FMR1*) gene that is insufficiently long to cause fragile X syndrome. Women with the premutation allele have a substantially increased risk of POI, possibly because raised intracellular mRNA concentrations might sequester CGG binding proteins that are important for RNA processing.

Gonadal dysgenesis also results from 46, XY complete gonadal dysgenesis and certain forms of autosomal aneuploidy.^{600,890,891} A rare cause is ERβ (ESR2) receptor inactivating mutation. A variable degree of ovarian dysgenesis occurs in trisomy 21; delayed menarche, anovulatory cycles, and primary gonadal failure are occasionally seen.⁸⁹² However, pregnancy

has been reported; trisomic offspring are common.⁸⁹³ Oocytes are virtually absent in trisomies 13 and 18. Ovarian dysgenesis also occasionally occurs as part of the Denys-Drash syndrome caused by a *WT-1* mutation.⁸⁹⁴ Gonadal dysgenesis also occurs in DNA-repair disorders that impair meiosis, such as ataxia-telangiectasia and Fanconi syndrome.^{895,896} Other autosomal genetic disorders causing premature ovarian failure include inactivating mutations of FOXL2, which are found in sporadic cases, as well in the autosomal dominant type 1 blepharophthalmos syndrome.⁸⁹⁷ POI also occurs in galactosemia,⁸⁹⁸ leukodystrophies, and myotonia dystrophica.^{899,900} Mutation of the inhibin alpha-subunit predisposes to POI, an effect that appears to vary depending on ethnicity; this is postulated to be caused by deficient paracrine interactions with TGFβ-family receptors.⁹⁰¹ Mutations in other autosomal genes that have been associated with POI include: *NOBOX*, *FSHR* (encoding the FSH receptor), and *TRIM37* (mutations of which cause Mulibrey nanism disorder).⁹⁰² Mutations in *NR5A1*, encoding the transcription factor SF-1, can result in 46, XY gonadal dysgenesis or POI, as well as being a cause of adrenal insufficiency because of impaired adrenal gland development.⁹⁰³ Variants in additional genes that have been identified in patients with POI include *PGRMC1* at Xq22-q24 (coding for a putative progesterone-binding membrane receptor) and the autosomal genes *SPIDR*, *GDF9*, *FIGLA*, *NANOS3*, *SYCE1*, *MCM8/9*, and *HFM1*.⁹⁰⁴ There are a number of other candidate genes with less clear evidence of causality,⁹⁰⁴ and mutations incriminated in the ovarian failure of mouse models⁹⁰⁵ or in genes involved in follicle development²⁶ may also be identified in the future as causes of human primary ovarian failure.

Injury to the ovary is a common cause of primary ovarian failure. Mumps oophoritis is a classic but rare cause of ovarian

failure. Very high-dose estrogen treatment in adolescence increases the risk of hypergonadotropic subfertility.⁸⁸⁶

Irradiation and chemotherapy for childhood neoplasia are frequent causes of primary ovarian failure now that life is effectively prolonged.^{890,906–909} Such treatments can cause either acute ovarian failure or a decreased follicular reserve. Acute ovarian failure results in a lack of pubertal development when it occurs prepubertally, or in arrested pubertal development or secondary amenorrhea when it occurs in pubertal or postpubertal girls. Decreased follicular reserve results in premature ovarian failure.⁹¹⁰ Ionizing radiation and alkylating agents damage DNA whether or not a cell is replicating.⁹¹¹ Therefore nonreplicating primordial follicles are not spared from these agents, although prepubertal ovaries may be less sensitive to damage than pubertal or postpubertal ovaries.^{910,912} The damage is dose related and the damage is worse with combined irradiation and chemotherapy compared with treatments with just one of these modalities. A radiation dose of 15 Gy or more causes acute ovarian failure in over 80% of prepubertal girls, whereas a dose more than 10 Gy will cause equivalent damage in postpubertal girls.⁹¹³ However, doses as low as 2 Gy will also impact long-term ovarian function by depleting the follicular pool by as much as 50%.⁹¹⁴ A cumulative cyclophosphamide dose⁹¹⁵ over 7.5 g/m² will cause acute ovarian failure in over 80% of females younger than 20 years.⁹¹³ Green et al. developed a method to quantitate a “cyclophosphamide equivalent dose (CED)” for other alkylating agents.⁹¹⁵ After prepubertal cancer treatment, 94% of girls can be anticipated to enter puberty and menstruate regularly, but 8% of these will develop nonsurgical premature menopause because of the reduced number of oocytes.⁹⁰⁸ The risk for premature menopause is 30% in those who have received both radiation and chemotherapy, 5% to 13% for those receiving either alone. Some with early hypergonadotropinism will experience ovarian recovery with normal pituitary-ovarian function after several years,⁹¹⁶ but infertility occurs. Several nonalkylating chemotherapies are also gonadotoxic (including platinum agents and anthracyclines), while others (such as methotrexate, fluorouracil, and vincristine) have very low or no gonadotoxic risk.^{917–920} However, data are scarce, and interactions among the various classes of chemotherapeutic agents is poorly understood. Radiation treatment for malignancy can also lead to infertility by causing hypogonadotropic hypogonadism, or through injury to the uterus. As a consequence of gonadotropin elevation when gonadal failure begins, puberty may progress rapidly.⁷⁷⁴

Sterilization by irradiation can be obviated by transposing the ovaries out of the irradiation field if possible. The evidence that the prepubertal ovary appears less sensitive to injury from irradiation and chemotherapy has suggested that GnRH agonist therapy might protect ovarian function in pubertal/postpubertal girls receiving such treatment. While there are some conflicting data, there is no clear benefit to such treatment.⁹¹² Imatinib (Gleevec[®]) is a potential oocyte-protective treatment, as it blocks an apoptotic pathway activated by radiation and cisplatin in mouse oocytes.⁹²¹

Gonadotropin resistance (Savage syndrome) can arise from autosomal recessive loss of function mutations of the LH or FSH receptor.^{917–919} The reported cases have had some degree of pubertal development followed by primary or secondary amenorrhea or oligomenorrhea. The ovaries of LH receptor mutants contain follicles in all stages of development, while those of FSH receptor mutants vary from hypoplastic to normal size, with antral follicle development varying from nil to 5 mm. Partial gonadotropin resistance is common in the Albright osteodystrophy form of pseudohypoparathyroidism, as part of the generalized defect in G-protein signal transduction.⁹²⁰

Autoimmune oophoritis is the basis of approximately half of spontaneous premature ovarian failure, although estimates vary from 5% to 85% in various series.⁹²² It is diagnosed by

its association with any of a variety of autoimmune endocrine or nonendocrine disorders, manifest or subclinical, that have in common defects in T cell suppressor function. Autoimmunity may be directed against the granulosa cell, oocyte, or theca cell. The clinical picture may resemble relatively selective resistance to FSH or, less frequently, to LH. The latter results from lymphocytic destruction of theca cells with sparing of granulosa cells from small antral follicles, which lack substrate to form estradiol and can only respond to the compensatory increase of FSH by producing inhibin-B.⁹²³ These patients have autoantibodies to steroidogenic cells and are at risk for adrenal failure. Usually these antibodies are directed against 21-hydroxylase, less frequently to side chain cleavage enzyme or 17-hydroxylase, seldom to 3 β -HSD. Replacement glucocorticoid therapy may temporarily ameliorate the immune oophoritis in such cases.⁹²⁴ A case with autoantibodies to testosterone has been reported.⁹²⁵ AIRE gene mutations have been identified as causative of type 1 polyendocrine failure. Ultrasonographic and histological findings are variable in premature ovarian failure and include large or small ovaries, inactive or polyfollicular ovaries, loss or preservation of primordial follicles, and infiltration by lymphocytes or plasma cells.⁸⁴⁶

Functional ovarian failure can also result from specific autosomal recessive defects in the biosynthesis of sex steroids. Both androgen and estrogen deficiency occur in lipid CAH (StAR and side chain cleavage mutations, see Fig. 16.22), 17 α -hydroxylase deficiency, P450 oxidoreductase deficiency, and 17,20-lyase deficiency.^{926,927} Thus affected genetic males may present with a female phenotype. Hypoestrogenism is associated with virilization in aromatase and 3 β -HSD deficiency. Aromatase is unique among the ovarian steroidogenic defects in not being associated with CAH. Congenital lipid adrenal hyperplasia is unique in that underlying StAR deficiency has too little direct impact on ovarian function to interfere with the early phases of puberty, but the gradual buildup of intraovarian lipid deposits resulting from enzyme deficiency—a “second hit”—causes ovarian damage with anovulation and late ovarian failure. SF-1 (NR5A1) deficiency can cause primary ovarian failure in the absence of adrenal insufficiency.⁹⁰³

Estrogen resistance caused by inactivating mutation of ER α has been reported.^{928,929} Hypoestrogenism and hyperestrogenemia were profound, the ovaries were enlarged and multicystic, and gonadotropin and testosterone levels were marginally to clearly elevated.

Gonadotropin Deficiency (Hypogonadotropic Hypogonadism). Congenital gonadotropin deficiency can occur in association with cerebral, hypothalamic, or pituitary dysfunction as an isolated defect.⁹³⁰ Congenital defects in hypothalamic-hypophyseal formation may be associated with midline facial defects. Congenital hypothalamic dysfunction may be associated with other neurological or endocrine dysfunction, such as in the Prader-Willi syndrome (congenital hypotonia, and neonatal failure to thrive followed by hypothalamic obesity, sometimes with hypopituitarism)⁹³¹ or the Laurence-Moon-Biedl syndrome (retinitis pigmentosa, obesity, mental deficiency).

Congenital hypogonadotropic hypogonadism often results from mutations (Table 16.6).^{212–214,220,222,226,235,719,932–942} The autosomal recessive forms of congenital combined pituitary hormone deficiency caused by PROP1, HESX1, LHX3, and OTX-2 mutations are associated with gonadotropin deficiency. Leptin and leptin receptor inactivating mutations cause gonadotropin deficiency in combination with moderate or extreme obesity.^{941,943} The report of a natural pregnancy in a woman with a homozygous mutation in the leptin receptor lends controversy to the current concept that leptin function is essential for reproduction.⁹⁴⁴

Gonadotropin deficiency may be associated with anosmia (olfactory-genital dysplasia or Kallmann syndrome).²¹⁴

TABLE 16.6 Gene mutations causing congenital hypogonadotropinism

Gene Mutation	Disorder
<i>CCDC141, CHD1, DUSP6, FGF8 and 17, FEZF1, FGFR1, FLRT3, IGFS10, IL17RD, KAL1, HS6ST1, KLB, PROK2, PROKR2, SEMA3A and 3E, SMCHD1, SPRY4, WDR11</i>	Disrupted migration of gonadotropin-releasing hormone (GnRH) neurons (Kallmann syndrome)
<i>DAX1 (NROB1), HESX-1, LHX3, NR5A1, PROP-1, SOX2</i>	Abnormal development of the hypothalamus and pituitary
<i>LEP, LEPR, PC1</i>	Associated with obesity
<i>GNRH1, KISS1, KISS1R, TAC3, TAC3R</i>	Abnormal GnRH pulsatility
<i>GNRHR, FSHB, LHB</i>	Abnormal gonadotropes
<i>DMXL2</i>	Associated with type 1 diabetes mellitus, central hypothyroidism, developmental delay, peripheral neuropathy
<i>OTUD4, PHPLA6, RNF216, STUB1</i>	Gordon Holmes syndrome (cerebellar ataxia, retinal dystrophy)
<i>POLR3A and 3B</i>	4H syndrome (hypomyelination, hypodontia, hypogonadotropic hypogonadism)
<i>RAB18, 3GAP1 and 3GAP2</i>	Warburg Micro syndrome (microcephaly, developmental delay, microcornea, optic atrophy)

This syndrome is 1/10th as frequent in females as in males. Mutations in the *KAL-1* gene in the pseudoautosomal region of the X-chromosome, which encodes anosmin, a key protein for GnRH neuronal migration, cause the highly penetrant X-linked form and rarely affect females. Inactivating mutations of other genes in the anosmin signaling pathway (*FGF8/FGFR1, PROK2/R2, NELE, and CHD7*) account for the vast majority of female cases; these are inherited as autosomal dominant or recessive (heterozygous, compound heterozygous, or digenic) traits with variable penetrance.^{214,719,933,934,945} Neurological and somatic abnormalities, such as synkinesia, cerebellar ataxia, sensorineural deafness, mental retardation, unilateral renal agenesis, and cleft palate, are variably associated genetic features of Kallmann syndrome. Rare affected individuals have only delayed puberty. These same mutations sometimes are responsible for normosmic idiopathic GnRH deficiency; *CHD7* mutations typically have features of CHARGE syndrome. Research is increasingly revealing new elements in the GnRH developmental and signaling pathway. For example, a single-nucleotide polymorphism in the *EAP1* gene has been associated with amenorrhea/oligomenorrhea in primates.⁹⁴⁶

GnRH receptor mutations account for about half of autosomal recessively inherited cases of isolated, normosmic gonadotropin deficiency.⁹³⁵ The degree of hypogonadism is variable, even within a family, with delayed puberty and delayed menarche as presentations.^{936,937,947}

Loss-of-function mutations of GnRH⁹⁴⁸ and in signaling systems that modulate GnRH release (*KISS/KISS1R, NKB/TAC3R*) are rare causes.^{222,235} The hypogonadism of most subjects with neurokinin B/TAC3R mutations is reversed by sex steroid therapy, which suggests that this signaling system is important for the start-up of puberty, but not its maintenance. Isolated hypogonadotropic hypogonadism also has been reported in a woman homozygous for a nonsense mutation of the X-linked orphan nuclear receptor *DAX1* gene, which was associated with X-linked adrenal hypoplasia congenita in her brothers.⁹⁴⁹

Isolated FSH deficiency caused by mutation in the β -subunit has been reported to cause primary amenorrhea in association with a unique test panel—low FSH, elevated LH, and low

testosterone levels.^{139,919} Carbohydrate-deficient glycoprotein syndrome (phosphomannomutase deficiency) is characterized by high levels of immunoreactive, but bioinactive gonadotropins, mimicking primary ovarian insufficiency.⁹⁵⁰ Isolated LH deficiency because of *LH β* gene inactivating point mutations has been reported to lead to secondary amenorrhea following normal pubertal development, but undetectable LH, high FSH, low estradiol, and macrocystic ovaries.⁹⁵¹ However, some inactivating LH mutations in men have high or low immunoreactive LH levels.⁹¹⁹ Mutations in genes responsible for anterior pituitary development may cause gonadotropin deficiency resulting in pubertal delay or CHH. LIM Homeobox 3 (*LHX3*), SRY-Box 2 (*SOX2*), and HESX homeobox 1 (*HESX1*) are responsible for early forebrain patterning and pituitary cell development.⁹⁵² Paired like homeodomain factor 1 (*PROP1*) is important for the development of somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs⁹⁵³ and patients with *PROP1* mutations have variable phenotypes ranging from CDGP to CHH.⁹⁵² Gonadotropin deficiency may also be associated with other conditions, particularly with neurological phenotypes. Mutations in RNA polymerase III subunit A and B (*POLR3A/B*) result in the 4H syndrome (hypomyelination, hypodontia, and hypogonadotropic hypogonadism)⁹⁵⁴ Ring finger protein 216 (*RNF216*), OTU deubiquitinase 4 (*OTUD4*) and Patatin-like phospholipase domain containing 6 (*PNPLA6*) CHH, and ataxia (also known as *Gordon-Holmes syndrome*).^{955,956} *DMX* like 2 (*DMXL2*) mutations are associated with CHH, other endocrine deficiencies, and polyneuropathies.⁹⁵⁷ Mutations in RAB3 GTPase activating protein catalytic subunit 1 (*RAB3GAP1*) result in dysregulation of the RAB3 cycle, leading to Warburg Micro syndrome with ocular, neurodevelopmental, and central reproductive defects.^{958,959}

Acquired gonadotropin deficiency can be a consequence of tumors, trauma, autoimmune hypophysitis,^{960,961} degenerative disorders involving the hypothalamus and pituitary,⁹⁶² irradiation,⁹⁶³ chemotherapy,⁹⁶⁴ or chronic illness.⁹⁶⁵ Hypogonadotropic hypogonadism will develop in about one-third of those receiving 20 to 30 Gy cranial irradiation, whereas it is typical in those receiving >50 Gy.^{762,966} Pituitary adenoma, craniopharyngioma, and dysgerminoma are the most common neuroendocrine neoplasms responsible for hypopituitarism in children. Most “nonfunctioning” pituitary adenomas are gonadotrope adenomas, which secrete gonadotropin subunits in response to TRH.⁹⁶⁷ A case of hypothalamic tumor is presented with Fig. 16.41. Pinealomas most commonly cause sexual infantilism. They may act by secreting an inhibitory substance, rather than by compressing key areas of the hypothalamus.⁷⁶⁹

Anorexia nervosa is the prototypic form of eating disorders, and is a common cause of hypogonadotropinism in teenagers. It is a syndrome of undernutrition because of voluntary starvation with a particular psychological dysfunction that results in amenorrhea.^{968–970} These patients uniformly consider themselves too fat in the face of objective evidence that they are underweight. The psychiatric criteria that distinguish this disorder from food faddism and fear of obesity consist of: (1) restriction of energy intake relative to requirements, leading to a significantly low body weight; (2) intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight; and (3) disturbance in the way in which one’s body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight. A 10th percentile BMI should be the initial maintenance goal.⁹⁷¹

Bulimia nervosa, the binge-eating/purging variant eating disorder, is similar in the overevaluation of body shape and weight and the use of extreme weight control behaviors. Physical activity tends to be high. These disorders may be manifest at an early stage as atypical eating disorders, before weight or

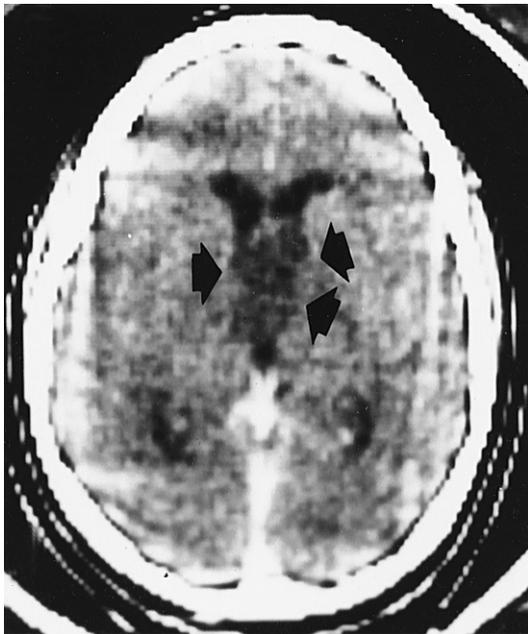


Fig. 16.41 Computed tomography of the brain of a 16-year-old girl with hypothalamic astrocytoma. The low-density tumor mass (arrows) extends superiorly from the hypothalamus, obliterates the third ventricle, and partially compresses the frontal horns of the lateral ventricles (particularly the right). This patient presented with secondary amenorrhea. Menarche had occurred at age 13 years, and menses were normal until 15.3 years. The patient then became amenorrheic in association with lethargy, episodic headaches, polyuria, and weight gain—despite little change in appetite. Physical examination was negative. The skull radiograph, electroencephalogram, visual fields, and serum prolactin and thyroxine levels were normal—and urine-specific gravity was 1.016. After biopsy of the cyst wall, studies revealed her to have gonadotropin, growth hormone, and partial antidiuretic hormone deficiencies.

amenorrhea criteria are met or when the binge is subjective. The cognitive defect that weight can serve as the predominant value in judging self-worth is central to anorexia nervosa. In contrast to other depressive individuals, these patients are generally content with themselves in the areas of intellectual and vocational achievement.

The cause is multifactorial. It involves a genetic predisposition. Concordance rates for the anorexic type are about 50% for monozygous twins, compared with about 5% for dizygotic twins. Many other risk factors have been implicated. Familial factors also include eating disorders of any type, depression, substance abuse, and adverse family interactions. Premorbid experiences, such as sexual abuse or social pressures, or premorbid characteristics, such as low self-esteem, compulsiveness, and perfectionism, are also important. Dieting meets a need for approval in our culture with its emphasis on dietary restriction and thinness as goals for women. Anorexia is often precipitated in vulnerable children by a new experience, such as puberty, leaving home or beginning college, or by adverse life events. The disorder is perpetuated by the complications of starvation, such as depression and reduced gastric emptying.

The onset tends to be at 12 years of age or later. Earlier onset is associated with growth arrest, delay of puberty, and primary amenorrhea.⁹⁷² Dysfunction of the hypothalamic-pituitary axis in anorexia nervosa includes not only hypogonadotropic hypogonadism, but GH deficiency^{973,974} and hypercortisolemia;

GH resistance and nonthyroidal illness also occur as consequences of malnutrition.⁹⁷⁵

The medical complications of anorexia nervosa are serious. The risk of death is approximately 10-fold increased: electrolyte imbalance, hypoglycemia, cardiovascular instability, bone marrow hypocellularity predisposing to silent infection, and renal failure account for about half of the mortality, suicide for the rest.

The weight changes leading to cessation or restoration of menstrual cycles are in the range of 10% to 15% of body weight. Recovery is associated with achieving a critical level of body fat stores above the 10th percentile (over approximately 20% body fat) (see Fig. 16.42), at a BMI approximately 20, that is, midnormal.^{976,977} There is an inverse relationship between body weight and the maturity of gonadotropin release in these patients. The 24-hour pattern of gonadotropin release tends to be immature (prepubertal or pubertal), and the diurnal LH pattern becomes mature upon recovery from undernutrition.⁹⁷⁸ LH pulsatility is low and may be restored by opiate antagonists.⁹¹ The gonadotropin response to GnRH and ovulatory response to clomiphene citrate are blunted in the malnourished state and become normal with weight gain to about 80% of ideal.^{979,980} Leptin levels are significantly decreased and are a major contributor to both the gonadotropin deficiency and to changes in the thyroid and GH axes.⁹⁸¹

Mild hypercortisolism is frequent and may contribute to the anovulation by mechanisms discussed further under Hypothalamic Anovulation.⁹⁸² Afternoon ACTH and cortisol levels are significantly higher and the response to CRH is significantly lower than normal. In contrast to Cushing syndrome, DHEAS levels tend to be blunted as a consequence of undernutrition.⁹⁸³

A fundamental neuropsychological flaw or hypothalamic disturbance⁹⁸⁴ has been suspected because some patients become amenorrheic before losing weight, and about half of the cases remain amenorrheic after treatment. The serotonergic systems implicated in the regulation of feeding and mood seem to remain altered even after weight restoration. Evidence exists for marked individual differences in reactivity of the neuroendocrine system to stress.⁹⁸⁵ The authors favor the concept that psychological problems lead to amenorrhea only in women who are predisposed by a unique preexisting hypothalamic dysfunction.

A number of features attributed to hypothalamic dysfunction, such as cold intolerance, may be caused by the subtle hypothyroid state that is secondary to the malnutrition.⁹⁸² Serum triiodothyronine levels are consistently low, serum thyroxine levels tend to be lower than average (although usually within normal limits), the pattern of TSH release indicates TRH deficiency, and the state of deep tendon reflexes and metabolism is consistent with hypothyroidism. Hypothyroidism may in part complicate the GH resistance of malnutrition that occurs as a consequence of the interference with IGF-1 generation: low IGF-1 initiates GH excess, compensatory somatostatin release, and subsequent inhibition of the thyrotropin response to TRH. Undernutrition also diverts the generation of thyroxine metabolites away from triiodothyronine toward reverse triiodothyronine.

Hyperprolactinemia is a potentially reversible cause of gonadotropin deficiency.⁹⁸⁶ Galactorrhea is present in about half of the patients, particularly those with residual estrogen production. The causes of hyperprolactinemia are diverse, including hypothalamic or pituitary disorders, drugs, hypothyroidism, renal or liver failure, peripheral neuropathy, stress, autoimmune, macroprolactinemia, genetic, and idiopathic.^{987–990} Elevated serum prolactin levels occur with a variety of tumors that cause functional or anatomic pituitary stalk section, thereby preventing dopaminergic inhibitory pituitary control. About

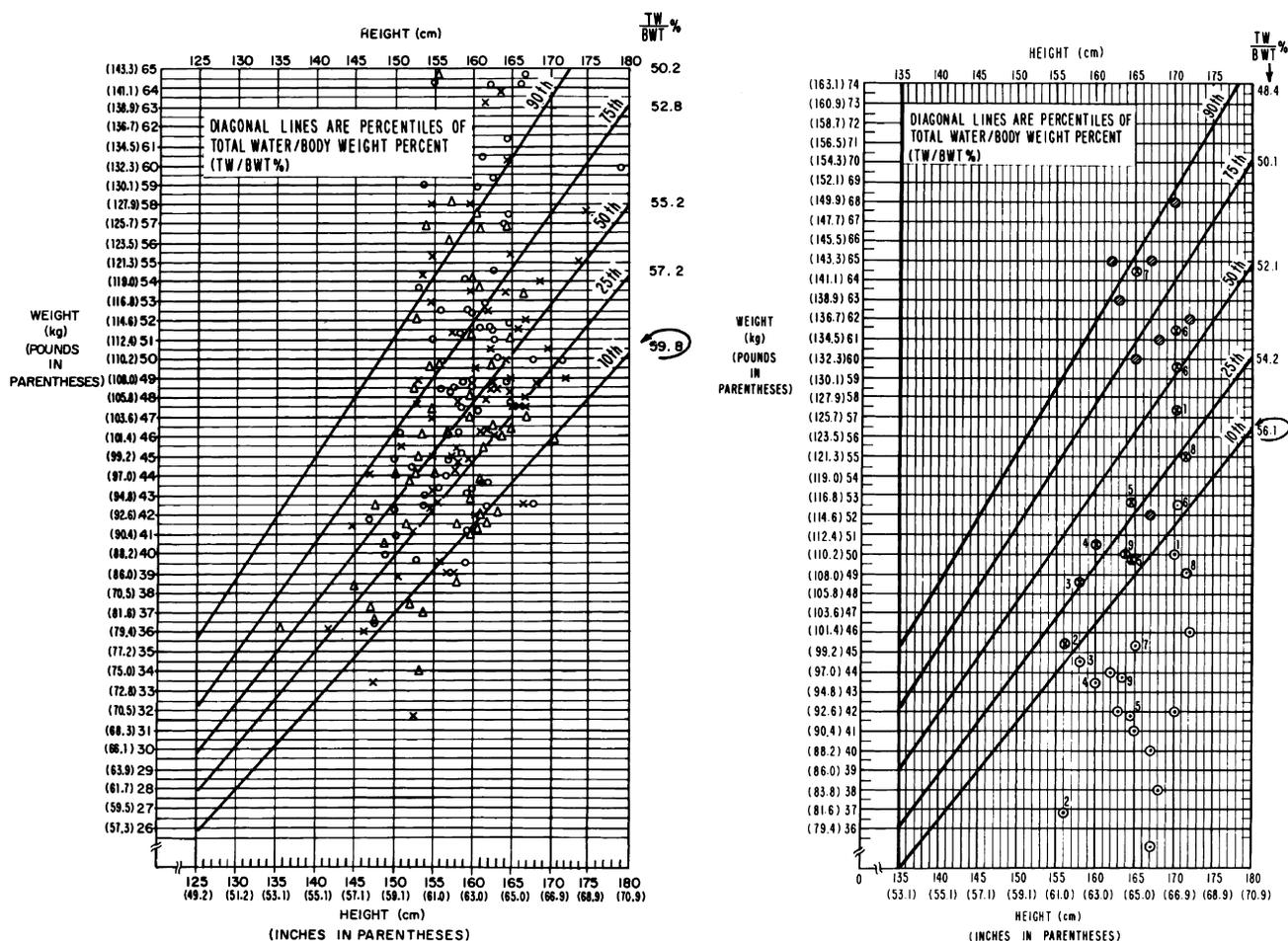


Fig. 16.42 Percentiles of fatness (*diagonal lines*) for white girls at menarche (left) and after menarche (right) equated with computed percentiles of total water as a percentage of total body weight. The minimal weight necessary at a particular height for the onset or maintenance of menses is very close to the 10th percentile of fatness on these respective charts. Data for anorexia nervosa cases are shown on the right-hand chart: ● at presentation; x at resumption of menses. (From Frisch, R.E., McArthur, J.W. (1974). Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*, 185, 949. Copyright © by the American Association for the Advancement of Science.)

one-third of hyperprolactinemic women have an identifiable pituitary adenoma. Prolactinomas less than 1 cm in diameter (microadenomas) cause no problems by local extension. Prolactinoma may be associated with multiple endocrine neoplasia type 1.⁹⁹¹ In about a quarter of adult hyperprolactinemia, the malfunction is caused by the ingestion of drugs, such as phenothiazines, estrogen, or cocaine.⁹⁹² Considerable hyperprolactinemia is idiopathic: decreased sensitivity to dopaminergic inhibition may underlie such cases.⁹⁹³

Macroprolactinemia is caused by a variant molecule or autoantibody formation comprised of prolactin and plasma proteins, most often caused by immunoglobulin (Ig)G prolactin-autoantibodies.⁹⁹⁴ In this situation, direct immunoassay indicates elevated levels of prolactin. However, the biologically available or active prolactin level is normal; thus there is no physiological consequence to the macroprolactinemia.

Hyperprolactinemia results in LH pulses that tend to be infrequent and LH secretion that is variable in response to GnRH.⁹⁹⁵ Selective prolactin excess causes variable degrees of gonadotropin deficiency, ranging from severe to partial (hypothalamic amenorrhea). Adrenal hyperandrogenism, hirsutism, and seborrhea are common.³⁶⁷

Frank virilization as a result of very high androgen levels suppresses gonadotropin levels and so causes defeminization.

However, the moderately hyperandrogenic disorders discussed later, which are more common, are associated with normal estrogenization.

Differential Diagnosis

The differential diagnosis of hypogonadism is included in [Box 16.5](#). Investigation should begin for hypogonadism when puberty is delayed or does not progress normally. Delayed puberty is indicated by lack of thelarche by the chronological or bone age of 13 years. Abnormal progression of puberty is suggested by failure of menses to occur within 3.0 years of the onset of thelarche or if secondary amenorrhea or oligomenorrhea has persisted for 1 year. As discussed earlier, normal menstrual frequency varies with the time after menarche, but an interval persistently greater than normal ([Box 16.2](#)) should prompt an evaluation.⁹⁹⁶ A family history of delayed puberty is compatible with the delay being constitutional rather than having an organic basis. The history should include a thorough past medical history and systems review, including intracranial, visual, olfactory, emotional, abdominal, or pelvic symptoms and systemic symptoms that might indicate chronic endocrine, metabolic, or systemic disorders that delay puberty. Upon examining the patient, the height and weight should be

carefully measured and growth rate and appropriateness of weight for height determined (see Fig. 16.42). Careful categorization of the stage of breast and sexual hair development are essential. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary for diagnosis.⁹⁹⁷ Examination of the mature breast should include an attempt to express milk from the ducts to the nipple. The finding of a structural genital abnormality may indicate that amenorrhea is caused by abnormal genital tract development, while clitoromegaly⁹⁹⁸ is a clue to a virilizing disorder. Neurological examination should include evaluation of eye movements, visual fields, and optic fundi, as well as a search for anosmia and midline defects.

If a disorder or syndrome associated with hypogonadism is recognized in the newborn period, advantage may be taken of the mini-puberty of the newborn to attempt to make a diagnosis during the first few months of life. For example, the hypergonadotropic hypogonadism of Turner syndrome and the hypogonadotropic hypogonadism of hypopituitarism may be documented during this critical period before the physiological restraint of puberty during late infancy and childhood makes it difficult to do so before the normal age of puberty.^{999–1002}

An algorithmic approach to the workup of patients with menstrual disorders is shown in Figs. 16.43 to 16.45.¹⁰⁰³ The laboratory workup depends on the degree of estrogenization, as initially assessed from the stage of breast development: it includes a bone age radiograph in adolescents who are not sexually mature and generally begins with a chronic disease panel, and determination of gonadotropins, estradiol, and testosterone level. A pregnancy test is indicated in a sexually mature adolescent. The diagnostic considerations differ in the anovulatory girl without FSH elevation, depending upon whether she is hypoestrogenic or estrogenized (see Box 16.5 and see Figs. 16.43 and 16.44).

FSH elevation indicates primary ovarian failure (see Figs. 16.43–16.45). Chromosome abnormalities are ordinarily the first consideration because the most common cause is Turner syndrome and its variants. Those individuals with primary ovarian failure that is not caused by Turner syndrome and its variants should be investigated for the fragile X premutation, autoimmune oophoritis, and steroidogenic defects.

Lack of FSH elevation in a prepubertal patient does not rule out primary ovarian failure if bone age is below 11 years because neuroendocrine puberty may not have occurred; in this situation, primary ovarian failure is not hypergonadotropic (see Table 16.5).⁸⁸² If FSH is not elevated and bone age has reached 11 years, in a prepubertal girl without a growth-attenuating or retarding disorder, one is dealing either with constitutional delay of puberty or isolated gonadotropin deficiency (see Fig. 16.43). “Constitutional” delay of puberty is the most likely diagnosis until the bone age reaches 11 to 13 years (see Table 16.5).⁸⁸² Its distinction from isolated gonadotropin deficiency may be difficult. The features that help to distinguish it from isolated gonadotropin deficiency are listed in Box 16.6 and discussed in Fig. 16.43 footnotes. The single most useful test is the LH level in response to GnRH testing because random LH levels in hypogonadotropic patients often overlap those of pre- and midpubertal normal children.¹⁰⁰ GnRH agonist testing may discriminate between these disorders better because the LH response at 3 to 4 hours is the best indicator of gonadotropin secretory reserve and because this test permits assessing the gonadal secretory response to the secreted gonadotropins at 24 hours.⁸³⁸ Kisspeptin was given in an experimental protocol to children with CDGP to determine whether it may be a diagnostic test to predict entry into puberty. Children showed a wide range of responses, ranging from a robust response to little to no response. Hence its utility as a predictor of future reproductive development remains questionable.¹⁰⁰⁴

BOX 16.6 Features That Distinguish Gonadotropin Deficiency From Constitutional Delay of Puberty

In a healthy delayed prepubertal girl with BA >11 y and prepubertal FSH, gonadotropin deficiency is:

- Possible if:
 - Weight loss greater than 5% to 8% (BMI <10th–15th percentile for height age)
 - Midline facial defect
 - CNS dysfunction
 - CT or MRI brain scan abnormal
- Probable if:
 - BA > 13 years and LH <0.15 U/L in early daytime
 - Anosmia or panhypopituitarism
- Diagnostic if:
 - Sleep-associated increase in LH lacking
 - GnRH agonist test subnormal response
 - Chronological age >16 y

BA, Bone age; BMI, body mass index; CNS, central nervous system; CT, computed tomography; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MRI, magnetic resonance imaging.

(Modified from Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491–505. With permission.)

The assessment of an adolescents’ degree of estrogenization is often difficult. Breast development indicates that there has been estrogen exposure, but does not mean that it is current. Determination of serum estradiol is the simplest test, but diurnal and cyclic variations must be considered. Determination of hormonal effects on vaginal cytology is the most indicative of overall estrogen exposure (see Fig. 16.28), but less well accepted by patients. A progestin challenge test is often helpful. A female who does not experience progestin withdrawal bleeding (see Fig. 16.44) probably has an ambient estradiol level of less than about 40 pg/mL.¹⁰⁰⁵ If bleeding does not occur in response to this maneuver, the integrity of the uterus can be demonstrated by eliciting withdrawal bleeding after a 3-week course of estrogen-progestin, most conveniently administered in the form of birth control pills.

A prolactin level is indicated in the initial workup of normogonadotropic patients, regardless of their estrogen status (Fig. 16.45). The prolactin level correlates with the size of prolactinomas, and a level over 200 ng/mL is typical of a macroprolactinoma. A prolactin level that does not correlate with the size of a large pituitary tumor suggests either that the tumor is not a prolactinoma and is causing a functional pituitary stalk section or it is a macroprolactinoma elaborating such high levels of prolactin as to artefactually lower the immunoassayable prolactin level by a “hook effect.”¹⁰⁰⁶ Very high blood or cerebrospinal fluid prolactin levels suggest invasiveness. The workup for this should include formal testing of visual fields (Goldman perimetry or evoked response). Pituitary microadenomas may be “incidentalomas” of no clinical significance, judging from an approximate 10% incidence in autopsy material.¹⁰⁰⁷ However, they require careful assessment of pituitary function and follow-up.¹⁰⁰⁸ Macroprolactinemia should be considered in the absence of clearly related symptoms and when MRI is negative or in the setting of autoimmune disease.^{988,989} Macroprolactinemia is confirmed when the prolactin level measured after precipitation of serum using polyethylene glycol is normal (or substantially reduced compared with the level measured in untreated serum).

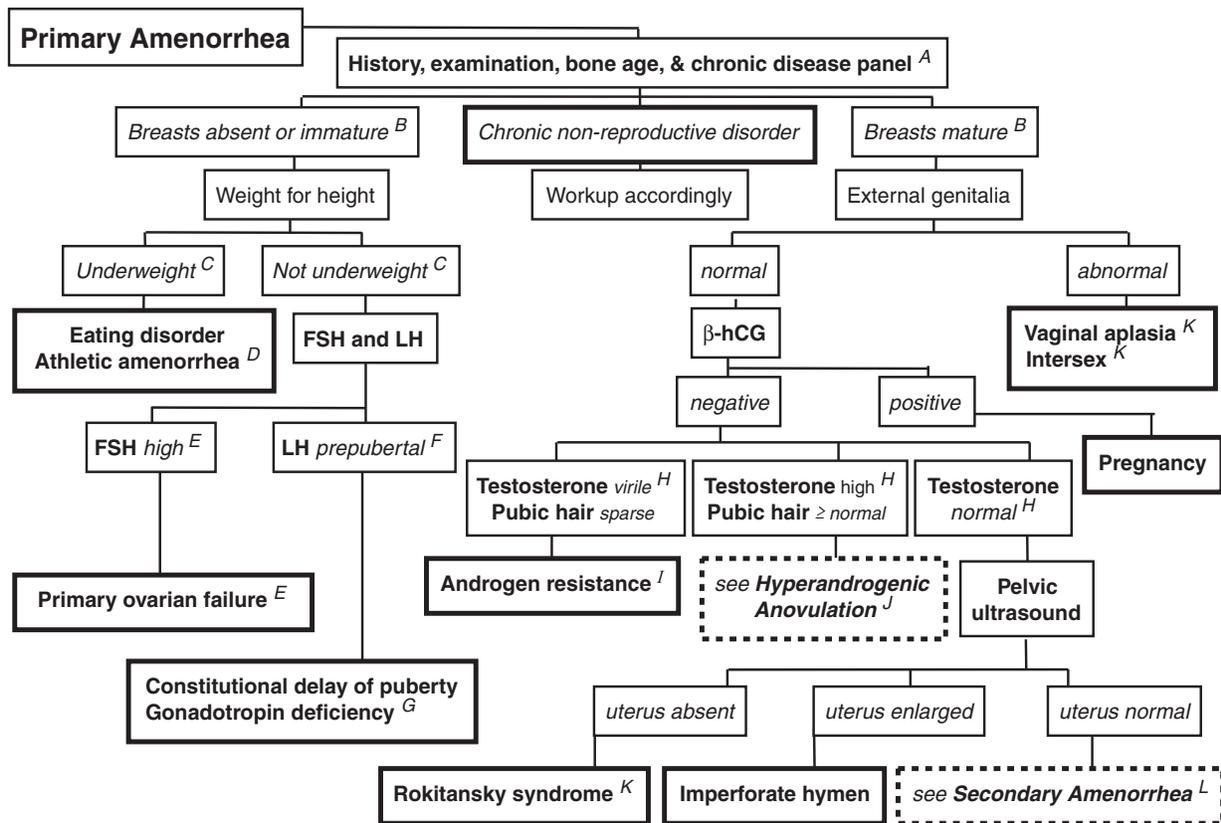


Fig. 16.43 Differential diagnosis of primary amenorrhea. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J., Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- A. Prime among the causes of primary amenorrhea are growth-retarding or growth-attenuating disorders. In the absence of specific symptoms or signs to direct the workup, laboratory assessment for chronic disease typically includes bone age radiograph if the adolescent is not sexually mature and a chronic disease panel (complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis).
 - B. Breast development ordinarily signifies the onset of pubertal feminization. However, mature breast development does not ensure ongoing pubertal estrogen secretion.
 - C. Body mass index (BMI) under the 10th percentile generally corresponds to body composition <20% body fat, which is the critical factor.
 - D. BMI may not accurately reflect body fat in serious athletes (who have a disproportionately greater muscle mass) or bulimia nervosa.
 - E. Follicle-stimulating hormone (FSH) is preferentially elevated over luteinizing hormone (LH) in primary ovarian failure. The most common cause of primary amenorrhea caused by primary ovarian failure is gonadal dysgenesis caused by Turner syndrome, but acquired causes must be considered (such as cytotoxic therapy). The workup of primary ovarian failure is considered in detail in the next algorithm (secondary amenorrhea and oligomenorrhea). Lack of FSH elevation virtually rules out primary ovarian failure only when the bone age is appropriate for puberty (≥ 11 years).
 - F. “Pediatric” gonadotropin assays sensitive to 0.15 U/L or lower are critical to the accurate diagnosis of gonadotropin deficiency and delayed puberty. A low LH level is more characteristic of these disorders than a low FSH level. Congenital gonadotropin deficiency is closely mimicked by the more common extreme variation of normal, constitutional delay of puberty.
 - G. History and examination may yield clues to the cause of hypogonadotropic hypogonadism, such as evidence of hypopituitarism (midline facial defect, extreme short stature, visual field defect) or anosmia (Kallmann syndrome) or functional hypothalamic disturbance (bulimia, excessive exercise). Random LH levels in hypogonadotropic patients are usually below 0.15 IU/L, but often overlap those of normal pre- and midpubertal children. The gonadotropin-releasing hormone (GnRH) test, measuring the gonadotropin response to a 50- to 100-mcg bolus, in the premenarcheal teenager strongly suggests gonadotropin deficiency if the LH peak is less than 4.2 IU/L by monoclonal assay. Inhibin-B under 20 pg/mL or responses to GnRH agonist testing (e.g., leuprolide acetate injection 10 mcg/kg SC) are reported to discriminate well between gonadotropin deficiency and constitutional delay (see text). It may not be possible to definitively establish the diagnosis of gonadotropin deficiency until puberty fails to begin by 16 years of age or progress at a normal tempo.
 - H. Plasma total testosterone is normally 20 to 60 ng/dL (0.7–2.1 nM) in women, 300 to 1200 ng/dL in men, but varies somewhat among laboratories. Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner.
 - I. Androgen resistance is characterized by a frankly male plasma testosterone level when sexual maturation is complete, male karyotype (46, XY), and absent uterus. External genitalia may be ambiguous (partial form) or normal female (complete form).
 - J. The differential diagnosis of hyperandrogenism is shown in a later algorithm.
 - K. Vaginal aplasia in a girl with normal ovaries may be associated with uterine aplasia (Rokitansky-Kustner-Hauser syndrome). When the vagina is blind and the uterus aplastic, this disorder must be distinguished from androgen resistance. If the external genitalia are ambiguous, it must be distinguished from other disorders of sex development (intersex).
 - L. Secondary amenorrhea differential diagnosis is presented in Fig. 16.43.
- hCG*, Human chorionic gonadotropin.

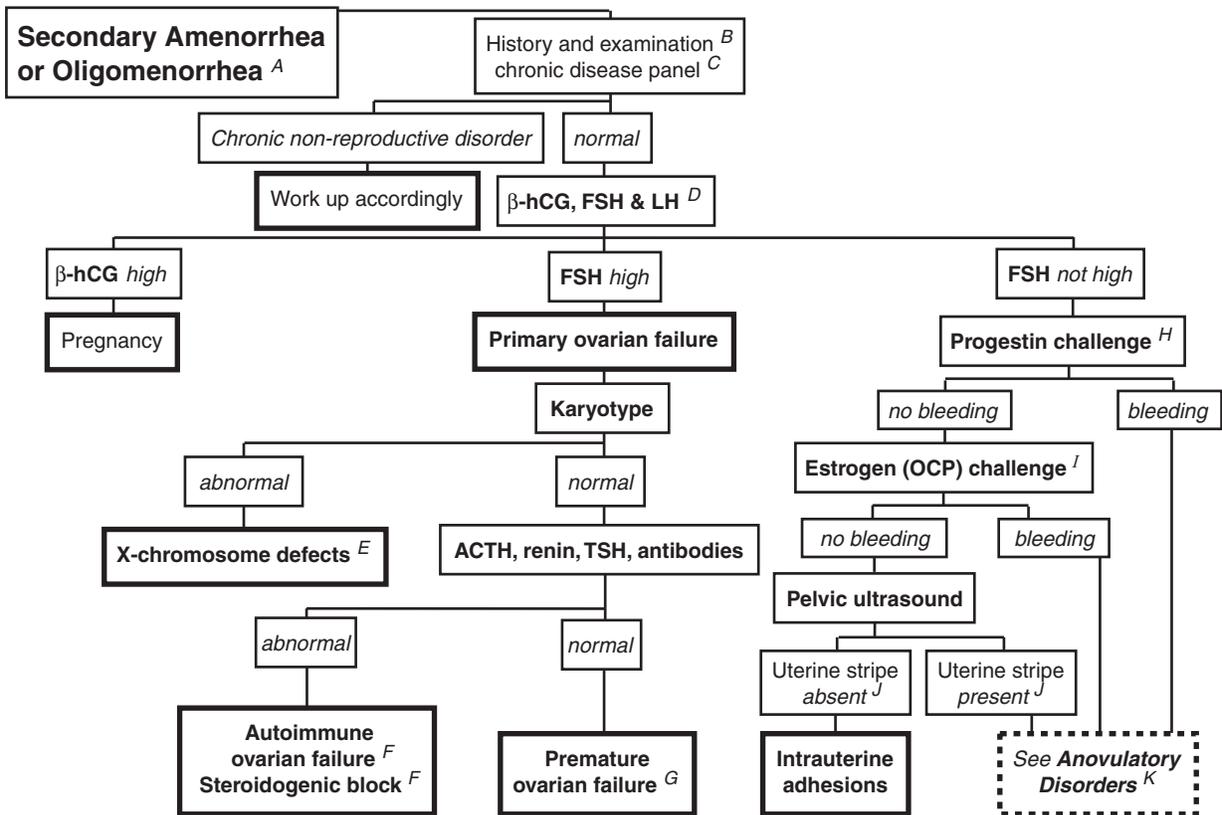


Fig. 16.44 Differential diagnosis of secondary amenorrhea or oligomenorrhea. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J., Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- A. Mature secondary sex characteristics are characteristic because the occurrence of menarche indicates that a substantial degree of development of the reproductive system has taken place; however, their presence does not rule out subsequent regression of gonadal function.
 - B. Diverse disorders of many systems cause anovulation. The history may reveal excessive exercise, symptoms of depression, gastrointestinal symptoms, radiotherapy to the brain or pelvis, or rapid virilization. Physical findings may include hypertension (forms of congenital adrenal hyperplasia, chronic renal failure), short stature (hypopituitarism, Turner syndrome, pseudohypoparathyroidism), abnormal weight for height (anorexia nervosa, obesity), decreased sense of smell (Kallmann syndrome), optic disc or visual field abnormality (pituitary tumor), cutaneous abnormalities (neurofibromatosis, lupus), goiter, galactorrhea, hirsutism, or abdominal mass.
 - C. In the absence of specific symptoms or signs to direct the workup, evaluation for chronic disease in a sexually mature adolescent typically includes complete blood count and differential, sedimentation rate, comprehensive metabolic panel, celiac panel, thyroid panel, cortisol and insulin-like growth factor-I levels, and urinalysis.
 - D. “Pediatric” gonadotropin assays sensitive to 0.15 U/L or lower are critical to the early diagnosis of many anovulatory disorders. Luteinizing hormone (LH) enters into the differential diagnosis of hypogonadotropic anovulatory disorders, which is outlined in Fig. 16.45.
 - E. Patients missing only a small portion of an X-chromosome may not have the Turner syndrome phenotype. Indeed, among 45,X patients the classic Turner syndrome phenotype is found in less than one-third (with the exception of short stature in 99%). Ovarian function is sufficient for about 10% to undergo some spontaneous pubertal development and for 5% to experience menarche. If chromosomal studies are normal and there is no obvious explanation for the hypogonadism, special studies for fragile X premutation and autoimmune oophoritis should be considered.
 - F. Autoimmune ovarian failure may be associated with tissue-specific antibodies and autoimmune endocrinopathies, such as chronic autoimmune thyroiditis, diabetes, adrenal insufficiency, and hypoparathyroidism. Nonendocrine autoimmune disorders may occur, such as mucocutaneous candidiasis, celiac disease, and chronic hepatitis. Rare gene mutations causing ovarian insufficiency include steroidogenic defects that affect mineralocorticoid status (17-hydroxylase deficiency is associated with mineralocorticoid excess and lipid adrenal hyperplasia with mineralocorticoid deficiency) and mutations of the gonadotropins or their receptors. Ovarian biopsy is of no prognostic or therapeutic significance. LH is disproportionately high in steroidogenic defects or autoimmune disease specifically affecting theca cells.
 - G. The history may provide a diagnosis (e.g., cancer chemotherapy or radiotherapy). Other acquired causes include surgery and autoimmunity. Chromosomal causes of premature ovarian failure include X-chromosome fragile site and point mutations. Other genetic causes include gonadotropin-resistance syndromes, such as LH or follicle-stimulating hormone (FSH) receptor mutation and pseudohypoparathyroidism. A pelvic ultrasound that shows preservation of ovarian follicles carries some hope for fertility.
 - H. Withdrawal bleeding in response to a 5- to 10-day course of progestin (e.g., medroxyprogesterone acetate 10 mg HS) suggests an overall estradiol level greater than 40 pg/mL. However, this is not entirely reliable and thus in the interest of making a timely diagnosis it is often worthwhile to proceed to further studies.
 - I. A single cycle of an oral contraceptive pill (OCP) containing 30 to 35 mcg ethinyl estradiol generally suffices to induce withdrawal bleeding if the endometrial lining is intact.
 - J. A thin uterine stripe suggests hypoestrogenism. A thick one suggests endometrial hyperplasia, as may occur in polycystic ovary syndrome.
 - K. The differential diagnosis of other anovulatory disorders continues in Fig. 16.45.
- ACTH, adrenocorticotropic hormone; hCG, Human chorionic gonadotropin; TSH, thyroid-stimulating hormone.

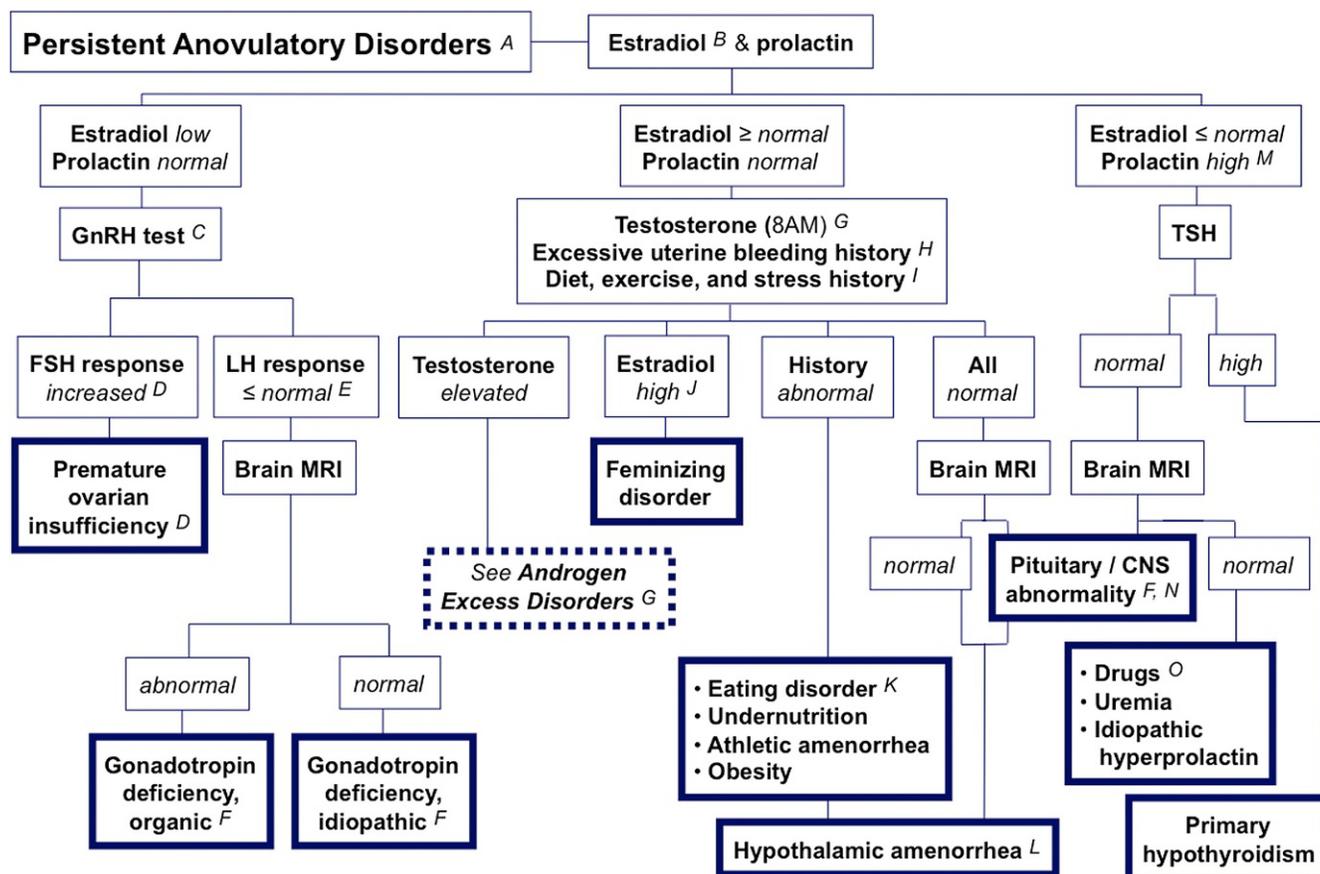


Fig. 16.45 Differential diagnosis of persistent anovulatory disorders. (Modified from Rosenfield, R.L. (2003). Menstrual disorders and hyperandrogenism in adolescence. In: Radovick, S., MacGillivray, M.H. eds. Pediatric Endocrinology a Practical Clinical Guide. Totowa, N. J., Humana Press, Inc., p. 451–478. With permission.)

Footnotes:

- Anovulatory disorders should be considered in any girl with persistent unexplained amenorrhea or oligomenorrhea, irregular menstrual bleeding, short cycles, or excessive menstrual bleeding. Physiological adolescent anovulation, a transient variation of normal, is the most common cause of menstrual dysfunction in the early postmenarcheal years. The workup in this algorithm progresses from negative studies in the preceding algorithm.
- Once breast development has matured, the breast contour does not substantially regress if hypoestrogenism develops. Hypoestrogenism is suggested if plasma estradiol is persistently under 40 pg/mL in a “pediatric” assay sensitive to less than 10 pg/mL. However, a single estradiol level may be misleading because of cyclic or episodic variations.
- Gonadotropin-releasing hormone (GnRH) testing is usually performed by assaying luteinizing hormone (LH) and follicle-stimulating hormone (FSH) before and 0.5 hour after the administration of 1 mcg/kg GnRH intravenously. GnRH agonist testing may alternatively be performed by administering 10 mcg/kg leuprolide acetate subcutaneously and assaying LH and FSH at 3 to 4 hours to assess gonadotropin reserve and at 18 to 24 hours to assess the ovarian steroid response to endogenous gonadotropin release.
- Baseline gonadotropin levels may be normal as the ovary begins to fail, as in early menopause, but an exaggerated FSH response to GnRH and subnormal estradiol response to the gonadotropin elevation induced by acute GnRH agonist challenge are characteristic. See also Premature Ovarian Failure in preceding figure.
- LH responses to GnRH may vary from nil to normal in gonadotropin deficiency: normal LH and FSH responses in the presence of hypoestrogenism indicate inadequate compensatory hypothalamic GnRH secretion.
- Gonadotropin deficiency may be congenital or acquired, organic or functional. Congenital causes include midline brain malformations or specific genetic disorders, such as Prader-Willi syndrome, Laurence-Moon-Biedl syndrome, or Kallmann syndrome. Kallmann, the association of anosmia with gonadotropin deficiency, occurs in both the X-linked and autosomal-recessive forms. Special magnetic resonance imaging (MRI) views often demonstrate absence of the olfactory tracts. Acquired gonadotropin deficiency may be secondary to a variety of organic central nervous system (CNS) disorders, varying from hypothalamic-pituitary tumor to radiation damage to empty sella syndrome. Autoimmune hypophysitis is a rare disorder, sometimes accompanying a polyendocrine deficiency syndrome. The prototypic form of functional gonadotropin deficiency is anorexia nervosa. Idiopathic hypogonadotropic deficiency may sometimes occur in families with anosmia, suggesting a relationship to Kallmann syndrome.
- Plasma free (or bioavailable) testosterone is about 50% more sensitive than total testosterone in detecting hyperandrogenemia. However, there are many pitfalls in testosterone assays at the low levels of women, reliable testosterone assays are not available to many physicians, and assaying the free testosterone introduces other potential sources of error. Therefore it is reasonable to begin the evaluation with a total testosterone determination if a free testosterone test in a reliable specialty laboratory is not available to the practitioner. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high-risk for congenital adrenal hyperplasia, such as Ashkenazi Jews. Initial assessment for differentiating among the causes of androgen excess is outlined in Fig. 16.49.
- Excessive (“dysfunctional”) uterine bleeding not controlled by progestin or oral contraceptive pill therapy additionally requires a pelvic ultrasound examination (for genital tract tumor or feminizing tumor), coagulation workup (which includes platelet count, prothrombin time, thromboplastin generation test, bleeding time, and von Willebrand factor), and consideration of the possibility of sexual abuse.

(Legend continuous on next page)

- I. The equivalent of 20 miles per week or more is generally required before body fat stores fall to the point where amenorrhea occurs. Physical or psychosocial stress may cause amenorrhea.
- J. The normal range for estradiol over the menstrual cycle is wide: values over 95 pg/mL usually indicate the preovulatory or luteal phase, but are compatible with a feminizing disorder.
- K. Low body fat caused by mild forms of stress disorders (anorexia nervosa, bulimia nervosa, and athletic amenorrhea) may be associated with acquired functional hypothalamic amenorrhea rather than frank gonadotropin deficiency. The low body fat content of athletic amenorrhea may not be reflected by weight for height because of high muscularity. Dual-photon absorptiometry scan may be useful in documenting body fat below 20%. Patients with anorexia nervosa may become amenorrheic before or when weight loss begins, indicating an important psychological component to the etiology. Obesity is also associated with anovulatory cycles and raises the possibility of Cushing syndrome.
- L. Hypothalamic amenorrhea is a diagnosis of exclusion. It is a form of partial gonadotropin deficiency in which baseline estrogen secretion is normal but a preovulatory LH surge cannot be generated. It may result from organic CNS disorders or be functional, caused by stress, undernutrition or obesity, diverse types of endocrine dysfunction, chronic disease, or idiopathic. It may be difficult to distinguish from hyperandrogenemia.
- M. Hyperprolactinemia is heterogeneous in its presentation. Some have normoestrogenic anovulation, which may be manifest as hypothalamic anovulation, hyperandrogenism, dysfunctional uterine bleeding, or short luteal phase. On the other hand, some are hypoestrogenic; these do not have galactorrhea.
- N. Large hypothalamic-pituitary tumors or other types of CNS injury cause variable pituitary dysfunction, which may include complete or partial gonadotropin deficiency and various manifestations of hypopituitarism (including secondary hypothyroidism). If they interrupt the pituitary stalk, hyperprolactinemia ensues. Hyperprolactinemia may also be caused by prolactinomas.
- O. Drugs, particularly neuroleptics of the phenothiazone or tricyclic type, may induce hyperprolactinemia.

Imaging studies are important ancillary measures. Pelvic ultrasound may demonstrate hypoplastic ovaries, endometrial disorders, or polycystic ovaries. MRI of the hypothalamic-pituitary area is important in the workup of gonadotropin deficiency, hyperprolactinemia, and hypothalamic anovulation. MRI may not be necessary if the patient presents with a history suggesting functional hypothalamic amenorrhea but is indicated in those cases with associated symptoms of headache, vomiting, vision change, alteration in thirst or urinary frequency, an abnormal neurological examination, or evidence of other pituitary hormonal abnormalities.⁹⁹⁶

Anorexic patients require psychiatric evaluation and consideration of brain tumor and partial bowel obstruction. Diet fadism and athletic addiction may be difficult to distinguish from anorexia nervosa. Constitutionally thinness is a variant of normal with normal menses and a distinctive hormonal profile.¹⁰⁰⁹ It is unclear whether the superior mesenteric artery syndrome is a primary disorder that mimics anorexia nervosa or is a complication of it.¹⁰¹⁰

Management

Underlying disorders must be treated appropriately. For example, tumors require surgery and/or radiotherapy.

For prolactinoma, dopaminergic treatment is the initial treatment of choice unless the patients' condition or eyesight is critical.^{991,1011} Hyperprolactinemia will be maximally suppressed within 1 month and the menstrual cycle normalized within 3 months by an effective dopaminergic agonist regimen. Cabergoline 0.5 to 1.0 mg once or twice weekly will usually control galactorrhea and shrink prolactinomas.^{987,1012} To minimize nausea, it is best to start with a low dose at bedtime. Bromocriptine does not activate the serotonin 5-hydroxytryptamine (2B) receptor, the proposed mechanism through which cabergoline is thought to stimulate cardiac valve dysfunction in the elderly, and so bromocriptine does not seem to be associated with an increased risk of cardiac valve regurgitation, and may be considered as an alternative to cabergoline treatment, albeit a less effective one. The usual bromocriptine maintenance dose is 0.25 to 0.5 mg twice daily. After 2 years of treatment, if the prolactin level is normal and there is no tumor evident on MRI, dopamine agonist may be tapered and possibly discontinued. Prolactin levels are then measured to monitor for recurrence.¹⁰¹³

Anorexia nervosa is best managed by an experienced multidisciplinary team. Refeeding is the first priority, and once steady weight gain is evident the psychodynamic issues can be addressed.¹⁰¹⁴ Family therapy of medically uncomplicated cases of anorexia nervosa on an outpatient basis generally yields the best results, with good improvement in over half of patients. Inpatient care is needed if there is severe bradycardia, hypotension or orthostasis, or electrolyte imbalance,⁹⁹⁶ and may be needed if there is failure of weight gain during outpatient therapy. Menses generally resume when psychotherapy is effective and body fat is restored to normal (see Fig. 16.42). The induction of menses by estrogen-progestin replacement is usually injudicious because it provides a false sense of recovery and does not yield the recovery of bone loss that occurs with weight gain.¹⁰¹⁵ However, in those adolescents with low bone density who have not had return of menses after a reasonable trial of nutritional and psychological intervention (perhaps 6–12 months), short-term treatment with transdermal estradiol and cyclic oral progestin (not oral contraceptives or ethinyl estradiol) can be considered to protect the patient's bone health.⁹⁹⁶ Although the acute episode can usually be successfully treated, there is a high rate of ongoing psychiatric disability and medical complications. Anorexia nervosa "by proxy" has been described in the offspring of former patients.¹⁰¹⁶

There are two aspects of therapy that are uniformly involved in managing hypogonadism—psychological support and hormone administration. Patients with delayed development that is a variation of normal should be reassured that there is nothing wrong, only a delay in timing of the onset of puberty. The wide normal variation in the pattern and time of the pubertal growth spurt should be explained in detail and the girl should be informed of her predicted eventual height. The majority of children with delayed puberty do not have overt psychological symptoms. Complex compensations and sublimations obviously occur. However, peer group pressures may make adjustment to sexual infantilism especially difficult when the age of 13 years is approached,¹⁰¹⁷ and a poor self-image may lead to social withdrawal and feelings of hopelessness. Physical immaturity may prolong psychological immaturity. A 6- to 12-month course of physiological sex hormone therapy at this time may help alleviate these anxieties. The physician should discuss the fact, when the evidence favors it, that the odds are overwhelmingly in favor of the "timer in the subconscious area of the brain" eventually turning on. When this will happen can be approximated from the skeletal age. One should not

hesitate to advise more intensive psychological counseling if it becomes apparent that the concern about puberty is but one aspect of a more general maladjustment. Ultimately, the decision as to whether to undertake treatment for delayed puberty is up to the patient and her family.

Assure the teenager with an organic basis for hypoestrogenism that feminization will occur, although in response to appropriate hormone treatment. Some genetic forms of gonadotropin deficiency are actually reversed by sex steroid therapy.²³⁵ However, most will require lifelong hormone replacement therapy. It should be kept in mind that attainment of normal breast development in the girl with panhypopituitarism requires replacement of GH and cortisol deficits. It is difficult however to induce secondary sex characteristics in some patients with systemic chronic inflammatory disease, such as lupus erythematosus.

In patients in whom short stature is an important concern, as in Turner syndrome, growth potential must be considered before undertaking estrogen replacement. GH therapy improves the adult height potential of patients with Turner syndrome, especially when started as soon as growth failure becomes apparent.¹⁰¹⁸ GH therapy in the United States is generally initiated at the US Food and Drug Administration (FDA)-approved dose of 0.375 mg/kg·wk. If adult height is likely to be unsatisfactory, concomitant treatment with oxandrolone 0.03–0.05 mg/kg/day should be considered.⁵⁷¹ Clitoromegaly is ordinarily negligible on this dosage; liver function should be monitored.

Two controlled studies have shown that pubertal estrogen replacement therapy is safe and efficacious in maximizing growth potential and age-appropriate feminization when started as young as 11 to 12 years of age using very low estrogen doses, far below those that are available by prescription, in conjunction with GH therapy in Turner syndrome.^{1020,1021} The estrogen content of oral contraceptive pills (OCPs) is supraphysiological for induction of breast development or for linear growth.

We favor use of one of the following hormone replacement regimens.^{571,1022} Whichever form of estrogen is used, pubertal development and growth should be monitored every 6 months, with bone age determinations at 6- to 12-month intervals to avoid unanticipated loss of growth potential.

IM depot estradiol in a starting dose of 0.2 mg/month will usually induce breast budding; the dose should be increased by 0.2 mg every 6 months.¹⁰²⁰ A midpubertal dose of 1.0 to 1.5 mg monthly, which is half the adult replacement dose, typically induces menarche within 1 year. An alternative oral regimen begins with 5 mcg/kg micronized estradiol (Estrace[®], 0.25 mg for a 50 kg girl) daily; the adult replacement dose is 1 to 2 mg/day.¹⁰²³

Transdermal estradiol is a convenient, physiological form of therapy¹⁰²⁴ that appears to have long-term health advantages over commonly used oral estrogens,^{1025,1026} but there are little data on their use for inducing puberty. We suggest starting transdermal feminization with 14 mcg daily for 1 week per month, a marginally feminizing dose that is in accord with current guidelines,^{571,1018} and escalating at 6-month intervals to an adult dose at 3 years. A suggested protocol for female pubertal induction for hypogonadal patients is provided in Table 16.7.¹⁰²³

For girls with hypogonadism and an intact uterus, cyclic progestin should be added after 2 years of estrogen therapy or when bleeding begins to occur at unpredictable times. A simple regimen is to use 100 mg of micronized progesterone (Prometrium[®]) at bedtime for 7 to 14 days during the second to third week of estrogen therapy or equivalent doses of medroxyprogesterone acetate (5–10 mg/day) or norethindrone acetate (5 mg/day). This will bring about normal menstruation during

TABLE 16.7 A Pubertal Transdermal Estradiol Replacement Regimen Beginning at 11 Years of Age^a

Age	Estradiol Dose
0–6 months	14 mcg, day 1–7 each month
6–12 months	14 mcg, day 1–14 each month
1–1.5 years	14 mcg, day 1–21 each month
1.5–2 years	25 mcg, day 1–21 each month
2–2.5 years	37.5 mcg, day 1–21 each month
2.5–3 years	28-day cycle: 50 mcg day 1–21 and Prometrium [®] 100 mg ^b day 12–21 every 28 days
	OR
	Continuous: 50 mcg day 1–14, then Combipatch [®] (50 mcg estradiol/0.14 mg norethindrone) day 16–28 every 28 days
3–3.5 years	28-day cycle: 75 mcg day 1–21 and Prometrium [®] 100 mg day 12–21 every 28 days
	OR
	Continuous: 75 mcg day 1–14 then Combipatch [®] (50 mcg estradiol/norethindrone) day 16–28 every 28 days
3.5–4 years	28-day cycle: 100 mcg day 1–21 and Prometrium [®] 100mg day 12–21 every 28 days
	OR
	100 mcg day 1–14 then Combipatch [®] (50 mcg estradiol/norethindrone) day 16–28 every 28 days
>4.0 years	Continue regimen or offer oral contraceptive pill

^aIn children ≥ 13 years old, consider starting with 25 mcg for 2–3 weeks monthly and increasing the dose at shorter intervals (e.g., 3 months).

^bIf inadequate bleeding, increase Prometrium to 200 mg day 12–21 or change to Combipatch 50 mcg estradiol/0.25mg norethindrone. (Modified from Klein, K.O., Rosenfield, R.L., Santen, R.J., et al. (2018). Estrogen replacement in Turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab*, 103, 1790–1803.)

the week preceding resumption of estrogen therapy. The addition of progestin will decrease the risk of endometrial hyperplasia and endometrial carcinoma, but premenstrual symptoms should be anticipated.

Once optimal height is achieved, most patients prefer to switch to combined oral contraceptive pills (OCP) as a convenient form of estrogen-progestin therapy. The pills containing the lowest dose of estrogen that will result in normal menstrual cycles are advisable. The potential risks of oral contraceptives must be kept in mind when counseling adolescents.¹⁰²⁷ The lowest estrogen dosages currently available in combination contraceptive pills in the United States contain 20 mcg (Mircette[®]) to 30 mcg (Yasmin[®]) ethinyl estradiol. Low-dose androgen replacement has been controversial, but may confer benefits to body composition, cognition, bone mineralization, and libido.¹⁰²⁸

Hypogonadotropic patients can achieve ovulation with gonadotropin therapy. Hypothalamic GnRH deficiency can be successfully treated by pulsatile GnRH.^{91,1029} Recently, kisspeptin has been considered as a therapeutic modality for the treatment of CHH; however, its short half-life has hampered efforts. Hence kisspeptin analogs were designed to decrease proteolytic degradation and renal clearance. These analogs induced direct excitatory action on GnRH neurons, advanced puberty, and synchronized ovulations in sheep. The potential for treatment of reproductive disorders in humans remains undefined.¹⁰³⁰ Because several genes in the GnRH signaling system are expressed in the gonads, some hypogonadotropic patients have primary defects in gonadal function.^{931,1031} Induction of ovulation is best carried out by a gynecologist specializing in reproductive endocrinology.

Fertility preservation is possible in some patients with primary ovarian failure. Oocyte cryopreservation for later in vitro

fertilization is feasible in pubertal girls after ovulation induction.^{887,890,909,1032} The girls must be pubertal for successful ovulation induction. In addition, cryopreservation of embryos requires sperm, likely limiting the appropriateness of this in pediatric patients. Cryopreservation of ovarian tissue^{922,1033} is a promising technique that may be useful for fertility preservation in prepubertal girls with ovarian failure, although this is currently an investigational approach. Finally, activation of residual follicle growth in autografts has recently been accomplished in adults with POI, with successful pregnancy after embryo transfer.¹⁰³⁴ Female germline stem cell research holds promise as a future fertility treatment.¹⁰³⁵ Up-to-date information for physicians and patients can be found through the Oncofertility Consortium (www.myoncofertility.org).

In girls with Turner syndrome, fertility preservation using either oocyte or embryo cryopreservation are currently only of use in those who have undergone spontaneous puberty. These girls should be counseled about these possibilities for fertility preservation.¹⁰³⁶ In these girls, monitoring AMH levels may be useful as a marker of ovarian function.¹⁰³⁷ On the other hand, primordial follicles have been found in ovarian tissue collected for cryopreservation from girls with mosaic or nonmosaic Turner syndrome, although the likelihood of finding follicles was much lower in those with a 45,X karyotype (10.7%) than in those with 46,XX/45,X mosaicism (86%).¹⁰³⁷ As noted earlier, however, ovarian cryopreservation remains an investigational procedure. In the rare spontaneous pregnancies that have occurred in women with Turner syndrome, the rate of congenital and chromosomal abnormalities in the fetus is approximately 50%,¹⁰³⁷ which must be considered when using autologous oocytes from any approach. Finally, women with Turner syndrome are at high risk of fetal and maternal pregnancy complications because of uterine anomalies, carbohydrate intolerance, and potential cardiovascular complications including aortic rupture. (For discussion of other aspects in the care of girls with Turner syndrome see [Chapter 17](#).)

Nonhypoestrogenic Menstrual Disturbances

Hypothalamic Anovulation

Hypothalamic anovulation causes menstrual disturbances in sexually mature women through a deficiency in GnRH secretion that is too subtle to cause frank hypoestrogenism. The neuroendocrine system stimulates ovarian estrogen secretion to a level normal for an early- or midfollicular phase female, but follicular development is inadequate for a normal dominant follicle to emerge. Amenorrhea or oligomenorrhea may result. However, in some patients, sufficient estrogenization occurs to cause dysfunctional uterine bleeding, which is discussed in the next section.

Reduced LH pulsatility¹⁰³⁸ and/or failure to generate a mid-cycle LH surge^{1039,1040} are characteristic. The pathophysiology seems to be mediated primarily by undernutrition and/or CRH excess. Negative energy balance may be present even in patients of normal, but less than average, weight and fat stores.^{497,981} Leptin deficiency is an important determinant of the decreased LH pulsatility. Ghrelin may play a role in LH inhibition.¹⁰⁴¹ The anovulation of psychic or physical stress seems to involve CRH excess.⁹⁸² In the brain, CRH releases β -endorphin from proopiomelanocortin, and the endorphin in turn inhibits GnRH release. Naloxone blockade of opioid action normalizes gonadotropin secretion.⁹¹ In the pituitary, CRH increases the set-point for ACTH release. This brings about a new steady state of increased cortisol secretion. Further ACTH response to CRH is blunted by the negative feedback of this cortisol excess. The result is a mildly cushingoid cortisol rhythm. Cortisol excess itself can contribute to the amenorrhea by inhibiting the

response to GnRH,¹⁰³³ as well as antagonizing some sex hormone actions. Adrenal androgens are elevated in competitive athletes who maintain body fat stores.¹⁰⁴²

Causes. Functional hypothalamic amenorrhea (FHA) describes hypothalamic anovulation that is unexplained by an identifiable organic CNS cause or chronic disease.⁹⁹⁶ These patients have a normal or slightly low serum LH, normal FSH, and estradiol in the early-mid follicular phase range (20–50 pg/mL). It is caused by energy deficit (because of weight loss and/or vigorous exercise) or psychological stress or a combination thereof. As with patients with anorexia nervosa and the athletic or psychogenic types of hypothalamic anovulation, they often have significant hypercortisolemia. However, there are patients with FHA who cannot be identified as having one of these causes. Some 24% of such women in one series had a history of delayed menarche.⁷²⁰ A primate model indicates that hypothalamic anovulation develops in stress-sensitive individuals from an innocuous combination of mild stress and mild caloric restriction.¹⁰⁴³ Heterozygosity for genes associated with Kallmann syndrome has been identified as a predisposing factor in 13% of cases; more than half of this subgroup had a family history of hypothalamic amenorrhea or delayed puberty.⁷²⁰

Athletic amenorrhea is the term given to hypothalamic anovulation associated with excessive exercise and low body fat stores. Running 20 miles or more per week is associated with increased incidence of amenorrhea.¹⁰⁴⁴ The female athletic triad consists of menstrual disturbance, eating disorder, and osteoporosis.¹⁰⁴⁵ Primary or secondary amenorrhea, oligomenorrhea, or short luteal phase are common in athletes.¹⁰⁴⁶ Ovarian function decreases approximately in proportion to the amount of physical activity and dietary restriction. Weight-bearing exercise is only partially protective of the effects of hypoestrogenism on weight-bearing bone. There is concern that amenorrheic athletes may be left with a permanent deficit in bone mass.¹⁰⁴⁷ Weight loss to 10% below ideal body weight and body fat less than 12% are risk factors for amenorrhea. BMI does not accurately reflect body fat stores in athletes because of their muscularity.¹⁰⁴⁸ Energy balance seems to be more critical than low body fat stores in mediating the anovulation.^{1046,1049} Menarche may occur or menses resume when the athlete's activity level suddenly decreases and before weight gain occurs. Other factors also contribute to cause amenorrhea. Nutritional deficiencies may coexist. Chronic undernutrition may suppress thyroid function as in anorexia nervosa.⁹⁸² Athletic amenorrhea resembles anorexia nervosa in patients' obsession with weight control.^{1046,1050}

Psychogenic amenorrhea from severe psychic stress has long been known (e.g., "boarding-school amenorrhea").¹⁰⁵¹ The onset of psychogenic amenorrhea may be identified as being associated with a discrete event, but the ovarian dysfunction tends to be long-lasting. Subtle nutritional deficits contribute.⁴⁹⁷

Epilepsy causes menstrual disturbances that seem to result from abnormal neuroendocrine regulation independently of drug treatment.¹⁰⁵²

Pseudocyesis is an extremely rare form of psychogenic amenorrhea that is caused by persistence of the corpus luteum. This syndrome tends to occur in infertile women with an overwhelming desire for pregnancy and conversion hysteria. Prolactin and LH excess appear to mediate this rare syndrome.¹⁰⁵³

Differential Diagnosis. Disorders outside the neuroendocrine-gonadal axis may cause or mimic hypothalamic anovulation. These include pregnancy, nutritional disturbance, glucocorticoid excess, disturbed thyroid function, drug abuse, chronic illness, hyperprolactinemia, and ectopic gonadotropin secretion.

Pregnancy must be excluded in all sexually mature adolescents with amenorrhea. An elevation of the serum β -hCG level is the earliest laboratory sign.¹⁰⁵⁴ Placental hCG initially drives constant overproduction of estrogens and progestins by the maternal corpus luteum, then production of estrogen and other sex steroids shifts to the fetoplacental unit and suppresses maternal pituitary gonadotropin release.

Optimal fat mass is necessary for normal gonadotropin levels in sexually mature women, and both obesity and undernutrition suppress gonadotropins: thus the gonadotropin response to relative adipose mass seems biphasic.³⁰⁸ Obesity is associated with blunted LH pulse amplitude that is partially attributable to increased LH clearance rate. Overproduction of estrogen from plasma precursors in adipose tissue⁴⁵⁷ may play a role in suppressing LH pulsatility.¹⁰⁵⁵ The extent to which sleep disruption may contribute to LH suppression is unclear.¹⁰⁵⁶ The effect of undernutrition seems to be mediated by factors related to energy balance, as discussed in the section Hypothalamic Anovulation.

Cushing syndrome (glucocorticoid excess) of any etiology causes anovulation by inhibiting the gonadotropin response to GnRH.¹⁰³³ Thyroid hormone deficiency suppresses gonadotropin release via gonadotrophin-inhibitory hormone,¹⁰⁵⁷ and interferes with gonadotropin action on the ovary.¹⁰⁵⁸ Drug abuse with tetrahydrocannabinol, ethanol, or opiates causes hypothalamic anovulation.^{1059,1060} Cocaine causes menstrual irregularity by suppressing gonadotropin secretion through mechanisms that include depletion of dopaminergic stores, resulting in hyperprolactinemia, and stimulation of CRH release.^{992,1061} Inflammatory illness acutely disrupts the estradiol-induced LH surge,¹⁰⁶² and chronic illness causes gonadotropin deficiency, which may be mediated partly by undernutrition and partly by cytokines.^{193,965} Disorders as diverse as diabetes mellitus and iron overload all impact GnRH secretion.^{1063,1064} Chronic renal failure causes complex dysfunction of the reproductive system, including poor clearance of gonadotropins and prolactin in the presence of inhibition of gonadotropins by a nondialyzable factor.¹⁰⁶⁵

Hyperprolactinemia occasionally causes secondary amenorrhea without frank hypoestrogenism.¹⁰⁶⁶ This situation probably results from a mild diminution in FSH secretion that only inhibits the emergence of a dominant follicle and, therefore, ovulation.

Postpill amenorrhea has been a term applied to the amenorrhea that sometimes follows the long-term use of hormonal contraceptives. This in the past was attributed to oversuppression, but oversuppression should not be expected to be the case with the current generation of oral contraceptives.³³⁴ About one-third of patients with secondary amenorrhea after discontinuation of estrogen and progestin-containing pills have a history of previous menstrual disturbance and ongoing menstrual problems.¹⁰⁶⁷ Another third can expect spontaneous remission of the amenorrhea. About half of the remainder of cases will have resolution of their menstrual disturbance after induced pregnancy. The most common cause of postpill amenorrhea is probably hyperprolactinemia because over 20% of such cases have galactorrhea. How often this antedates ingestion of the contraceptive pill is unknown. Menses may be restored in normoprolactinemic cases by dopaminergic treatment, which suggests that in such cases there is excessive pituitary prolactin secretion that is too subtle to be detected by measurement of serum levels.¹⁰⁶⁸ The anovulation resulting from depot-medroxyprogesterone acetate contraception is related to the extremely slow rate of absorption and metabolism of this steroid; menses return when the blood levels of this progestin fall below the threshold for suppression of the LH surge,¹⁰⁶⁹ and only rarely has it been associated with disturbed prolactin secretion.¹⁰⁷⁰

Gonadotropin or hCG secretion by a tumor can cause normoestrogenic or hyperestrogenic anovulation.^{1071,1072} In one

LH-producing tumor, sex steroid levels were normal; the lack of virilization was attributed to ovarian desensitization to LH,¹⁰⁷¹ while in another, virilization occurred, which was attributed to preexisting polycystic ovary syndrome-hyperthecosis and extreme LH elevation.¹⁰⁷³ Other hyperestrogenic disorders that cause anovulatory bleeding are discussed under Precocious Puberty.

Hypothalamic anovulation is ordinarily a diagnosis of exclusion. The medical evaluation should be performed as discussed in the preceding section, with particular attention to the possibilities of emotional stressors, excessive exercise, the use of birth control pills or other drugs, and state of health. The physical examination should be particularly directed to the state of nutrition, the possibilities of intracranial or systemic disease, galactorrhea, thyroid dysfunction, glucocorticoid excess, hirsutism, and obesity. If this workup is negative, an MRI of the hypothalamic-pituitary area is indicated. Hypothalamic anovulation may be documented by demonstrating subnormal LH pulse frequency, but this is not generally practical. Leptin levels tend to be low but nondiagnostic.⁹⁸¹ The response to a GnRH agonist test is normal but seems to lack the normal priming response to repeat testing.¹⁰⁷⁴ Excessive uterine bleeding from hypothalamic anovulation must be distinguished from that caused by other causes (see next section).

Management. Many patients with hypothalamic anovulation will benefit from nutritional counseling. Diet faddists and athletes should be advised about the necessity of optimal body energy reserves for the maintenance of normal menstrual cycles (see Fig. 16.42). The teleological significance of this may be pointed out, namely, that inherent in the evolutionary process is the inhibition of pregnancy in times of inadequate food supplies. Ongoing psychological counseling is advisable for patients who cannot change their dietary or exercise patterns because of an abnormal body image. Estrogen replacement only partially corrects bone mineralization unless nutrition is optimized.^{996,1075} However, if spontaneous menses do not return after a 6- to 12-month trial of improved nutrition, modification of the patient's exercise regimen, and/or psychological counseling, short-term use of transdermal estradiol and cyclic oral progestins (not oral contraceptives or ethinyl estradiol) should be considered in those with low bone density to protect the patient's long-term bone health.⁹⁹⁶ Obese girls should be advised that there is a substantial possibility that reduction to a normal weight will result in restoration of menses and improved probability of fertility.

Mature teenagers whose amenorrhea is unexplained should be assured that they have a high likelihood of fertility with appropriate endocrinological treatment. However, such treatment is unlikely to be of any benefit to them until such time as they desire to become pregnant. Meanwhile, the main objective of therapy is to normalize the endometrial cycle by periodic progestin administration. For this purpose, progestin (micronized progesterone 100–200 mg orally at bedtime for 14 consecutive days) usually is effective in inducing withdrawal periods. During the first few years after menarche, it is reasonable to administer this treatment on alternate months to allow detection of late maturation of a regular menstrual cycle.

Induction of an ovulatory cycle has been reported to occasionally result in resumption of spontaneous normal menses.¹⁰⁶⁷ An ovulatory cycle can normally be induced by the administration of clomiphene citrate once nightly for five doses. If treatment is successful, menses generally occurs about 1 month from commencement of the treatment. One should start with the 50-mg dose because larger doses may cause hyperstimulation of the ovaries with the development of ovarian cysts. For this reason, one should perform an ultrasound examination to

rule out cystic ovaries before going successively to 100 to 150 mg dosage. This treatment is not generally recommended in the teenage years, however. Dopaminergic therapy has been reported to be successful in causing the resumption of ovulation in postpill amenorrhea, modest undernutrition, and other unexplained cases of secondary amenorrhea. Otherwise, induction of ovulation is best left to the endocrinological gynecologist to supervise at such time as the woman wishes to conceive. The vast majority of patients with no obvious cause for their secondary amenorrhea will become pregnant after appropriate treatment with estrogen, clomiphene, dopaminergic agonist, human menopausal gonadotropins, or pulsatile GnRH therapy.

Excessive Uterine Bleeding Caused by Ovulatory Dysfunction

Causes. Heavy uterine bleeding may be abnormally frequent, as indicated by intervals less than 21 days, excessively prolonged, as indicated by menstrual flow that lasts more than 7 days, or heavy, as indicated by soaking more than one pad or tampon every 2 hours, passing large clots, or experiencing a sensation of gushing (Box 16.2).⁷⁴¹ It is usually caused by bleeding from a hyperplastic endometrium.⁸⁸² It is a manifestation of ovulatory dysfunction,¹⁰⁷⁶ which may result in anovulatory or immature ovulatory cycles.^{735,736,738} This “dysfunctional uterine bleeding” (DUB) is most often a manifestation of physiological adolescent anovulation (Box 16.7). Hyperandrogenism, particularly polycystic ovary syndrome, and coagulopathy are the most common causes of severe anemia, which can be life-threatening. Less common causes are hypothalamic anovulation, estrogen-

producing cysts or tumors, hypothyroidism, hyperprolactinemia, and incipient premature ovarian failure. The workup should therefore include measurement of serum androgen, estradiol, prolactin, thyroid, gonadotropin levels and an evaluation for bleeding disorders.

Luteal Phase Defects

A minimum serum level of progesterone must be generated by the corpus luteum for the endometrium to sustain sufficient development to support implantation.^{1077,1078} A lesser level is a cause of “luteal insufficiency” as a cause of infertility. Luteal insufficiency may present as short cycles (<21 days) with frequent menstrual bleeding or as infertility with normal menstrual cycles in which the short luteal phase follows a prolonged follicular phase (see Fig. 16.35). It is normal during the early post-menarcheal years.⁷³⁸

A major determinant of normal corpus luteum formation and function is optimal development of the corpus luteum predecessor, the dominant follicle. Luteal insufficiency¹⁰⁷⁷ may arise from subtle deficiency of inhibin, LH or FSH during the follicular phase, an inadequate preovulatory LH surge, or corpus luteum unresponsiveness to LH.^{1077,1079,1080} These abnormalities all cause incomplete emergence of a dominant follicle and the subsequent formation of an inadequate corpus luteum. Luteal insufficiency is common during early postmenarcheal cycles,^{736,738} where it is considered to be caused by ovulatory immaturity (see Fig. 16.35) and may otherwise be the result of hyperprolactinemia,¹⁰⁸¹ obesity,¹⁰⁵⁵ hypothalamic amenorrhea, or hyperandrogenism.

Daily measurements indicate that the minimum peak serum progesterone level normally reached during the middle of the normal luteal phase ranges from 900 to 3000 ng/dL (28–95 nmol/L).^{1077,1082} Both cyclic and hourly variations in progesterone secretion make it difficult to document the minimally adequate level. Thus current clinical practice commonly defines evidence of ovulation as a progesterone level over 300 to 500 ng/dL (9–15 nmol/L),^{735,1083} approximately sufficient to raise the basal body temperature:¹⁸⁸ the lower of these cutoffs is about twice the normal maximum the day before ovulation,^{110,188} and the highest of these does not necessarily indicate a normal mature ovulatory cycle. However, a progesterone value greater than 165 ng/dL in an adolescent indicates that ovulation has occurred, although not necessarily a mature luteal phase.⁷³⁸

Differential Diagnosis. DUB must be distinguished from the other causes of abnormal genital bleeding listed in Box 16.7.^{882,1076,1084,1085} The possibility that it is pregnancy related must be considered and a pregnancy test performed. Sexual abuse is a prime consideration in recurrent vaginal bleeding. Genital tract or feminizing tumors characteristically cause bleeding that cannot be controlled with cyclic progestin or estrogen-progestin therapy. Abnormally heavy menstrual flow in adolescents is often idiopathic (“essential menorrhagia”); it is theorized to result from imbalance of vasodilating and vasoconstricting prostanoid action on the endometrium. However, pathological causes must be considered because bleeding disorders are present in about 20% of adolescents with menorrhagia requiring hospitalization and in 50% of those presenting at menarche. Patients requiring hospitalization for abnormal bleeding should have a platelet count, prothrombin time, partial thromboplastin time, bleeding time, and von Willebrand factor level performed.^{1084,1085} Transvaginal ultrasound, which is not often feasible in the virginal adolescent, is as reliable as hysteroscopy in determining whether or not the endometrial cavity is normal.

Failure of serum progesterone to rise over 500 ng/dL during the luteal phase is diagnostic of corpus luteum insufficiency with 71% accuracy.¹⁰⁷⁸ However, a higher progesterone level than this must be sustained to transform the endometrium sufficiently to support implantation.

BOX 16.7 Differential Diagnosis of Excessive Genital Bleeding in Adolescence

- Ovulatory dysfunction (“dysfunctional”) uterine bleeding
 - Physiological anovulation (perimenarcheal)
 - Hyperandrogenism
 - Polycystic ovary syndrome
 - Hyperestrogenism
 - Feminizing tumor
 - Hypothyroidism
 - Hypothalamic anovulation
 - Hyperprolactinemia
 - Chronic disease
 - Incipient premature ovarian failure
 - Luteal phase defects
- Pregnancy-related uterine bleeding
 - Threatened, missed, or incomplete abortion
 - Molar pregnancy
 - Ectopic pregnancy
- Uterine tumor, polyp, adenomyosis
- Coagulopathy
- Endometrial
 - Idiopathic (“essential menorrhagia”)
 - Intrauterine device
- Iatrogenic
 - Breakthrough bleeding (intrauterine device or contraceptive pills)
- Vaginal bleeding
 - Trauma
 - Tumor
 - Foreign body
 - Infection

(From Rosenfield, R.L., Barnes, R.B. (1993). Menstrual disorders in adolescence. *Endocrinol Metab Clin North Am*, 22, 491.)

Management. Nonsteroidal antiinflammatory agents may lessen DUB. An estrogen-progestin oral contraceptive with 35 mcg ethinyl estradiol is first-line treatment to stop dysfunctional bleeding that is acute or associated with anemia. For active bleeding, the dosage is advanced rapidly until bleeding stops, up to 4 times daily, and then sustained for 7 days. Treatment is then stopped for 5 days, and the patient warned that heavy withdrawal bleeding with cramps may occur. Therapy with a low-dose pill, given as for contraception, is then begun to prevent recurrence of dysfunctional bleeding and is continued for about 3 cycles. Hemoglobin should be monitored and supplemental iron prescribed.

Cyclic progestin can be used as an alternative to the OCP to prevent recurrent dysfunctional bleeding in a patient who is not sexually active. Micronized progesterone 100 to 200 mg/day for 1 week is given at 3- to 4-week intervals. After the third-month, therapy is stopped and the patient is observed for 1 to 2 months for spontaneous bleeding. If none occurs the progestin can be given every other month (e.g., medroxyprogesterone 5–10 mg) for 7 to 14 days to prevent recurrent dysfunctional bleeding. If in the progestin-free month a normal spontaneous menstrual period occurs, progestins are withheld in the subsequent month to determine if the patient has developed regular ovulatory cycles.

Hemodynamic instability due to severe and/or acute blood loss is an indication for hospitalization and treatment with an antifibrinolytic drug (e.g., aminocaproic acid 100 mg/kg or tranexamic acid 10 mg/kg) begun IV, and intravenous fluids and blood products as necessary. Premarin[®] can be administered in a dose of 25 mg intravenously every 3 to 4 hours for 3 to 4 doses. When medical management fails, a bleeding diathesis or uterine structural abnormality should be considered (see later). If heavy bleeding persists, in spite of hormonal and antifibrinolytic treatment, surgical intervention is indicated by a gynecologist.^{1084,1085}

Unexplained (“essential”) menorrhagia is treated much the same way as dysmenorrhea. OCP therapy will decrease menstrual blood loss by about 50% in these women. Antiprostaglandins, such as naproxen 500 mg twice a day, decrease blood loss nearly as effectively.

Perimenstrual Symptoms

Dysmenorrhea. Uterine cramping is characteristic of normal ovulatory cycles, apparently as the result of prostaglandin release within the endometrium upon the withdrawal of progesterone. Pain with menses becomes a source of morbidity in 14% of adolescents.¹⁰⁸⁶ When pain is acute and qualitatively different from the usual menstrual pain, ectopic pregnancy must be considered.^{1054,1087} An ectopic pregnancy often causes vaginal bleeding that occurs 2.5 weeks later than the time of the expected next menstrual period and is typically light. However, the bleeding may be heavy and so resemble an episode of dysfunctional uterine bleeding. Ectopic pregnancy is usually diagnosable by a combination of ultrasonography, a serum β -hCG level over 1000 IU/L, and a progesterone level of less than 2500 ng/dL.

In patients with chronic pelvic pain unresponsive to anti-prostaglandins or OCP psychological overlay is possible, but attention should be directed to the possibility of endometriosis, uterine outlet obstruction, gynecological tract masses, or the poorly defined entity of *vulvodinia*.¹⁰⁸⁸ Ultrasonography and laparoscopy may be indicated to further evaluate these patients. Endometriosis is an estrogen-dependent disorder that accounts for approximately half of the cases of chronic pelvic pain in teenagers.¹⁰⁸⁹ Genetic factors and congenital obstruction of the genital tract predispose to endometriosis,

and aberrant estradiol formation in endometrial stroma has been incriminated in the pathogenesis.¹⁰⁹⁰ GnRH agonist therapy is approved to provide symptomatic relief, but adolescents are at particular risk for bone loss, and progestin therapy may be an effective alternative.

Dysmenorrhea may be ameliorated by antiprostaglandin therapy. Naproxen (275 mg 4 \times daily [QID] after a 550 mg loading dose) has been shown to be superior to aspirin (650 mg QID) or placebo when begun 2 days before the anticipated onset of menses.¹⁰⁹¹ The OCP is an alternative that will relieve dysmenorrhea in about 90% of cases by reducing endometrial mass.⁸⁸² Because smoking, alcohol intake, and excessive weight are risk factors, lifestyle counselling is advisable.

Premenstrual Syndrome. This is the term applied when cyclic mood changes confined to the second half of the menstrual cycle become debilitating.¹⁰⁹² It is often disruptive to women’s personal, social, and occupational function. If symptoms of marked mood swings, depressed mood, anxiety, and irritability occur, it is classified as premenstrual dysphoric disorder.¹⁰⁹³ Neuropsychiatric symptoms may include epilepsy¹⁰⁹⁴ and bizarre behavior.¹⁰⁹⁵ These seem to represent aberrant responses to normal cyclic hormonal changes.¹⁰⁹⁶ Subnormal activation of the hypothalamic-pituitary-adrenal axis in response to progesterone has been found.¹⁰⁹⁷ Some evidence indicates that variation in the degree of progesterone metabolism to neuroactive steroids affects the severity of symptomatology.¹⁰⁹⁸ Oral contraceptive therapy with the anti-mineralocorticoid progestin drospirenone is indicated if psychotropic therapy is unsuccessful. Downregulation of pituitary gonadotropin secretion by GnRH agonist therapy is efficacious, but its usefulness is limited by the side effects of estrogen deficiency. The relationship of premenstrual syndrome to other luteal phase symptomatology, such as recurrent fever and autoimmune symptoms, may be related to hyperresponsiveness of cytokines to progesterone.^{1099,1100}

Hyperandrogenism in Adolescence

Hyperandrogenism of a mild to moderate degree is the most common cause of persistent normoestrogenic menstrual abnormality and/or hirsutism. Hyperandrogenism developing during adolescence is usually caused by polycystic ovary syndrome (PCOS), but the differential diagnosis of hyperandrogenism includes other ovarian or adrenal disorders, abnormal peripheral formation of androgen, and drugs (see Box 16.8).^{40,1101}

Causes

The definition of PCOS has evolved since its description by Stein and Leventhal as a syndrome of amenorrhea and polycystic ovaries, often accompanied by hirsutism, acne, and/or obesity.¹¹⁰² Over recent decades, internationally accepted diagnostic criteria were developed for adults based on various combinations of otherwise unexplained hyperandrogenism, anovulation, and a polycystic ovary, which are all encompassed by Rotterdam consensus criteria.⁴⁰ These criteria generate four phenotypes, which are here listed in order of the decreasing diagnostic specificity of the phenotypes (Box 16.9) that underlies the controversies in the acceptance of the milder types. Insulin resistance, obesity, and LH excess, although not diagnostic features of the syndrome, are common and contribute to its pathogenesis; like hyperandrogenism, their severity has generally proven to correlate with specificity. In 2015 consensus diagnostic criteria for adolescent PCOS were developed by international pediatric subspecialty societies: these are an

BOX 16.8 Causes of Adolescent Hyperandrogenism

Physiological Adolescent Anovulation

Functional Gonadal Hyperandrogenism

- PCOS: Primary functional ovarian hyperandrogenism (common form of polycystic ovary syndrome)
- Secondary polycystic ovary syndrome
 - Virilizing congenital adrenal hyperplasia
 - Adrenal rests of the ovaries
 - Syndromes of severe insulin resistance
 - Acromegaly
 - Epilepsy ± valproic acid therapy
- Ovarian steroidogenic blocks
- Disorders of sex development
- Chorionic gonadotropin excess

Functional Adrenal Hyperandrogenism

- PCOS: Primary functional adrenal hyperandrogenism (uncommon form of polycystic ovary syndrome)
- Virilizing congenital adrenal hyperplasia
- Other glucocorticoid-suppressible functional adrenal hyperandrogenism
 - Prolactin excess
 - Cortisone reductase (and apparent cortisone reductase) deficiency
 - Apparent dehydroepiandrosterone sulfotransferase deficiency
 - Glucocorticoid-resistant functional adrenal hyperandrogenism
 - Cushing syndrome (endogenous: adrenal hyperplasia or neoplasm)
 - Glucocorticoid resistance

Peripheral Androgen Overproduction

- Idiopathic hyperandrogenism
- Obesity
- Portohepatic shunting

Virilizing Drugs

Androgenic Drugs

(Modified with permission from Buggs, C., Rosenfield, R.L. (2005). Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am*, 34, 677–705.)

age- and stage-appropriate modification of the “National Institutes of Health criteria” (i.e., otherwise unexplained persistent hyperandrogenic anovulation) (see [Box 16.9](#)).^{124,1101,1103}

The adolescent consensus criteria attempt to strike a balance between under- and overdiagnosis of the syndrome. Normal adolescents often have menstrual cycles that are abnormal by adult standards, so anovulatory criteria must be age- and pubertal stage-appropriate (see [Box 16.2](#)). Persistence of the menstrual abnormality is required to avoid misdiagnosing physiological adolescent anovulation as PCOS. Unlike adult standards, mild hirsutism is not a criterion for hyperandrogenism because half of mild hyperandrogenism without menstrual abnormality is a normal variant.⁶⁴⁷ Polycystic ovary morphology (PCOM) is not a criterion because many normal adolescents meet adult PCOM criteria and adolescent norms are not well defined.

Clinical Manifestations. PCOS in adolescents resembles that in adults, with similar clinical and endocrinological heterogeneity. The cardinal symptoms typically begin when mature gonadotropin levels are achieved, in the perimenarcheal stage, so PCOS has been documented in children as young as 10 years of age. Cases with “ovulatory PCOS” have a subtle ovulatory abnormality that escapes notice until presentation

BOX 16.9 Diagnostic Criteria for Polycystic Ovary Syndrome**ADULT DIAGNOSTIC CRITERIA (ROTTERDAM)****Otherwise Unexplained Alternative Phenotypes:**

- Phenotype 1 (“Classic polycystic ovary syndrome [PCOS]”/“Stein-Leventhal syndrome”)^a
 - Clinical and/or biochemical evidence of hyperandrogenism
 - Evidence of oligoanovulation
 - Ultrasonographic evidence of a polycystic ovary
- Phenotype 2 (Essential National Institutes of Health Criteria)^a
 - Clinical and/or biochemical evidence of hyperandrogenism
 - Evidence of oligo-anovulation
- Phenotype 3 (“Ovulatory PCOS”)
 - Clinical and/or biochemical evidence of hyperandrogenism
 - Ultrasonographic evidence of a polycystic ovary
- Phenotype 4 (Nonhyperandrogenic PCOS)
 - Evidence of oligoanovulation
 - Ultrasonographic evidence of a polycystic ovary

ADOLESCENT DIAGNOSTIC CRITERIA**Otherwise Unexplained Combination of:**

- Abnormal uterine bleeding pattern
 - Abnormal for age or gynecological age
 - Persistent symptoms for 1–2 years
- Evidence of hyperandrogenism
 - Persistent testosterone elevation above adult norms in a reliable reference laboratory is the best evidence
 - Moderate-severe hirsutism is clinical evidence of hyperandrogenism
 - Moderate-severe inflammatory acne vulgaris is an indication to test for hyperandrogenemia

(Modified and reprinted with permission from Rosenfield, R.L. (2015). The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*, 136, 1154–1165.)

in adulthood with unexplained infertility¹¹⁰⁴ or recurrent miscarriages.¹¹⁰⁵

Persistent symptomatic menstrual abnormality (primary or secondary amenorrhea, oligo-amenorrhea, or excessive uterine bleeding) (see [Box 16.2](#)) in a normally feminized girl may be the only manifestation of hyperandrogenism. In about one-third of cases, menstrual dysfunction occurs in the absence of cutaneous manifestations.

An abnormal menstrual pattern usually constitutes evidence of oligoanovulation. The menstrual pattern should be interpreted in the context of the patient’s gynecological age (see [Box 16.2](#)), as discussed earlier in the section Physiological Adolescent Anovulation. Although persistence of menstrual abnormality for 2 years is required for diagnosing PCOS in an adolescent (see [Box 16.9](#)), a provisional diagnosis can—and should—be made within 1 year in circumstances that require treatment.¹²⁴ PCOS is a state of relative, not absolute, infertility in which ovulation often occurs unpredictably and, on the other hand, in which menstrual regularity does not necessarily indicate ovulatory regularity.

The cutaneous signs of hyperandrogenism are variably expressed manifestations of androgen excess. They are present in about two-thirds of cases. Hirsutism is the most common pilosebaceous manifestation. However, acne, or uncommonly seborrhea or balding, may be the only pilosebaceous manifestation of hyperandrogenism.^{1106,1107}

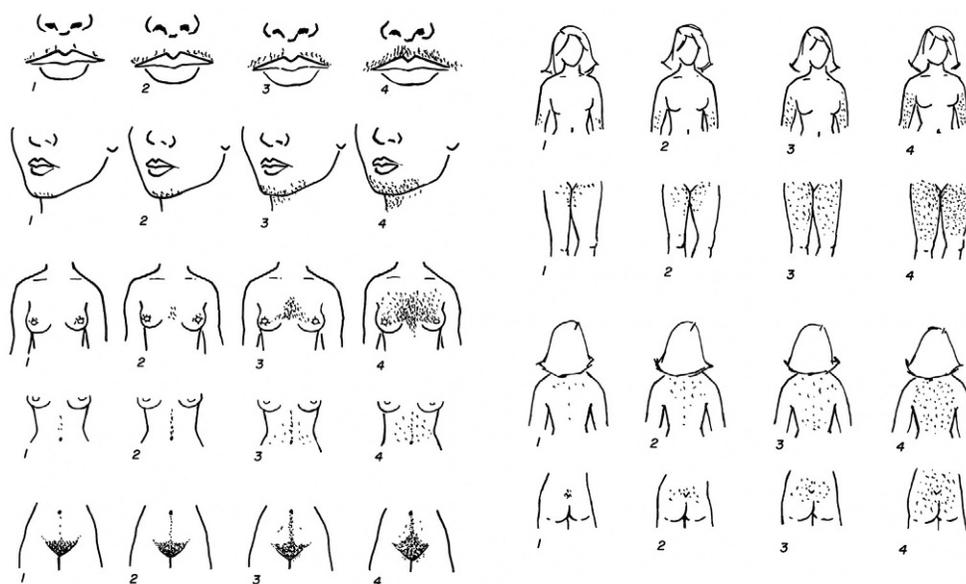


Fig. 16.46 Ferriman-Gallwey hirsutism scoring system. Each of the nine body areas that is most sensitive to androgen is assigned a score from 0 (no hair) to 4 (frankly virile), and these are summed to provide a hormonal hirsutism score. Generalized hirsutism (score ≥ 8) is abnormal in the general US population, whereas locally excessive hair growth (score 1–7) is a common normal variant that may be cosmetically important to patients. The normal score is lower in some Asian and higher in Mediterranean, Hispanic, and Middle Eastern populations (see text). (From Hatch, R., Rosenfield, R.L., Kim, M.H., Tredway, D. (1981). Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol*, 140, 816–830. With permission.)

Hirsutism is defined clinically as excessive terminal hair that appears in a male pattern (sexual hair).⁶⁴⁷ It is commonly graded according to the hormonal Ferriman-Gallwey system, which quantitates the extent of hair growth in the most androgen-sensitive areas (see Fig. 16.46). The normal score is lower in some Asian populations and higher in Mediterranean, Hispanic, and Middle Eastern populations.

The absence of hirsutism in approximately one-third of hyperandrogenic adults appears to be because of relatively low sensitivity of their pilosebaceous unit to androgens. Thus biochemical hyperandrogenemia may be entirely cryptic, manifesting neither cutaneous signs nor anovulatory symptoms. Conversely, hirsutism without elevated circulating levels of androgen or other clinical evidence of hyperandrogenism—“idiopathic hirsutism”—accounts for approximately one-half of mild hirsutism and one-sixth of moderate-severe hirsutism.⁶⁴⁷

Although comedonal acne is common in adolescent girls, moderate-severe (>10) inflammatory acneform lesions in any area of the skin is uncommon during the perimenarcheal years and is an indication for testing for hyperandrogenemia.¹²⁴

Acanthosis nigricans, a manifestation of insulin-resistant hyperinsulinism, and associated obesity are the presenting complaints in about one-third of cases, sometimes before menstrual abnormalities develop.^{1108,1109} About one-half of PCOS patients are obese.¹¹⁰¹ The obesity occasionally begins in mid-childhood. Central body fat content is increased independently of BMI, and at least one-third of normal-weight PCOS patients have increased intraabdominal fat.^{40,1110}

Laboratory Manifestations. Persistent elevation of serum testosterone above adult norms in a reliable reference laboratory is the best evidence of hyperandrogenism (Box 16.9).¹²⁴ The problematic nature of many androgen assays is discussed later in the section Differential Diagnosis. Elevated serum free testosterone is the single most sensitive indicator of hyperandrogenemia because the bioactive portion of the serum testosterone is the free fraction.⁶⁴⁷ SHBG serum concentrations govern the fraction of testosterone that is free; they are lowered by obesity

and androgen excess itself. Androstenedione and DHEAS serum levels are often elevated in PCOS and should be measured if androgen excess is strongly suspected in spite of normal total and free testosterone levels. Serum DHT levels are of little diagnostic value.

A unique type of functional ovarian hyperandrogenism (primary FOH) can be documented in 85% of PCOS cases by specific tests of ovarian androgenic function.⁴⁰ The GnRH agonist test and hCG tests evaluate the gonadal response to, respectively, endogenous gonadotropin release or exogenous administration of the LH analogue hCG. In two-thirds of PCOS patients, these tests show a distinctive pattern of ovarian steroidogenic hyperresponsiveness, of which 17-OHP hyperresponsiveness is the best marker; there is no evidence of a steroidogenic block, and, indeed, estradiol is significantly hyperresponsive. The dexamethasone androgen-suppression test (DAST)—predicated on the principle that residual concentrations of androgens after suppression of adrenal function by glucocorticoid administration ordinarily arise from the ovary—shows elevated testosterone post-DAST in 80% of PCOS patients: this includes most with 17-OHP hyperresponsiveness to gonadotropin stimulation and two-thirds of those who lack it. These tests are indicated only in the case of diagnostic uncertainty.

A related adrenal steroidogenic defect is found in 25% to 50% of PCOS: primary functional adrenal hyperandrogenism (FAH).⁴⁰ This is indicated by DHEA hyperresponsiveness to ACTH; this correlates ($r = 0.7$) with DHEAS elevation. Isolated FAH is the sole steroidogenic defect in about 5% of PCOS.

The identification and clinical significance of PCOM presents quandaries.¹²⁴ Ultrasonographically, PCOM has been defined in adults by consensus criteria as an ovary with a volume greater than 10.0 cc by a simplified formula or a small antral follicle (2–9 mm diameter) count of 12 or more per ovary. However, these criteria have become problematic in young adults, particularly because the newer high-definition vaginal imaging techniques show that small antral follicle counts up to 24 are normal. Adult PCOM criteria are especially problematic when applied to adolescents. For one thing, an

accurate antral follicle count cannot be defined by the abdominal ultrasonographic approach necessary in virginal adolescents. Furthermore, current data suggest that ovarian volume and antral follicle count are slightly higher in adolescents than in adults. Consequently, one-third to half of normal adolescents meet adult criteria for PCOM. Until further research establishes definitive criteria, current evidence suggests that a mean ovarian volume of 12 cc (or single ovary >15 cc) be considered enlarged in adolescents, and PCOM are not a diagnostic criterion for PCOS in adolescents.

PCOM is variably related to hyperandrogenism.^{40,124} On one hand, it is absent in 5% to 20% of adult PCOS. On the other, PCOM is a common finding among healthy women. When care has been taken to exclude those with PCOS features, about one-quarter of apparently normal volunteers with PCOM have mild subclinical androgenic ovarian dysfunction that is in the PCOS range; it has been postulated that these are carriers of PCOS or at risk for PCOS.

AMH levels are independently associated with PCOM, because they indicate an increased number of small growing follicles, and with hyperandrogenism.⁴⁰ Although a mildly increased AMH level is common in asymptomatic females with PCOM and has been suggested as a surrogate indicator of it, AMH elevation of twofold or more above the upper normal limit suggests PCOS with high specificity.

Serum LH and the LH/FSH ratio are increased in about half of PCOS subjects.⁴⁰ Accumulating evidence suggests that LH levels in PCOS are determined by the severity of hyperandrogenemia, moderate degrees of which stimulate LH production, and the extent of obesity, which suppresses LH levels.

Insulin resistance in PCOS is significant independently of obesity.⁴⁰ Insulin resistance interacts with obesity and age to cause metabolic syndrome, which confers risk for cardiovascular disease,^{40,1111} sleep-disordered breathing,^{1112,1113} and nonalcoholic fatty liver disease.¹¹¹⁴

Approximately one-half of PCOS patients have an abnormal degree of insulin resistance, and about one-half of these have metabolic syndrome. Insulin resistance, as determined by euglycemic clamp, is present in 50% of obese adolescents with PCOS compared with BMI-matched controls¹¹¹⁵ (75% compared with normal-BMI controls) and about one-half of such girls have metabolic syndrome.¹¹¹⁶ Adolescents with PCOS are thus at increased risk for glucose intolerance.¹¹¹⁷ They are also predisposed to the pancreatic beta-cell dysfunction of type 2 diabetes mellitus.¹¹¹⁸ Dyslipidemia prevalence is generally low, but it is related to the degree of obesity and insulin resistance.^{1116,1119–1121}

Pathophysiology. PCOS ordinarily seems to be caused by intraovarian androgen excess (see Fig. 16.47).⁴⁰ Primary FOH accounts for the vast majority (about 85%) of PCOS (Fig. 16.48).^{40,1101} Functionally typical PCOS, constituting two-thirds of cases, is caused by typical FOH, which occurs because of a unique type of steroidogenic dysfunction, dysregulation of steroidogenesis. A related dysregulation of adrenal dysregulation, FAH, occurs in both typical and atypical FOH.¹¹²²

Typical FOH is characterized by generalized steroidogenic hyperresponsiveness to LH, of which 17-OHP is the most consistent marker. One-third of PCOS is functionally atypical, in that the 17-OHP abnormality is not demonstrable. One-half of these have functionally atypical FOH, identifiable by subnormal androgen suppression by dexamethasone. In the remainder, which constitutes a small minority (15%) of PCOS cases, an ovarian source for androgen excess, is not demonstrable. The androgen excess of these latter functionally atypical PCOS cases appears to arise from isolated FAH in about one-third of cases and from obesity in most of the remainder. These are exceptions to the general rule that modest extraovarian androgen excess does not interfere with ovarian function.

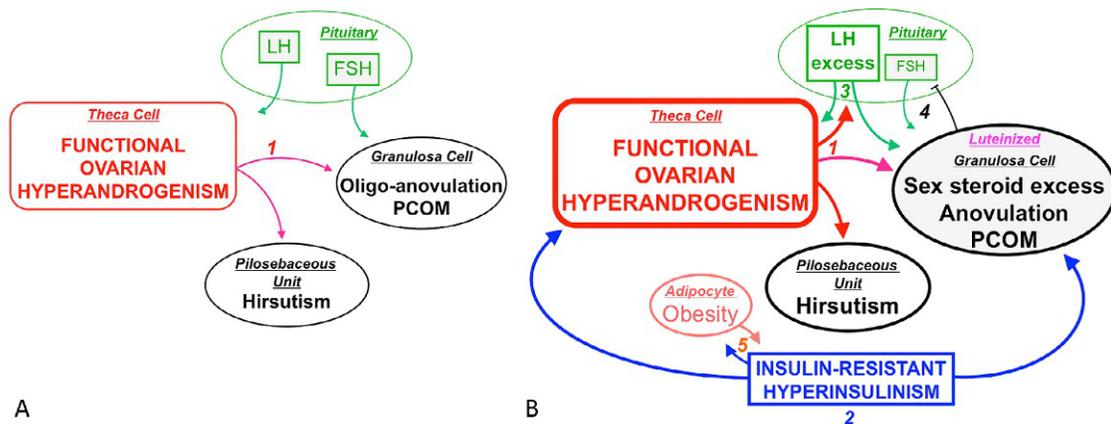


Fig. 16.47 Unified minimal model of polycystic ovary syndrome (PCOS) pathophysiology. Panel A: Functional ovarian hyperandrogenism (FOH) is present in nearly 90% of PCOS and can account for all the cardinal clinical features of the syndrome: hyperandrogenemia, oligoanovulation, and polycystic ovaries (step 1). Pituitary luteinizing hormone (LH) secretion is necessary to sustain the ovarian androgen excess, but it is not sufficient to cause it. Panel B: About one-half of patients with FOH have an abnormal degree of insulin-resistant hyperinsulinism (step 2). Insulin-resistant hyperinsulinism acts on theca cells to aggravate hyperandrogenism, synergizes with androgen in prematurely luteinizing granulosa cells, and stimulates fat accumulation. The increased hyperandrogenemia provokes LH excess, which then acts on both theca and luteinized granulosa cells to worsen hyperandrogenism (step 3). LH also stimulates luteinized granulosa cells to secrete estradiol (step 4), which suppresses follicle-stimulating hormone (FSH) secretion. These hyperinsulinism-initiated changes in granulosa cell function further exacerbate polycystic ovary morphology (PCOM) and further hinder ovulation. Obesity increases insulin resistance, and the resultant increased hyperinsulinism further aggravates hyperandrogenism (step 5). Boldness and enlarged font represents greater severity. Both FOH and insulin resistance typically have an intrinsic basis. This model does not exclude the possibility that the unknown intrinsic ovarian defects that underlay the ovarian steroidogenic dysfunction also involve granulosa cell folliculogenesis and other systems as well. The figure also does not depict other associated defects, such as the functional adrenal hyperandrogenism that often accompanies the ovarian hyperandrogenism and the contribution of excess adiposity to peripheral androgen production and gonadotropin suppression. (Modified from Rosenfield, R.L., Ehrmann, D.A. (2016). The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*, 37, 467–520. With permission.)

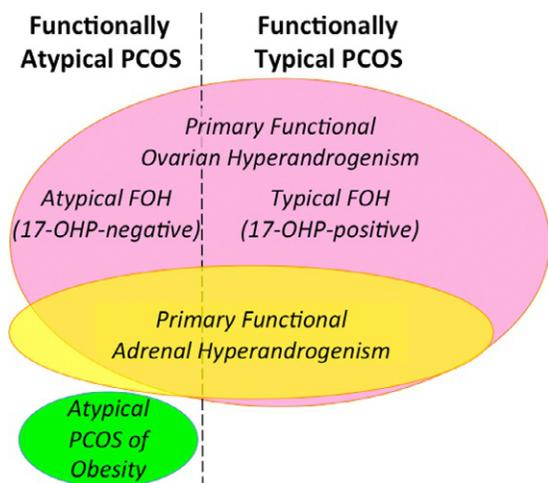


Fig. 16.48 Functional categorization of polycystic ovary syndrome (PCOS) in relationship to source of androgen and its prevalence. Most PCOS (85%) is caused by primary functional ovarian hyperandrogenism (FOH); about 30% of these have an associated primary functional adrenal hyperandrogenism. About two-thirds of PCOS have functionally typical PCOS, that is, they have typical FOH, which is characterized by hyperresponsiveness of 17-OHP to a GnRHag or hCG test. The remaining one-third of PCOS cases have functionally atypical PCOS, lacking 17-OHP hyperresponsiveness. Most of these have functionally atypical FOH, in which ovarian androgen excess is indicated by a dexamethasone androgen-suppression test. A small number (5%) are caused by isolated functional adrenal hyperandrogenism. In a minority of cases, the source of androgen cannot be identified as ovarian or adrenal: most of these have the functionally atypical PCOS of obesity (5%); the source of the androgen is unexplained (idiopathic) in less than 20% of the functionally atypical PCOS group ($\geq 5\%$ of PCOS). 17-OHP, 17-Hydroxyprogesterone. (Modified and reproduced with permission from Rosenfield, Polycystic ovary syndrome in adolescents. In: Rose BD, ed. *UpToDate*: 2014. Waltham, MA, www.uptodate.com/index.)

Dysregulation of steroidogenesis seems to result from imbalance among the various intrinsic and extrinsic factors involved in the modulation of trophic hormone action.⁴⁰ Within the ovary, there appear to be flaws in the processes that normally coordinate androgen and estrogen secretion in response to gonadotropin stimulation (see Fig. 16.19). Theca cells from polycystic ovaries of classic PCOS patients in long-term culture have an intrinsic steroidogenic dysregulation that can account for these steroidogenic abnormalities: these cells constitutively overexpress most steroidogenic enzymes, most prominently at the level of 17-hydroxylase and 17,20-lyase activities, both properties of P450c17, which are the rate-limiting steps in the biosynthesis of testosterone precursors. As in the ovary, dysregulation of local steroidogenic regulatory processes within the adrenal cortex appears to cause a characteristic type of primary FAH in which excessive dehydroepiandrosterone is formed in response to ACTH as a by-product of cortisol secretion.

As a consequence of dysregulated steroidogenesis, PCOS theca cells do not undergo the normal homologous desensitization to LH with downregulation of steroidogenesis in response to excess LH stimulation. Therefore, they are hypersensitive to LH stimulation. “Escape” from homologous desensitization to LH excess appears to be the basis for the distinctive pattern of steroidogenic hyperresponse to GnRH agonist or hCG testing, which is characterized by disproportionate

hyperresponsiveness of 17-OHP relative to other ovarian steroids without evidence of a steroidogenic block.

Granulosa cell functions are also defective in PCOS.⁴⁰ Folliculogenesis of small follicles is excessive, which has been attributed to androgen excess, although a primary granulosa cell defect cannot be ruled out, accounting for excess AMH production. Inhibin-B hyperresponsiveness to FSH also appears to aggravate theca androgen secretion via a paracrine action and also contributes to tamping down FSH production.

The reason for the high prevalence of obesity in PCOS is not entirely clear.⁴⁰ Obesity increases insulin resistance, and in turn the insulin-resistant hyperinsulinism seems to enhance obesity because insulin signaling in human subcutaneous adipose tissue is intact in PCOS. The mechanisms by which obesity causes insulin resistance are also unclear, but they include deficient insulin-sensitizing adipokines, such as adiponectin, and excessive proinflammatory cytokines, such as TNF- α , which both aggravate insulin resistance and hyperandrogenism, that are secreted by unique adipose tissue macrophages.^{40,1123} Hyperandrogenism also sensitizes circulating mononuclear cells to secrete inflammatory cytokines in response to glucose and saturated fat ingestion. Visceral fat contributes more to PCOS insulin resistance than does abdominal fat because its lipolytic response to catecholamines, the major lipolytic stimulus in man, is uniquely enhanced.⁴⁰

Insulin resistance is an inconsistent, aggravating factor in the pathogenesis of FOH that is present in about half of cases (Fig. 16.47, panel B). It is usually mild, particularly in the developmental (adolescent) phase of the syndrome. It does not account for the intrinsic theca cell dysfunction characteristic of ordinary PCOS and only plays a *primary* pathogenic role in the development of the PCOS that complicates severe or extreme insulin-resistance syndromes.

The insulin resistance of PCOS is selective for the metabolic effects of insulin.⁴⁰ This creates a paradox in which the hyperinsulinemia compensatory for resistance to the glucose-metabolic effect of insulin, nevertheless, elicits excess insulin action in some tissues. Thus the steroidogenic, mitogenic, and protein-metabolic actions of insulin remain intact.

This insulin-resistant hyperinsulinism (hyperinsulinism) aggravates the ovarian and adrenal steroidogenic dysregulation of PCOS.⁴⁰ It is closely associated with the anovulation of PCOS. Insulin, like IGFs, modulates gonadotropin and ACTH action. Consequently in excess it synergizes with them. In the ovary insulin synergizes with LH by increasing theca cell LH receptor site expression to cause “escape” from desensitization. Consequently, hyperinsulinism enhances theca cell androgen production and theca and stromal cell hyperplasia. Insulin excess also synergizes with androgen to prematurely luteinize granulosa cells by inducing LH receptor expression, thus enhancing estrogen, progesterone, and inhibin-B production and arresting dominant follicle development.

Hyperinsulinism also seems to *play* a role in mediating the development of obesity in PCOS.^{40,1124} The transcription factor KLF15 seems to play an important role in mediating insulin stimulation of both adipogenesis and testosterone formation.⁵⁶³

PCOS is gonadotropin dependent; gonadotropins are necessary for the expression of gonadal steroidogenic enzymes.⁴⁰ However, LH excess does not seem to ordinarily be the fundamental cause of the hyperandrogenism. LH excess is variable (found in 50%–75% of cases) and seems to depend on androgen-obesity balance, which, respectively, raise and lower gonadotropin levels. Homologous desensitization normally limits the androgenic response to LH elevation: thus LH excess alone seems unlikely to cause the hyperandrogenism of PCOS, although it may aggravate it in PCOS, where intrinsic ovarian dysfunction causes “escape” from desensitization. Evidence is accumulating that androgen excess causes LH excess by

interfering with progesterone-estrogen negative feedback effect on LH secretion. Nevertheless, the possibility of a primary role for LH excess remains, particularly in the PCOS that is secondary to congenital virilizing disorders.

A model of the pathophysiology of PCOS is shown in Fig. 16.47.⁴⁰ The high intraovarian androgen concentration arising from the thecal cell dysfunction of FOH can cause all the essential features of the syndrome (Fig. 16.47, panel A). Excess androgen secretion causes the pilosebaceous manifestations of the syndrome. The locally very high intraovarian androgen concentrations act on granulosa cells to hinder the emergence of a dominant follicle, causing oligoanovulation. This paracrine effect of great androgen excess is coupled with its stimulation of increased proliferation of small follicles causing PCOM. Insulin-resistant hyperinsulinism aggravates PCOS severity by acting in conjunction with androgen excess to prematurely luteinize follicles, causing thecal and stromal hyperplasia. The moderately increased hyperandrogenemia causes secondary LH elevation by interfering with female hormone negative feedback; meanwhile, estrogen and inhibin-B excess inhibit FSH secretion. In the presence of the escape from homologous desensitization induced by hyperinsulinism, this LH excess amplifies the ovarian dysfunction. The hyperinsulinism also promotes adiposity, which in turn aggravates the insulin-resistant state.

In primary FAH, the steroidogenic pattern of response to ACTH resembles an exaggerated adrenarche and in the past was confused with nonclassic 3 β -HSD deficiency.⁴⁰ This FAH has been associated with mild adrenal enlargement and a small degree of autonomous adrenocortical function in some cases.^{40,1125} The pattern of adrenal secretion is compatible with dysregulation of zona reticularis steroidogenesis prominent at the level of the 17-hydroxylase/17,20-lyase activities of P450c17 and it correlates with evidence of the related ovarian dysregulation.¹¹²⁶ Nevertheless, it is the only steroidogenic abnormality in a small minority of PCOS.

The remaining PCOS cases lack evidence of steroid secretory abnormalities. Most of these are obese (Fig. 16.48), a few are idiopathic. Excess adipose tissue appears sufficient to account for hyperandrogenic anovulation and thus cause the atypical PCOS of obesity.⁴⁰ Adipocytes form testosterone from androstenedione via type 5 17 β -hydroxysteroid dehydrogenase, which is upregulated by insulin. Obesity also suppresses ovulation and LH levels; blunted LH pulse amplitude is at least in part caused by accelerated metabolism of LH. The atypical PCOS of obesity is in general characterized by mild hyperandrogenemia (normal total testosterone, mildly elevated free testosterone, normal DHEAS, low SHBG), and most have normal-size ovaries, normal AMH levels, and normal LH levels; their insulin resistance is unremarkable for PCOS.

One of the great puzzles about PCOS pathogenesis is whether hyperandrogenism might cause insulin resistance. Androgens in vitro exert some antiinsulin effects.¹¹²⁷ A variety of studies indicate that administration of oral androgens or virilizing doses of androgen to humans causes insulin resistance.^{1127,1128} The induction of modest hyperandrogenism does not do this in normal women,¹¹²⁹ but in nonhuman primates it will induce insulin resistance when combined with an obesogenic diet.^{1130,1131} However, reversal of PCOS androgen excess has not ameliorated insulin resistance in the majority of studies.¹¹²⁷

Etiology. PCOS seems to arise as a complex trait that results from the interaction of diverse genetic and environmental factors once mature gonadotropin levels are achieved peripubertally.⁴⁰ These complex interactions generally mimic an autosomal dominant pattern of inheritance with variable penetrance. Heritability of PCOS has been estimated at over 70%, based on studies in identical twin sisters. Heritable factors

include maternal PCOS and PCOM, hyperandrogenemia, and metabolic syndrome.

GWAS identified overexpression of DENND1A.V2 in most PCOS populations.^{40,1132} This previously unsuspected protein is present in theca and zona reticularis cells and is overexpressed in PCOS. It facilitates steroidogenesis; the mechanism is unknown, but it has been speculated to upregulate LH receptor signaling by affecting receptor trafficking. Induced overexpression of it in normal theca cells has reproduced this PCOS genetic and biochemical phenotype in vitro.⁴⁰ A wide variety of other gene variants with linkage and/or association with PCOS have been identified by candidate gene and molecular genetic studies, and novel markers are reported regularly.⁴⁰ Variants discovered in fibrillin 3 have been proposed to dysregulate TGF- β signaling and account for ovarian stromal hyperplasia. AMH coding variants with decreased signaling that could potentially cause the syndrome have been reported in 6.7% of PCOS patients.¹⁷³

Environmental factors include prenatal androgen exposure and both undernutrition and overnutrition.⁴⁰ Virilizing CAH is sometimes complicated by PCOS in patients whose CAH is well controlled. Congenital virilization of several animal species, including nonhuman primates, has been shown to program hyperandrogenic anovulation and insulin resistance in offspring. These observations suggest that insults to the intrauterine environment induce epigenetic changes that lead to altered gene expression and disease in later life. Because in utero androgen excess from the fetal or maternal PCOS ovary is unlikely to account for this programming in ordinary PCOS,⁴⁰ the androgen programming models rather suggest that intrauterine alterations in intermediates in androgen signaling that are common to other effector pathways, such as prostaglandins, may play a role in the pathogenesis of PCOS by altering the epigenome.^{517,587,613}

Postnatal environmental risk factors can be viewed as a second "hit," which cause a latent heritable or congenitally programmed susceptibility trait to become manifest as PCOS. Postnatal factors include acquired obesity, insulin resistance, and hyperandrogenism of nonovarian origin. The variety of pathways involved and lack of a common thread attests to the multifactorial nature and heterogeneity of the syndrome.

Moderately severe insulin-resistant hyperinsulinism characterizes two syndromes of intractable obesity in childhood that herald PCOS in adolescence: pseudo-Cushing syndrome and pseudoacromegaly.⁴⁰ Premature adrenarche seems to pose a moderately increased risk (overall approximately twofold) for developing PCOS.⁴⁰ The nature of the association is unclear. We favor the concept that premature adrenarche is sometimes an early manifestation of the steroidogenic dysregulation that underlies PCOS, although in some populations low birth weight may be the underlying risk factor.

Other Causes of Functional Ovarian Hyperandrogenism.

In the early postmenarcheal years, physiological anovulation may present with mild hyperandrogenemia without clinical evidence of androgen excess in about one-quarter of cases, but the hyperandrogenic anovulation does not persist. Secondary PCOS can result from several disorders (see Box 16.8).⁴⁰ Virilizing CAH frequently causes ovarian hyperandrogenism. Three mechanisms are involved.¹¹³³ For one, poor control of adrenal hyperandrogenism causes polycystic ovaries and amenorrhea by direct effects on the ovary. Adrenal rests of the ovaries may cause polycystic ovaries and hyperandrogenism. Finally, patients with CAH, particularly those with classic CAH, are at high risk for the emergence of PCOS at puberty caused by developmental programming (see previous section Disorders of Sex Development).

All known forms of severe or extreme insulin resistance, including hereditary cases of insulin receptor mutations and acquired lipodystrophy, are accompanied by PCOS, possibly by acting through the IGF-1 signal transduction pathway to cause escape from desensitization to LH. Acromegaly itself is associated with PCOS. The antiepileptic drug valproic acid causes hyperandrogenism and polycystic ovaries, and an association of epilepsy itself with PCOS is possible.^{462,1052}

Rare causes of hyperandrogenism include primary ovarian steroidogenic blocks. These (aromatase deficiency⁹⁹⁹ and virilizing CAH caused by 3 β -HSD deficiency¹¹³⁴) cause hyperandrogenism in association with grossly polycystic ovaries and elevated LH levels. Ovarian 17-ketosteroid reductase deficiency has been reported to be responsible for a PCOS-like picture in two families, but there has been no molecular confirmation of an underlying mutation.¹¹³⁵ Functional ovarian hyperandrogenism may also result from an ovotesticular disorder of sex development. Excessive hCG stimulation mediates the hyperandrogenism of hyperreactio luteinalis and luteoma of pregnancy,¹¹³⁶ and excessive LH appeared to mediate hilus cell hyperplasia in a case of FSH-resistant ovarian follicles.¹¹³⁷ An extremely high level of hCG caused by tumor has been reported to virilize a nonpregnant woman with preexisting PCOS.¹⁰⁷³

Other Causes of Functional Adrenal Hyperandrogenism.

The PCOS type of primary FAH that appears to arise from dysregulation of adrenal steroidogenesis occurs as an isolated entity, not associated with FOH, in 5% of hyperandrogenic women.⁴⁰ This may sometimes be an outcome of premature adrenarche. This type of adrenal dysfunction was previously mistaken for nonclassical 3 β -HSD deficiency, which is now known to be a rare disorder.¹¹³⁸

Less than 10% of adrenal hyperandrogenism is caused by the other disorders listed in Box 16.8. The most common of these are nonclassic CAH, which accounts for 4.2% of hyperandrogenic women worldwide, second only to PCOS (prevalence >80%) as the most common cause of hyperandrogenism in adolescent and adult females.⁶⁴⁷ The prevalence varies from 0.1% to 2% in the general US population to 4% in Ashkenazi Jews, to 10% in Mediterranean-Middle East countries.⁸⁴¹ Adrenal hyperandrogenism can on rare occasions arise from other rare congenital disorders of adrenal steroid action or metabolism, such as glucocorticoid resistance, apparent cortisone reductase deficiency, and apparent sulfotransferase deficiency.^{1139–1142}

About 40% of hyperprolactinemic women have hyperandrogenism, sometimes in association with polycystic ovaries.^{367,1143} The great majority (about 85%) of these women have galactorrhea; the combination of hirsutism, galactorrhea, and amenorrhea comprises the Forbes-Albright syndrome. Prolactin excess causes adrenal hyperandrogenism because of multiple effects of prolactin excess on adrenal androgen production and androgen metabolism.³⁶⁷ Serum prolactin values more than 25 ng/mL in hyperandrogenic women usually have an identifiable cause, rather than PCOS itself: most common are pituitary adenoma and antidopaminergic drugs.¹¹⁴⁴

Peripheral Androgen Overproduction. In about 10% of PCOS patients, an ovarian or adrenal source cannot be ascertained by thorough testing (see Fig. 16.48). This “functionally atypical PCOS” seems in most cases to be caused by obesity: excess adipose tissue has the capacity to both cause anovulation and to form testosterone from androstenedione.⁴⁰ In about 20% of such cases (\leq 5% of PCOS), the source of the hyperandrogenemia is unexplained: this is termed *idiopathic hyperandrogenism* (as distinct from “idiopathic hirsutism”). Quirks in steroid peripheral metabolism have been suspected to be the cause.

PCOS has also been reported as a complication of portosystemic shunting.¹¹⁴⁵ Impaired steroid metabolism has been postulated as the mechanism.¹¹⁴⁶

Tumoral Hyperandrogenism. Virilizing tumors are rare, accounting for about 0.2% of hyperandrogenism; about half are malignant.⁶⁴⁷ About half are ovarian, half adrenal. Masculinizing sex cord-stromal tumors of the ovary are unusual before the teenage years. Leydig-Sertoli cell tumor (androblastoma, arrhenoblastoma) is the most common type. Virilizing granulosa-theca cell tumor (thecoma) is unusual before menopause.^{1073,1147} Dysgerminomas virilize only if they have interstitial cell elements. Lipid cell tumors tend to respond to ACTH, as well as LH and produce 17-OHP; thus they must be considered in the differential diagnosis of late-onset CAH.¹¹⁴⁸ The abnormal differentiation that underlies tumor formation typically leads to an abnormal pattern of steroid secretion with androstenedione greatly predominating over testosterone secretion.¹¹⁴⁹ However, some thecomas have been reported to predominantly secrete testosterone.^{1150,1151} Gonadoblastomas are virilizing tumors virtually confined to individuals with dysgenetic gonads with Y-chromosomal material in their genome. Some masculinizing ovarian sex cord-stromal tumors may be caused by activating mutations of stimulatory G-proteins.¹¹⁵² Leydig cell and adrenal rest tumors of the ovary are extremely rare causes of masculinization in childhood.^{20,436,1153} Adrenal virilizing tumors are rare in adolescence; their peak incidence is in early childhood and adulthood⁸²¹ (see sections Precocious Puberty and Incomplete precocity).

Androgenic Drugs. Drug-induced masculinization in adolescence is encountered most often in athletes. The medical history is important in detection because standard clinical laboratory tests for androgens are not helpful in the detection of either natural or artificial androgens.^{647,1154} This is also the case for masculinization that results from unintended contact with topical androgens used by a parent or by a sexual partner.^{647,828} Valproic acid use in epileptics raises testosterone levels and may mimic PCOS.⁴⁶²

Differential Diagnosis

Hyperandrogenism should be considered in any girl who presents with hirsutism or moderate-severe inflammatory acne, menstrual disturbance, or acanthosis nigricans with central obesity (waist circumference >88 cm) during puberty.

Hirsutism must be distinguished from locally excessive hair growth in the absence of an abnormal hirsutism score (“patient-important hirsutism”) and from hypertrichosis, the generalized excess growth of vellus hair that sometimes occurs on a hereditary basis or in patients taking glucocorticoids, phenytoins, diazoxide, or cyclosporine. Hypertrichosis is distributed in a nonsexual pattern (e.g., generalized distribution or more prominent distribution on the forehead or shoulders) and is not caused by excess androgen, although it may be aggravated by excess androgen.

The possibility of PCOS is heightened if hirsutism, treatment-resistant acne, or menstrual irregularity are associated with a history of prepupal risk factors for PCOS—congenital virilizing disorders; premature adrenarche; intractable prepupal obesity, particularly if associated with pseudo-Cushing syndrome or pseudo-acromegaly; or a family history of PCOS or metabolic syndrome.

Diagnostic Approach

The goals of the laboratory evaluation for hyperandrogenism are to obtain evidence of hyperandrogenemia and to determine the specific etiology. The diagnosis of hyperandrogenism is on the firmest grounds if hyperandrogenemia is demonstrated biochemically, rather than relying on hirsutism as a clinical

surrogate for it, although documentation of hyperandrogenemia can be problematic.

The evaluation starts with a thorough assessment of the clinical symptoms and signs suggestive of PCOS and for disorders that mimic it (Fig. 16.49).

The degree and distribution of sexual hair growth should be identified, usually done using the Ferriman-Gallwey score (see Fig. 16.46). Moderate or severe hirsutism (hirsutism score >15) constitutes clinical evidence of hyperandrogenism in an adolescent (see Box 16.9). The history should specifically explore

whether the patient is taking medications that may mask symptoms (e.g., depilatory use) or cause hirsutism (e.g., anabolic steroids, valproate).

Moderate or severe inflammatory acne (>10 lesions in any area, e.g., face, chest, back) or acne that is persistent and poorly responsive to topical dermatological therapy suggests the possibility of hyperandrogenism.

Assessment should also include evaluation for symptoms or signs that suggest a hyperandrogenic disorder other than PCOS, including virilization (e.g., rapidly progressive hirsutism,

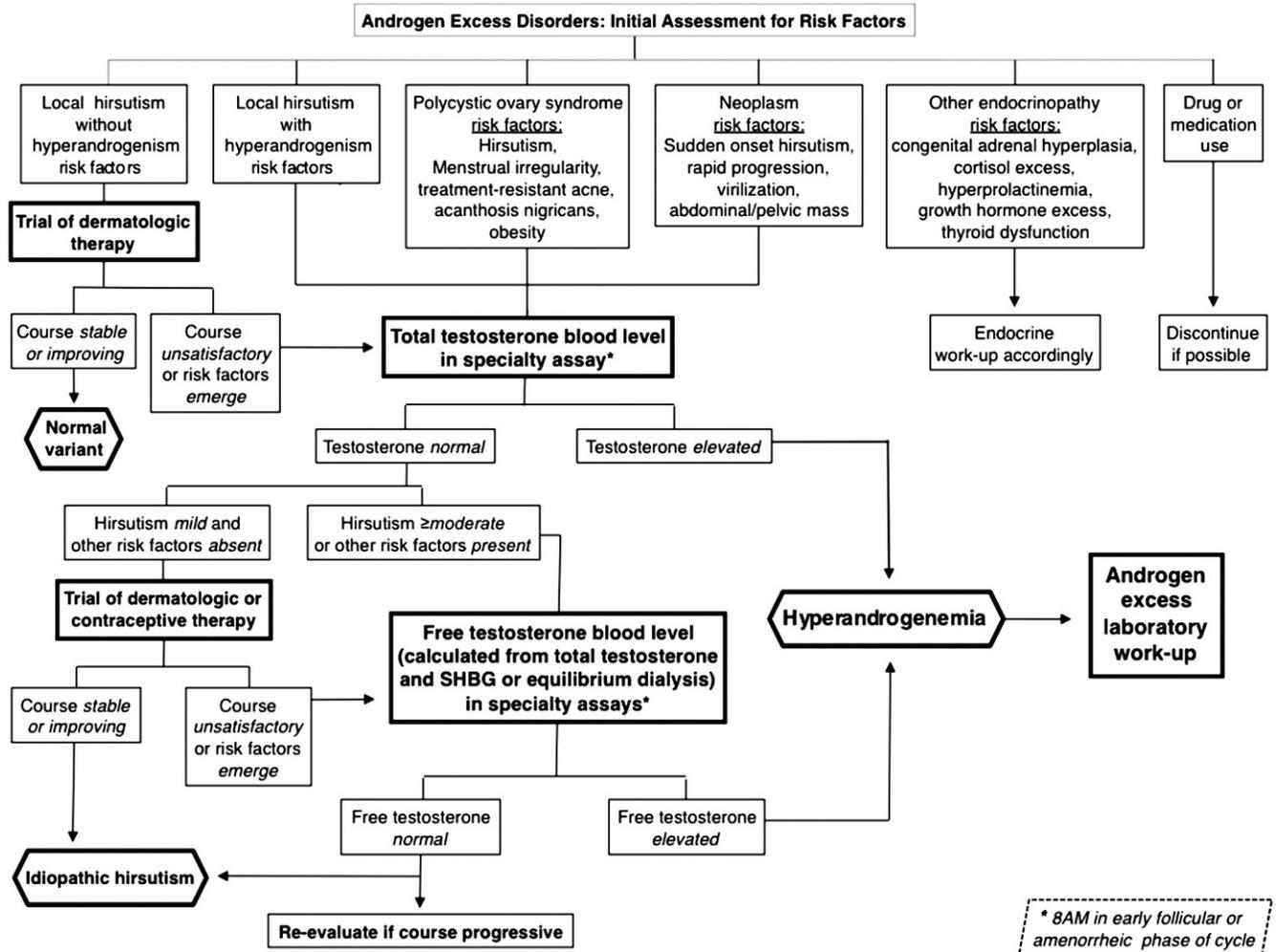


Fig. 16.49 Initial assessment of women for risk of androgen excess disorders. Patient-important localized areas of hair growth in the presence of a normal hirsutism score (“local hirsutism”), but not accompanied by other androgen excess risk factors, do not require an endocrine workup before embarking on local dermatologic therapy. In women with local hirsutism with other androgen excess risk factors or an elevated hirsutism score, androgen levels should be assessed. PCOS is the most common cause to be considered, but androgen-producing tumors, congenital adrenal hyperplasia, and diverse androgenic disorders, as shown, should be excluded. Drugs that cause hirsutism include anabolic or androgenic steroids (consider in athletes and patients with sexual dysfunction) and valproic acid (consider in neurologic disorders), which causes hyperandrogenemia. Serum testosterone is best assessed in the early morning, on days 4-10 of the menstrual cycle in eumenorrheic women or on random days in amenorrheic women, by an accurate and specific assay such as liquid chromatography/mass spectrometry. Females with mild hirsutism (score 8-15), normal total testosterone level, and no risk factors probably have idiopathic hirsutism, which may be responsive to endocrine therapy. Serum free testosterone should be measured as indicated by a specialty reference assay if the serum total testosterone is normal in the presence of risk factors or progression of hirsutism on therapy. Simultaneous assay of 17-hydroxyprogesterone is indicated in subjects at high risk for congenital adrenal hyperplasia. Some adolescents diagnosed with idiopathic hirsutism by this algorithm will have polycystic ovaries on ultrasound as adults, which is unlikely to be important unless fertility is an issue. Progression of hyperandrogenism in the presence of a normal serum free testosterone is very unusual; such patients should be thoroughly reevaluated, possibly also measuring other steroids related to androgen excess, such as 17-hydroxyprogesterone, androstenedione and DHEAS. The initial laboratory work-up for androgen excess is shown in Fig.16.50. (Modified with permission from Martin, et al. Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. Copyright The Endocrine Society.)

clitoromegaly), abdominal mass, galactorrhea, cushingoid or acromegaloid changes, evidence of thyroid dysfunction, or a family history of hyperandrogenic disorders. Inspection of the external genitalia is indicated, but an internal pelvic examination seldom is necessary.⁹⁹⁷

If hirsutism is local or mild (hirsutism score <8) and if menses are regular with no other clinical evidence of risk factors that would suggest an underlying cause, unwanted or excess sexual hair growth can be managed by dermatological means without pursuing further laboratory evaluation.^{462,647} Unless fertility becomes an issue in adulthood for subjects with local hirsutism or idiopathic hirsutism, demonstrating polycystic ovary morphology to diagnose ovulatory PCOS is unlikely to affect management.

Laboratory assessment for hyperandrogenism begins with measurement of serum testosterone: this is the single most important androgen to evaluate (Fig. 16.49).^{124,467,647} Although other androgens are present in blood, their assessment ordinarily makes little difference in diagnosis and management if serum free testosterone is normal. Serum free testosterone is about 50% more sensitive in detecting excessive androgen production because hyperandrogenic women have a relatively low level of SHBG. There are many pitfalls in testosterone assays at the low levels found in women and children, and reliable testosterone assays are not available in most local laboratories. Assays of high sensitivity and specificity, such as are provided by postchromatographic RIA or tandem mass spectrometry by specialty laboratories, are required. Assaying the free testosterone level introduces other potential sources of error. Direct assays of the free testosterone concentration are inaccurate and should be avoided. The best methods to calculate free testosterone as the product of the total testosterone and a function of SHBG: free testosterone = total testosterone × percent free testosterone, where percent free testosterone is most accurately determined by dialysis, or alternatively calculated from the SHBG concentration.¹¹⁵⁵ The combination of a high-normal total testosterone and a low-normal SHBG yields a high free testosterone concentration.

Exclusion of hyperandrogenic disorders that mimic PCOS (see Box 16.8) is important to meet diagnostic criteria. Because PCOS accounts for 95% of hyperandrogenisms, a diagnostic strategy is required that takes into account individual patient preference, including benefits in relation to economy of cost and of time. Guidelines vary slightly, but most suggest screening for nonclassic CAH, which accounts for most non-PCOS hyperandrogenism, hyperprolactinemia, thyroid disease, and virilizing tumor, which is the most serious although rare; testing for Cushing syndrome and acromegaly are generally suggested^{124,647,1107,1156} for those with suggestive clinical features. Normal results of a simple panel of endocrine screening tests to exclude these other causes of hyperandrogenemia (see Fig. 16.50) ensures the diagnosis of PCOS with about 99% reliability.

Practice varies as to the indications for ultrasonography in girls with confirmed hyperandrogenemia. The primary purpose of ultrasonography is to screen for the rare but serious adrenal or ovarian tumor, as it is not required for diagnosis. Ultrasonography also provides the opportunity for patient reassurance and education. For many women, the diagnosis of ovarian “cysts” raises a concern about tumors, so it is reassuring to know that a tumor has not been seen.

Some tests require consideration of the time of day or stage of cycle that sampling is performed. Serum testosterone levels are 20% higher in the morning than in the afternoon and double in midcycle; norms are based on midfollicular phase controls.⁶⁴⁷ An early morning 17-OHP level over 170 ng/dL (>5.1 nmol/L) is approximately 95% sensitive and 90% specific for nonclassic CAH, but they are insensitive by

midmorning.^{647,841} The most common cause of false positives is PCOS,¹⁹⁵⁷ the most serious cause is virilizing tumors.^{1148,1157} DHEAS elevations are usually caused by the FAH of PCOS; DHEAS greater than 700 mcg/dL suggests adrenal virilizing tumor or a rare type of CAH (3β-HSD deficiency). A cortisol level less than 10 mcg/dL (276 nmol/L) is reassuring evidence against endogenous Cushing syndrome unless the clinical index of suspicion is high.

The senior author's practice is to ordinarily begin the specific workup for PCOS at the initial evaluation for hyperandrogenic anovulation, an evaluation that includes testosterone (see Fig. 16.49), by adding a few tests for the common non-PCOS causes of hyperandrogenism (see Fig. 16.50).¹¹⁰¹ For practical reasons, sampling for early morning serum 17-OHP and ultrasound are ordinarily scheduled; at the same time, whatever further workup might be indicated by the initial test results (e.g., early morning free testosterone if midday testosterone was not clear-cut in the face of high clinical index of suspicion for hyperandrogenism).

A more comprehensive endocrine evaluation is sometimes indicated because the basic evaluation described earlier does not exclude rare virilizing disorders (see Box 16.8).^{647,1156} The approach to further studies to determine the source of hyperandrogenemia varies among subspecialists and with the needs of the individual patient.

Our preference is to use a DAST to attempt to make a positive diagnosis of the ovarian dysfunction of PCOS versus determining whether further workup is necessary for rare forms of CAH or other rare adrenal disorders (Fig. 16.51).^{1101,1122} Suppression of serum androgens and cortisol in response to a low-dose dexamethasone suppression test segregates patients diagnostically. Subnormal testosterone suppression with normal adrenocortical suppression indicates a source of androgen other than an ACTH-dependent adrenal one and is found in 80% of PCOS. However, tumor or other ovarian pathology must be excluded by ultrasound examination. If both cortisol and androgen suppression are subnormal, then the androgen excess may be secondary to dexamethasone noncompliance, endogenous Cushing syndrome, or glucocorticoid resistance. If testosterone suppression is normal, then ACTH (cosyntropin) stimulation testing to rule out nonclassic CAH is recommended. If both dexamethasone and ACTH testing are normal, the most likely diagnosis is the atypical PCOS of obesity or idiopathic hyperandrogenism.

A short (4-h) DAST, as described in the Fig. 16.51 legend, suffices in the absence of high suspicion for virilizing disorder. It is helpful in distinguishing the potentially reversible pseudo-PCOS of simple obesity, in which case testosterone suppresses normally, from the persistent ovarian dysfunction of ordinary PCOS.¹¹²² However, a more prolonged course of low-dose dexamethasone (long DAST, 4 days) is required to suppress the hyperandrogenism of CAH.

Further extensive diagnostic studies beyond those indicated in Fig. 16.50 are seldom indicated unless there is reason to suspect a virilizing tumor or a disorder of sexual differentiation. Computed tomography and MRI permit the best visualization and more detailed assessment of tumors, particularly of the adrenal gland. On rare occasions, ultrasonography has been insensitive in detecting a virilizing ovarian tumor in adults.^{847,1158} Further or alternative workup may include acute GnRH agonist testing, or assessment of the response to hormonal suppression treatment to determine the source of androgen.

Management

Management of hyperandrogenism is individualized according to symptoms and patient goals—hirsutism and acne;

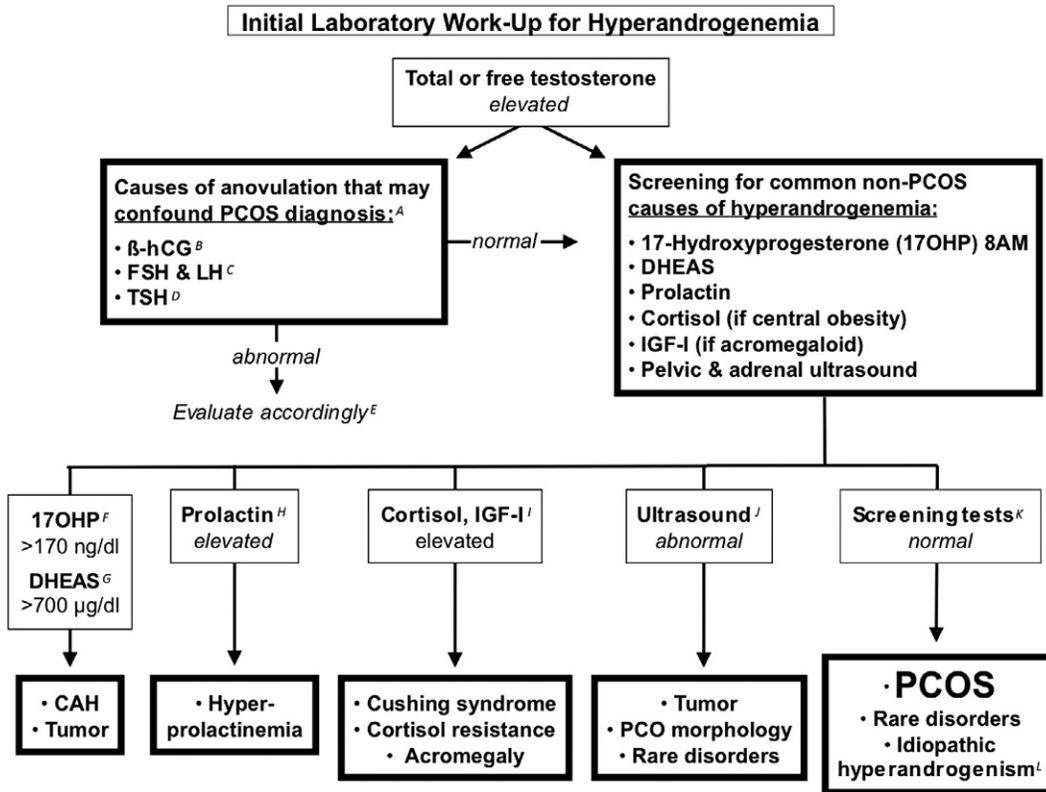


Fig. 16.50 Initial laboratory workup for cause of androgen excess. Polycystic ovary syndrome is a diagnosis of exclusion: this algorithm identifies the most common other causes of hyperandrogenemia and hyperandrogenic anovulation. (Modified from Rosenfield, R.L. (2018). Polycystic ovary syndrome in adolescence. Clinical features and diagnosis of polycystic ovary syndrome in adolescents. *UpToDate*. With permission.)

Footnotes:

- A. These tests for anovulatory disorders are advisable for hyperandrogenemic patients with menstrual abnormalities, because they may confound the diagnosis of polycystic ovary syndrome (PCOS).
- B. Pregnancy is associated with high testosterone levels.
- C. Slightly elevated luteinizing hormone (LH) and slightly low follicle-stimulating hormone (FSH) are common in PCOS. High FSH suggests primary hypogonadism. Low LH suggests hypogonadotropic hypogonadism.
- D. Thyroid dysfunction may alter the total testosterone level by altering sex hormone binding globulin. Hypothyroidism may cause multicystic ovaries and coarse hair that may be mistaken for hirsutism.
- E. See preceding algorithms for workup of anovulatory disorders.
- F. 8:0 AM 17-hydroxyprogesterone (17-OHP) over 170 ng/dL is approximately 95% sensitive and 90% specific for detecting common type (21-hydroxylase-deficient) nonclassic congenital adrenal hyperplasia (NCCAH) in anovulatory or early-midfollicular phase women. NCCAH is the most common disorder mimicking PCOS. Falsely positive 17-OHP elevation is found in PCOS, luteal phase women, and virilizing neoplasms.
- G. Dehydroepiandrosterone sulfate (DHEAS) greater than 700 mcg/dL suggests adrenal virilizing tumor or the rare 3β-hydroxysteroid dehydrogenase deficiency form of NCCAH.
- H. Hyperprolactinemia may cause either hypogonadotropic hypogonadism or hyperandrogenic anovulation (which is usually accompanied by galactorrhea, the Forbes-Albright syndrome).
- I. Optional tests are cortisol and insulin-like growth factor 1 (IGF-I). Endogenous Cushing syndrome should be considered in cases with central obesity. Plasma cortisol under 10 mcg/dL essentially rules out endogenous Cushing syndrome unless the clinical index of suspicion is high. Acromegaly should be ruled out by IGF-I screening if the patient has acromegaloid overgrowth.
- J. Ultrasonography screens for ovarian and adrenal tumor. Polycystic ovary morphology is supportive, but it is not specific for, nor is it a criterion for, the diagnosis of adolescent PCOS. Ultrasound may detect ovotesticular disorder of sex development (true hermaphroditism) or human chorionic gonadotropin (hCG)-related disorders of pregnancy.
- K. Exclusion of the preceding disorders in a hyperandrogenic patient with menstrual dysfunction meets standard diagnostic criteria for PCOS with approximately 99% reliability. However, this workup does not identify rare adrenal disorders (e.g., rare types of congenital adrenal hyperplasia [CAH] and adrenal steroid metabolic disorders, very small tumors, such as the rare testosterone-secreting neoplasm) or idiopathic hyperandrogenism.
- L. Idiopathic hyperandrogenism (documented hyperandrogenemia with no demonstrable source) occurs in approximately 1% of hyperandrogenic women. This is distinct from the more common “idiopathic hirsutism” (hirsutism without other clinical evidence of hyperandrogenism). TSH, Thyroid-stimulating hormone.

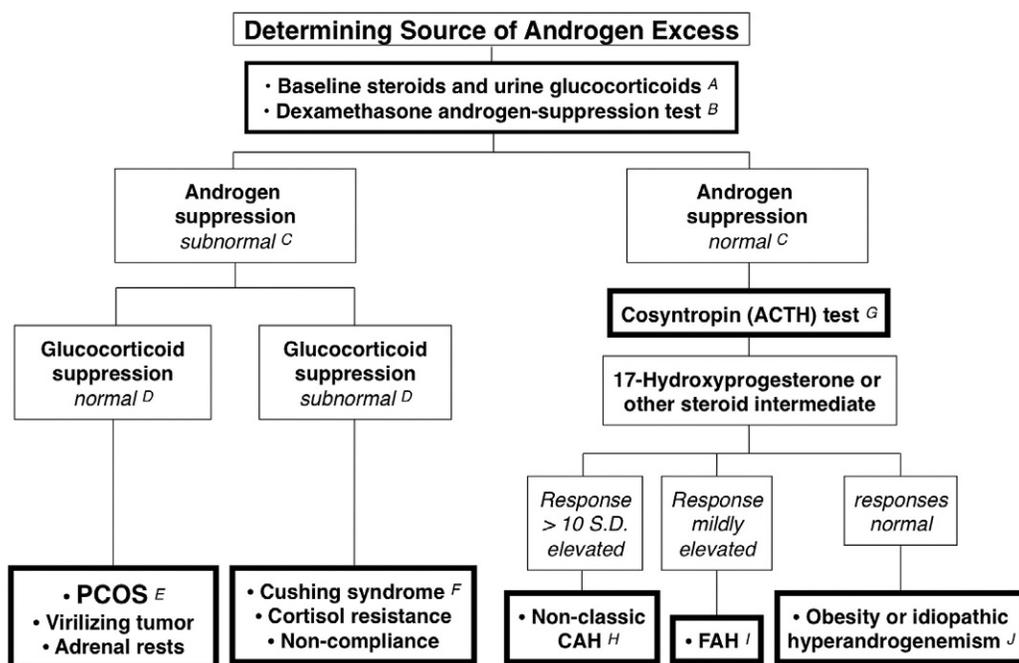


Fig. 16.51 An approach to determining the source of androgen excess. Determination of the source of excess androgen often permits a positive diagnosis of the characteristic ovarian and adrenal dysfunction of polycystic ovary syndrome (PCOS) and will elucidate rare disorders that mimic PCOS. (From Buggs C, Rosenfield RL. Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin North Am* 2005; 34:677.)

Footnotes:

- A. After obtaining an early morning blood sample for baseline steroid intermediates (e.g., 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, dehydroepiandrosterone, androstenedione) and a 24-hour urine for glucocorticoids (i.e., free cortisol and 17 α -hydroxycorticosteroids, as well as creatinine to control for completeness of collection), a dexamethasone androgen-suppression test (DAST) is performed.
- B. A short DAST (sampling blood 4 h after a single noontime 0.5 mg dexamethasone dose) maximally suppresses total and free testosterone and 17-hydroxyprogesterone, but dehydroepiandrosterone sulfate (DHEAS) and cortisol are not maximally suppressed in comparison to the 4-day DAST. A long DAST (4 days) is a definitive test: this consists of a 4-day course of dexamethasone 0.5 mg 4 times daily before an early morning posttest blood sample on day 5.
- C. Normal androgen suppression in response to the 4-day DAST is indicated in our laboratory by total testosterone less than 28 ng/dL (1.0 nmol/L), free testosterone under 8 pg/mL (28 pmol/L), DHEAS less than 40 mcg/dL (1.0 micromol/L) (>75 % fall), and 17-hydroxyprogesterone under 26 ng/dL (0.8 nmol/L).
- D. Normal glucocorticoid suppression is indicated by serum cortisol under 1.5 mcg/dL (40 nmol/L) and urinary cortisol under 10 mcg (27 nmol) per 24 hours by the second day of dexamethasone administration.
- E. Subnormal suppression of androgens and a normal suppression of cortisol and DHEAS is characteristic of PCOS, but the rare (<1% of cases) virilizing adrenal tumor or adrenal rests should be considered on the basis of clinical features. Virilizing tumors are often characterized by an abnormal pattern of steroid intermediate elevations that is atypical for congenital adrenal hyperplasia (CAH).
- F. Subnormal suppression of both cortisol and androgen is consistent with endogenous Cushing syndrome or cortisol resistance. Poor suppression can also result from noncompliance with the dexamethasone regimen.
- G. A cosyntropin (ACTH) stimulation test is appropriate if androgen suppression by dexamethasone is normal. The ACTH test is usually performed by administering 250 mcg cosyntropin by IV push and drawing blood for steroids 30 to 60 minutes later.
- H. The diagnosis of CAH is suggested if the response to ACTH is over 10 standard deviations (SDs) above the average: this corresponds to, for example, 17-hydroxyprogesterone greater than 1500 ng/dL (45 nmol/L), 17-hydroxypregnenolone greater than 5000 ng/dL (158 nmol/L).
- I. Primary functional adrenal hyperandrogenism (FAH) (suggested by a modest rise in 17-hydroxypregnenolone or 17-hydroxyprogesterone that does not meet the criteria for the diagnosis of CAH) is sometimes the only demonstrable source of androgen excess in PCOS. A rare mimic of FAH and idiopathic hyperandrogenemia is (apparent) cortisone reductase deficiency: baseline urinary glucocorticoids consist primarily of cortisone metabolites rather than cortisol metabolites, so 17 α -hydroxycorticosteroid excretion is elevated, but cortisol excretion is normal.
- J. When the source of hyperandrogenemic anovulation remains unexplained after intensive investigation (approximately 10% of cases), it usually appears to be caused by the atypical PCOS of obesity. Otherwise, the diagnosis is idiopathic hyperandrogenism (distinct from idiopathic hirsutism) if (apparent) cortisone reductase deficiency has been excluded.

menstrual irregularity; obesity—and the cause of androgen excess.^{706,1159,1160} Here, we will discuss the management of PCOS.

Hirsutism and Acne. Cosmetic measures are the cornerstone of care for unwanted hair.⁶⁴⁷ Bleaching and shaving suffice for many. Depilating agents and waxing treatments are useful, but prone to cause skin irritation. Repeated topical application

of eflornithine hydrochloride cream brings about significant reduction of local hirsutism within 6 to 8 weeks. It is a useful adjunct to photoepilation, providing a more rapid response. Topical benzoyl peroxide and retinoids are the cornerstone of care for acne; short-term oral tetracycline-derived antibiotics are appropriate for moderate-severe inflammatory acne.¹¹⁶¹

For hirsutism or acne that requires more than these dermatological measures, first-line treatment for both the hyperandrogenemia and menstrual abnormalities of PCOS is ordinarily OCPs, which correct both,⁶⁴⁷ with several being FDA approved for the treatment of acne. Antiandrogen treatment of cutaneous symptoms is suggested for most hyperandrogenic women before undertaking treatment with photoepilation⁶⁴⁷ or oral isotretinoin (Accutane®).

Endocrine therapy is directed at interrupting androgen production or action. This causes the pilosebaceous unit to revert toward the prepubertal vellus type (see Fig. 16.29). The maximal effect on the sebaceous gland occurs within 3 months, but that on sexual hairs requires 9 to 12 months of treatment, because of the long duration of the hair growth cycle. Hirsutism requires use of endocrine treatment as long as the patient wishes to maintain improvement.

Combination OCPs, the first-line endocrine treatment for women with the dermatological or menstrual abnormalities of PCOS, act by suppressing serum androgens, mainly by inhibiting ovarian function. They also raise SHBG, which suppresses the serum free fraction of testosterone, and modestly lower DHEA sulfate levels. They normalize androgen levels by 3 weeks of therapy.

All estrogen-progestin combinations in combination with cosmetic measures are similarly effective for women with acne or mild hirsutism.⁶⁴⁷ A 7-point improvement of hirsutism score can be anticipated on average. Compared with levonorgestrel or norethindrone, low-androgenic progestins, such as norgestimate, and antiandrogenic progestins, such as drospiro-none, successively better optimize serum lipid profiles, but successively increase venous thromboembolism (VTE) risk, although the risk is ordinarily low in adolescence and far less than that of pregnancy. VTE risk is also related to estrogen dose, but 20 to 35 mcg ethinyl estradiol-containing OCPs have generally favorable risk-benefit ratios. Drospirenone-containing OCPs also have antiminerocorticoid activity and thus may be associated with less weight gain than other OCPs.¹¹⁶²

Antiandrogens yield mild improvement in hirsutism beyond that attainable with OCPs.⁶⁴⁷ The combination can be expected to reduce the Ferriman-Gallwey score by 15% to 40%, although there is considerable individual variation. Antiandrogen use for this purpose is off-label because all carry the risk of causing undervirilization of the male fetus. Therefore all antiandrogens should be prescribed with a contraceptive, preferably an OCP. They may have a modest effect on the metabolic abnormalities associated with PCOS.¹¹⁶³

Spirolactone in high dosage is probably the safest most effective antiandrogen available in the United States.⁶⁴⁷ Guidelines recommend starting with 100 mg twice a day until the maximal effect has been achieved and then attempting to reduce the dose to 50 mg twice a day for maintenance therapy. Spirolactone usually is well tolerated, but it is contraindicated in patients with adrenal, hepatic, or renal insufficiency. Women are at risk of hyperkalemia if on potassium-sparing diuretics, potassium supplements, daily nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, heparin, or such drugs. Therefore electrolytes should be monitored. Alone, it tends to cause irregular bleeding.

Other antiandrogens have been extensively evaluated to treat hirsutism and hirsutism equivalents, including cyproterone acetate, flutamide, and finasteride. Cyproterone acetate is a potent progestational antiandrogen that is used with estrogen in a reverse sequential regimen: 50 to 100 mg is given during days 1 to 10 of cycles in which estrogen is given from days 1 to 21. Flutamide is a more specific antiandrogen with efficacy similar to that of cyproterone and spiro-lactone, but it is not recommended because of a seemingly idiosyncratic risk of fatal hepatocellular toxicity.⁶⁴⁷ Finasteride, a type 1 5-alpha-reductase inhibitor, tends to be

somewhat less effective than other antiandrogens in the treatment of hirsutism and pattern balding in females. Other more potent and selective antiandrogens are in earlier stages of evaluation.^{1164,1165} Although topical minoxidil is the only medication approved for alopecia treatment, antiandrogen-OCP therapy may be superior in those with PCOS.

GnRH agonist therapy with estrogen-progestin add-back therapy should be considered for the treatment of hirsute women only if they are intolerant of OCPs or if OCPs are ineffective for severe hyperandrogenemia.⁶⁴⁷ Glucocorticoids are of limited efficacy in the management of the hirsutism of the FAH of PCOS.⁶⁴⁷

Menstrual Irregularity. OCPs, discussed in some detail earlier as the usual first-line endocrine treatment of hirsutism and acne unresponsive to cosmetic measures, are also ordinarily the first-line treatment for menstrual irregularity. Beyond hygienic concerns, menstrual irregularity requires treatment because chronic anovulation is associated with increased risk of developing endometrial hyperplasia and carcinoma. Although lower estrogen doses are the safest, 30 to 35 mcg ethinyl-estradiol is often necessary in larger girls to provide menstrual regularity.

There are several limitations to the use of OCPs in the management of PCOS in adolescents. OCPs may be contraindicated in patients who are at high risk for venous thrombosis, and they should be used with caution and in the lowest estrogen dose possible in patients with migraine headaches. OCPs must be discontinued when patients desire conception. OCPs will bring growth to end in perimenarcheal girls. The long-term consequences of these agents on fertility are unknown; while there is the theoretic possibility of postpill amenorrhea, very high-dose estrogen begun in early adolescence increases the risk of primary ovarian insufficiency rather than hypogonadotropinism.⁸⁸⁶

It is advisable to recheck patients after 3 months of therapy to assess the efficacy of treatment and normalization of androgen levels. To document persistence of PCOS and to curtail mistaken beliefs that the treatment is curative, it is advisable to withdraw OCPs for approximately 3 months when the patient is gynecologically mature to determine persistence of hyperandrogenic anovulation. In doing so, however, one must keep in mind that the anovulatory infertility of PCOS is relative, not absolute, so contraceptive counseling is indicated.

Progestin monotherapy is an alternative to OCPs for the control of menstrual irregularities. Micronized progesterone (Prometrium®) 100 to 200 mg daily at bedtime for 7 to 10 days induces withdrawal bleeding in the majority of patients, but some do not respond, apparently because of an antiestrogenic effect of androgen excess on the endometrium, and breakthrough bleeding is more likely than with OCPs. Progestin therapy has the appeal of permitting the detection of the emergence of normal menstrual cyclicity. However, it is ineffective for improving androgen levels or hirsutism. The perimenarcheal girl who responds well to progestin therapy can be maintained at approximately 6-week cycles to permit the detection of spontaneous menses. Side effects of progestin include mood symptoms (depression), bloating, and breast soreness. Patients must be informed that oral progestin dosed in this way is not a means of contraception.

Obesity and Metabolic Syndrome. An oral glucose tolerance test, more sensitive for the detection of prediabetes than hemoglobin A1c, and lipid panel, is suggested in obese PCOS patients.^{1103,1166} Those with metabolic syndrome should be screened for sleep-disordered breathing and fatty liver disease. We also advise screening primary relatives for metabolic syndrome and PCOS, because PCOS is closely related to parental

metabolic syndrome and maternal PCOS.¹¹⁶⁶ Women with PCOS are at significantly increased risk of depression, anxiety, and bipolar, autism spectrum, and attention deficit hyperactivity disorders and should be screened for these disorders.¹¹⁶⁷

Insulin-lowering treatments of obesity, from weight loss to drug treatment, uniformly but modestly improve hyperandrogenism. Lifestyle modification is the first-line treatment for the overweight and obesity of PCOS.^{1103,1156,1168} Calorie reduction is indicated, as with any obese subject.^{1168,1169} Obese patients experience improvement in their anovulatory symptoms in approximate proportion to the amount of weight loss.¹¹⁷⁰ Improvement in hirsutism is minimal, however.^{647,1171}

Bariatric surgery has led to improvement in hirsutism, androgen levels, and menses in the vast majority of obese adults with PCOS.¹¹⁷² Much of this seems to be caused by the atypical form of PCOS caused by obesity.^{40,1055} Bariatric surgery is suggested only for select adolescents with extremely high BMI and access to highly specialized centers.¹¹⁶⁸

Metformin is often used as an adjunct to the management of obesity and insulin-resistant metabolic abnormalities in patients with PCOS, although abnormal glucose tolerance is the only approved indication for it. It is usually administered by escalating doses over 3 to 4 weeks from 500 mg to 2000 mg in two divided doses (or as a single dose of the extended release form) daily, as tolerated, to minimize anorexia and nausea. Such effects contribute to weight loss but are also the cause of about a 15% drop-out rate. Metformin effectiveness for PCOS is minimized in the absence of weight control.¹¹⁷³ Three randomized, double-blind, placebo-controlled trials compared metformin to lifestyle counseling for 3 to 6 months in adolescents with PCOS.^{1174–1176} Metformin was found to significantly improve HDL cholesterol levels. It increased the likelihood of menses significantly in only the shortest of the three studies, with only nonsignificant tendencies to an increase in ovulatory cycles in the others. It did not lower testosterone levels or weight significantly better than lifestyle/placebo, which likely explains its inconsistent effects on insulin levels in these trials.¹¹⁰³ These results are less salutary than those of open-label trials.

Available randomized trials comparing metformin to OCPs in adolescents have not been blinded, which limits their quality.¹¹⁷⁷ Although the mean suppression of free testosterone levels is greatest on OCPs the relatively small size of the studies has made it difficult to demonstrate statistical differences between treatments.¹¹⁷⁵ When metformin and OCPs are used in combination, the positive metabolic effects of metformin are blunted by the negative effects of OCPs on lipids.¹¹⁷⁸ Metformin has been reported to ameliorate the negative effect of a COC on thrombin generation.¹¹⁷⁹

It is advisable to obtain a baseline comprehensive metabolic panel to confirm normal hepatic and renal function before institution of metformin therapy. Although extremely rare, lactic acidosis is a potential complication of metformin use.

Other metabolic therapies have a place in the management of PCOS only to the extent that they may be indicated for control of coexistent diabetes mellitus or hyperlipidemia. Because of the limited efficacy and concern for weight gain and toxicity, thiazolidinediones are not generally advisable in adolescents with PCOS.¹¹⁰¹

FUTURE DIRECTIONS

Tremendous advances continue to occur in our understanding of puberty. The identification of genes involved in ovarian differentiation, the discovery of new hormones and hormone receptors, new insights into the regulation of gene transcription and signal transduction, further identification of the role of genetic factors and prenatal epigenomic imprinting on pubertal

disorders, and advances in the application of mass spectrometry to steroid assays can also be anticipated to occur in the next 5 years. We are in the midst of an explosion of information in the biological sciences. It is becoming clear that the body puts a wide but finite repertoire of hormones and growth factors to myriad and unexpected uses. Many concepts that we hold dear at this moment are at the best likely to be shown to be oversimplifications, at worst wrong. New information comes to light faster than we can assimilate it. The understanding of the interactions of the human genome with environmental factors can be expected to yield new insights into our understanding of puberty and its disorders.

REFERENCES

1. Donovan B, van der Werff, ten, Bosch J. *Physiology of Puberty*. London: Edward Arnold Ltd; 1965.
2. Wilkins L. *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. 3rd ed.; 1965. Springfield.
3. Ramirez D, McCann S. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. *Endocrinology*. 1963;72:452.
4. Mills I, Brooks R, Prunty F. The relationship between the production of cortisol and androgen by the human adrenal. In: Currie A, Symington T, Grant J, eds. *The Human Adrenal Cortex*. Baltimore: Williams & Wilkins; 1962.
5. Krsmanovic LZ, Hu L, Leung PK, Feng H, Catt KJ. The hypothalamic GnRH pulse generator: multiple regulatory mechanisms. *Trends Endocrinol Metab*. 2009;20:402–408.
6. Grumbach M, Roth J, Kaplan S, Kelch R. Hypothalamic-pituitary regulation of puberty in man: Evidence and concepts derived from clinical research. In: Grumbach M, Grave C, Mayer F, eds. *The Control of the Onset of Puberty*. New York: John Wiley & Sons; 1974:115–207.
7. Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes F. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*. 1975;40:545–551.
8. Arey L. *Development of the Female Reproductive System*. 6th ed. Philadelphia: WB Saunders; 1974.
9. Schwanzel-Fukuda M, Jorgenson K, Bergen H, Weesner G, Pfaff D. Biology of normal luteinizing hormone-releasing hormone neurons during and after their migration from olfactory placode. *Endocr Rev*. 1992;13:623.
10. Clements J, Reyes F, Winter J, et al. Ontogenesis of gonadotropin-releasing hormone in the human fetal hypothalamus. *Proc Soc Exp Biol Med Soc Exp Biol Med*. 1980;163:437.
11. Kaplan S, Grumbach M, Aubert M. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. *Recent Prog Horm Res*. 1976;32:161.
12. King J, Gerall A. Localization of luteinizing hormone-releasing hormone. *J Histochem Cytochem*. 1976;24:829.
13. Raisman G, Field P. Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. *Brain Res*. 1973;54:1.
14. Gorski R. Sexual differentiation of the brain: possible mechanisms and implications. *Can J Physiol Pharmacol*. 1985;63:577–594.
15. Rosenfield RL. Identifying children at risk of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2007;92:787–796.
16. Foecking EM, Szabo M, Schwartz NB, Levine JE. Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression, and acceleration of the gonadotropin-releasing hormone pulse generator. *Biol Reprod*. 2005;72:1475–1483.
17. Tapanainen J, Koivisto M, Vihko R, Huhtaniemi I. Enhanced activity of the pituitary-gonadal axis in premature human infants. *J Clin Endocrinol Metab*. 1981;52:235–238.
18. Kuiri-Hanninen T, Kallio S, Seuri R, et al. Postnatal developmental changes in the pituitary-ovarian axis in preterm and term infant girls. *J Clin Endocrinol Metab*. 2011;96:3432–3439.
19. Gulyas B, Hodgen G, Tullner W, et al. Effects of fetal or maternal hypophysectomy on endocrine organs and body weight in infant

- rhesus monkeys (*Macaca mulatto*): with particular emphasis on oogenesis. *Biol Reprod*. 1977;16:216.
20. Merrill J. Ovarian hilus cells. *Am J Obstet Gynecol*. 1959;78:1258.
 21. Carney JA. Unusual tumefactive spindle-cell lesions in the adrenal gland. *Hum Pathol*. 1987;18:980–985.
 22. Rabinovici J, Jaffe R. Development and regulation of growth and differentiated function in human and subhuman primate fetal gonads. *Endocr Rev*. 1990;11:532.
 23. Hummertsch K, Irving-Rodgers HF, Hatzirodos N, et al. A new model of development of the mammalian ovary and follicles. *PLoS One*. 2013;8. e55578.
 24. Cox JJ, Willatt L, Homfray T, Woods CG. A SOX9 duplication and familial 46,XX developmental testicular disorder. *N Engl J Med*. 2011;364:91–93.
 25. Nicol B, Yao HH. Gonadal identity in the absence of pro-testis factor SOX9 and pro-ovary factor beta-catenin in mice. *Biol Reprod*. 2015;93:35.
 26. Richards JS, Pangas SA. The ovary: basic biology and clinical implications. *J Clin Invest*. 2010;120:963–972.
 27. Chassot AA, Gillot I, Chaboissier MC. R-spondin1, WNT4, and the CTNBN1 signaling pathway: strict control over ovarian differentiation. *Reproduction*. 2014;148. R97–110.
 28. Lim HN, Hawkins JR. Genetic control of gonadal differentiation. *Baillière's Clin Endocrinol Metab*. 1998;12:1–16.
 29. Peters H. Migration of gonocytes into the mammalian gonad and their differentiation. *Philos Trans R Soc London (Biol)*. 1970;259:91.
 30. Baker T. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci*. 1963;158:417–433.
 31. Peters H, Byskov A, Grinstead J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab*. 1978;7:469.
 32. Payne AH, Jaffe RB. Androgen formation from pregnenolone sulfate by the human fetal ovary. *J Clin Endocrinol Metab*. 1974;39:300–304.
 33. George FW, Wilson JD. Conversion of androgen to estrogen by the human fetal ovary. *J Clin Endocrinol Metab*. 1978;47:550–555.
 34. Cole B, Hensinger K, Maciel GA, Chang RJ, Erickson GF. Human fetal ovary development involves the spatiotemporal expression of p450c17 protein. *J Clin Endocrinol Metab*. 2006;91:3654–3661.
 35. Fowler PA, Anderson RA, Saunders PT, et al. Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary. *J Clin Endocrinol Metab*. 2011;96:1754–1762.
 36. Ross GT, Schreiber JR. The ovary. In: Yen SSC, Jaffe R, eds. *Reproductive Endocrinology*. Philadelphia: WB Saunders Company; 1978.
 37. Peters H. The human ovary in childhood and early maturity. *Eur J Obstet Gynecol Reprod Biol*. 1979;9:137.
 38. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*. 1996;17:121–155.
 39. Yin O, Cayton K, Segars JH. In vitro activation: a dip into the primordial follicle pool? *J Clin Endocrinol Metab*. 2016;101:3568–3570.
 40. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev*. 2016;37:467–520.
 41. Carr D, Naggar R, Hart A. Germ cells in the ovaries of XO female infants. *Am J Clin Pathol*. 1968;19:521.
 42. Block E. A quantitative morphological investigation of the follicular system in newborn female infants. *Acta Anat*. 1953;17:201–206.
 43. Ellinwood WE, McClellan MC, Brenner RM, Resko JA. Estradiol synthesis by fetal monkey ovaries correlates with antral follicle formation. *Biol Reprod*. 1983;28:505–516.
 44. Zachos NC, Billiar RB, Albrecht ED, Pepe GJ. Developmental regulation of follicle-stimulating hormone receptor messenger RNA expression in the baboon fetal ovary. *Biol Reprod*. 2003;68:1911–1917.
 45. Richardson S, Senikas V, Nelson J. Follicular depletion during the menopausal transition: Evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab*. 1987;65:1231.
 46. Gartler S, Andina R, Cant N. Ontogeny of X chromosome inactivation in the female germ line. *Exp Cell Res*. 1975;91:454.
 47. Zinn AR. The X chromosome and the ovary. *J Soc Gynecol Investig*. 2001;8. S34–36.
 48. Liu C, Peng J, Matzuk MM, Yao HH. Lineage specification of ovarian theca cells requires multicellular interactions via oocyte and granulosa cells. *Nat Comm*. 2015;6:6934.
 49. Lee YM, Kumar BM, Lee JH, et al. Characterisation and differentiation of porcine ovarian theca-derived multipotent stem cells. *Vet J*. 2013;197:761–768.
 50. Schmidt D, Ovitt CE, Anlag K, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development*. 2004;131:933–942.
 51. Yang H, Yao X, Tang F, Wei Y, Hua J, Peng S. Characterization of female germline stem cells from adult mouse ovaries and the role of rapamycin on them. *Cytotechnology*. 2018;70:843–854.
 52. Klinger FG, Rossi V, De Felici M. Multifaceted programmed cell death in the mammalian fetal ovary. *Int J Dev Biol*. 2015;59:51–54.
 53. Robles R, Morita Y, Mann KK, et al. The aryl hydrocarbon receptor, a basic helix-loop-helix transcription factor of the PAS gene family, is required for normal ovarian germ cell dynamics in the mouse. *Endocrinology*. 2000;141:450–453.
 54. de Bruin JP, Dorland M, Bruinse HW, Spliet W, Nikkels PG, Te Velde ER. Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev*. 1998;51:39–46.
 55. Sir-Petermann T, Hittchfeld C, Codner E, et al. Gonadal function in low birth weight infants: a pilot study. *J Pediatr Endocrinol Metab*. 2007;20:405–414.
 56. Liew SH, Nguyen QN, Strasser A, Findlay JK, Hutt KJ. The ovarian reserve is depleted during puberty in a hormonally driven process dependent on the pro-apoptotic protein BMF. *Cell Death Dis*. 2017;8. e2971.
 57. Gagnon R. Fetal endocrine adaptation to placental insufficiency. *Endocrinologist*. 1998;8:436–442.
 58. Winter J, Faiman C. Pituitary-gonadal relations in female children and adolescents. *Pediatr Res*. 1973;7:948–953.
 59. Winter JSD, Hughes IA, Reyes FL, Faiman C. Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J Clin Endocrinol Metab*. 1976;42:679.
 60. Danon M, Velez O, Ostrea T, Crawford JD, Beitins IZ. Dynamics of bioactive luteinizing hormone-human chorionic gonadotropin during the first 7 days of life. *Pediatr Res*. 1988;23:530–533.
 61. Bangham DR, Berryman I, Burger H, et al. An international collaborative study of 69-104, a reference preparation of human pituitary FSH and LH. *J Clin Endocrinol Metab*. 1973;36:647–660.
 62. Greaves RF, Hunt RW, Chiriano AS, Zacharin MR. Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. *Pediatrics*. 2008;121. e574–580.
 63. Perlman M, Schenker J, Glassman M, Ben-david M. Prolonged hyperprolactinemia in preterm infants. *J Clin Endocrinol Metab*. 1978;47:894–897.
 64. Bouvattier C, Carel JC, Lecointre C, et al. Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the AR gene. *J Clin Endocrinol Metab*. 2002;87:29–32.
 65. Sullivan SD, Moenter SM. Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci U S A*. 2004;101:7129–7134.
 66. Bidlingmaier F, Knorr D. Oestrogens: physiological and clinical aspects. *Pediatr Adolesc Endocrinol*. 1978;4:43–84.
 67. Betend B, Claustrat B, Bizollon C, Ehre C, Francois R. Etude de la fonction gonadotrope hypophysaire par le test a la LH-RH pendant la premiere année de la vie. *Ann Endocrinol (Paris)*. 1975;36:325.
 68. Rosenfield RL, Burstein S, Cuttler L, et al. Use of nafarelin for testing pituitary-ovarian function. *J Reprod Med*. 1989;34:1044–1050.
 69. Chellakootty M, Schmidt IM, Haavisto AM, et al. Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. *J Clin Endocrinol Metab*. 2003;88:3515–3520.
 70. Conte FA, Grumbach MM, Kaplan SL, Reiter EO. Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients: Relation to the restraint of puberty. *J Clin Endocrinol Metab*. 1980;50:163–168.

71. Penny R, Olambiwonnu N, Frasier S. Serum gonadotropin concentrations during the first four years of life. *J Clin Endocrinol Metab.* 1974;38:320.
72. Elsholz DD, Padmanabhan V, Rosenfield RL, Olton PR, Phillips DJ, Foster CM. GnRH agonist stimulation of the pituitary-gonadal axis in children: age and sex differences in circulating inhibin-B and activin-A. *Hum Reprod.* 2004;19:2748–2758.
73. Bourguignon JP, Hoyoux C, Reuter A, Franchimont P. Urinary excretion of immunoreactive luteinizing hormone-releasing hormone-like material and gonadotropins at different stages of life. *J Clin Endocrinol Metab.* 1979;48:78–84.
74. Rosenfield RL. Hormonal events and disorders of puberty. In: Givens J, ed. *Gynecologic Endocrinology*. Chicago: Year Book Medical Publishers; 1977:1–19.
75. Attardi B, Ohno S. Androgen and estrogen receptors in the developing mouse brain. *Endocrinology.* 1976;99:1279.
76. Rifkind AB, Kulin HE, Ross GT. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the urine of prepubertal children. *J Clin Invest.* 1967;46:1925–1931.
77. Apter D, Butzow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab.* 1993;76:940–949.
78. Wilson ME, Fisher J, Chikazawa K. Estradiol negative feedback regulates nocturnal luteinizing hormone and follicle-stimulating hormone secretion in prepubertal female rhesus monkeys. *J Clin Endocrinol Metab.* 2004;89:3973–3978.
79. Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *J Clin Endocrinol Metab.* 2000;85:1074–1080.
80. Merrill JA. The morphology of the prepubertal ovary: relationship to the polycystic ovary syndrome. *Southern Med J.* 1963;56:225–231.
81. Kraus F, Neubecker R. Luteinization of the ovarian theca in infants and children. *Am J Clin Pathol.* 1962;37:389–397.
82. Stanhope R, Adams J, Jacobs H, Brook C. Ovarian ultrasound assessment in normal children, idiopathic precocious puberty, and during low dose pulsatile gonadotrophin releasing hormone treatment of hypogonadotropic hypogonadism. *Arch Dis Child.* 1985;60:116.
83. Bridges NA, Cooke A, Healy MJR, Hindmarsh PC, Brook CGD. Standards for ovarian volume in childhood and puberty. *Fertil Steril.* 1993;60:456–460.
84. Buzi F, Pilotta A, Dordoni D, Lombardi A, Zaglio S, Adlard P. Pelvic ultrasonography in normal girls and in girls with pubertal precocity. *Acta Paediatr.* 1998;87:1138–1145.
85. de Vries L, Horev G, Schwartz M, Phillip M. Ultrasonographic and clinical parameters for early differentiation between precocious puberty and premature thelarche. *Eur J Endocrinol.* 2006;154:891–898.
86. Badouraki M, Christoforidis A, Economou I, Dimitriadis AS, Katzos G. Evaluation of pelvic ultrasonography in the diagnosis and differentiation of various forms of sexual precocity in girls. *Ultrasound Obstet Gynecol.* 2008;32:819–827.
87. Dickerman Z, Prager-Lewin R, Laron Z. Response of plasma LH and FSH to synthetic LHRH in children at various pubertal stages. *Am J Dis Child.* 1976;130:634–638.
88. Rettig K, Duckett CE, Sweetland M, Reiter EO, Root AW. Urinary excretion of immunoreactive luteinizing hormone-releasing hormone-like material in children: Correlation with pubertal development. *J Clin Endocrinol Metab.* 1981;52:1150–1155.
89. Barnea A, Cho C, Porter J. A role for the ovaries in maturational processes of hypothalamic neurons containing luteinizing hormone-releasing hormone. *Endocrinology.* 1979;105:1303.
90. Knobil E. The neuroendocrine control of the menstrual cycle. *Recent Prog Horm Res.* 1980;36:53–88.
91. Marshall J, Kelch R. Gonadotropin-releasing hormone: role of pulsatile secretion in the regulation of reproduction. *N Engl J Med.* 1986;315:1459–1468.
92. Crowley Jr WF, Filicori M, Santoro NF. GnRH secretion across the normal menstrual cycle. In: Crowley WJ, Hofler J, eds. *The Episodic Secretion of Hormones*. New York: John Wiley/Churchill Livingstone; 1987:219–231.
93. Rosenfield RL, Bordini B, Yu C. Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. *J Clin Endocrinol Metab.* 2013;98:1591–1601.
94. Hansen JW, Hoffman HJ, Ross GT. Monthly gonadotropin cycles in premenarcheal girls. *Science.* 1975;190:161–163.
95. Collett-Solberg P, Grumbach M. A simplified procedure for evaluating estrogenic effects and the sex chromatin pattern in exfoliated cells in urine: Studies in premature thelarche and gynecomastia of adolescence. *J Pediatr.* 1965;66:883–990.
96. Kapen S, Boyar R, Finkelstein J, Hellman L, Weitzman E. Effect of sleep-wake cycle reversal on luteinizing hormone secretory pattern in puberty. *J Clin Endocrinol Metab.* 1974;39:293–299.
97. Norjavaara E, Ankargerg C, AAlbertsson-Wikland K. Diurnal rhythm of 17 β -estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay. *J Clin Endocrinol Metab.* 1996;81:4095–4102.
98. Boyar RM, Wu RHK, Roffwarg H, et al. Human puberty: 24-Hour estradiol patterns in pubertal girls. *J Clin Endocrinol Metab.* 1976;43:1418–1421.
99. Lucky AW, Rich BH, Rosenfield RL, Fang VS, Roche-Bender N, L.H. bioactivity increases more than immunoreactivity during puberty. *J Pediatr.* 1980;97:205.
100. Rosenfield RL, Helke J. Is an immunoassay available for the measurement of bioactive LH in serum? *J Androl.* 1992;13:1–10.
101. Beitins I, Padmanabhan V. Bioactivity of gonadotropins. *Endocrinol Metab Clin N Am.* 1991;20:85–120.
102. Legro RS, Lin HM, Demers LM, Lloyd T. Rapid maturation of the reproductive axis during perimenarche independent of body composition. *J Clin Endocrinol Metab.* 2000;85:1021–1025.
103. Avendaño MS, Vazquez MJ, Tena-Sempere M. Disentangling puberty: novel neuroendocrine pathways and mechanisms for the control of mammalian puberty. *Hum Reprod Update.* 2017;23(6):737–763.
104. Xia L, Van Vugt D, Alston E, Luckhaus J, Ferin M. A surge of gonadotropin-releasing hormone accompanies the estradiol-induced gonadotropin surge in the Rhesus monkey. *Endocrinology.* 1992;131:2812–2820.
105. Gasc JM, Baulieu EE. Regulation by estradiol of the progesterone receptor in the hypothalamus and pituitary: an immunohistochemical study in the chicken. *Endocrinology.* 1988;122:1357–1365.
106. Lin W, Ramirez V. Effect of pulsatile infusion of progesterone on the in vivo activity of the luteinizing hormone-releasing hormone neural apparatus of awake unrestrained female and male rabbits. *Endocrinology.* 1988;122:868.
107. Moll Jr GW, Rosenfield RL. Direct inhibitory effect of estradiol on pituitary luteinizing hormone responsiveness to luteinizing hormone releasing hormone is specific and of rapid onset. *Biol Reprod.* 1984;30:59.
108. Young JR, Jaffe RB. Strength-duration characteristics of estrogen effects on gonadotropin response to gonadotropin-releasing hormone in women. II. *J Clin Endocrinol Metab.* 1978;42:432–442.
109. March CM, Goebelsmann U, Nakamura RM, Mishell Jr DR. Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle-stimulating hormone surges. *J Clin Endocrinol Metab.* 1979;49:507–513.
110. Chang R, Jaffe R. Progesterone effects on gonadotropin release in women pretreated with estradiol. *J Clin Endocrinol Metab.* 1978;47:119–125.
111. Advis J, Krause J, McKelvy J. Evidence that endopeptidase-catalyzed luteinizing hormone releasing hormone cleavage contributes to the regulation of median eminence LHRH levels during positive steroid feedback. *Endocrinology.* 1983;112:1147–1149.
112. Harlow C, Shaw N, Hillier S, Hodges J. Factors influencing follicle-stimulating hormone-responsive steroidogenesis in marmoset granulosa cells: Effects of androgens and the stage of follicular maturity. *Endocrinology.* 1988;122:2780.
113. Richards J, Bogovich K. Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. *Endocrinology.* 1982;111:1429–1438.
114. Jia X-C, Kessel B, Welsh TNJ, Hsueh AJ. Androgen inhibition of follicle-stimulating hormone-stimulated luteinizing hormone

- receptor formation in cultured rat granulosa cells. *Endocrinology*. 1985;117:13–22.
115. Hsueh AJW, Adashi EY, Jones PBC, Welsh TNJ. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocrinol Rev*. 1984;5:76–127.
 116. Findlay J. Peripheral and local regulators of folliculogenesis. *Reprod Fertil Dev*. 1994;6:1–13.
 117. White SS, Ojeda SR. Changes in ovarian LHRH receptor content during the onset of puberty in the female rat. *Endocrinology*. 1981;108:347.
 118. Eckstein B, Shani J, Ravid R, Goldhaber G. Effect of androstenediol sulfates on luteinizing hormone release in ovariectomized rats. *Endocrinology*. 1981;108:500.
 119. Rovner P, Keltz J, Allshouse A, et al. Induction of the LH surge in premenarchal girls confirms early maturation of the hypothalamic-pituitary-ovarian axis. *Reprod Sci*. 2018;25:33–38.
 120. Aono T, Minagawa J, Kinugasa T, Tanizawa O, Kurachi K. Response of pituitary LH and FSH to synthetic LH-releasing hormone in normal subjects and patients with Sheehan's syndrome. *Am J Obstet Gynecol*. 1973;117:1046.
 121. Polhemus D. Ovarian maturation and cyst formation in children. *Pediatrics*. 1953;11:588–593.
 122. Adams J, Franks S, Polson DW, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. *Lancet*. 1985;326:1375–1379.
 123. Venturoli S, Porcu E, Fabbri R, et al. Longitudinal change of sonographic ovarian aspects and endocrine parameters in irregular cycles of adolescence. *Pediatr Res*. 1995;38:974–980.
 124. Rosenfield RL. The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*. 2015;136:1154–1165.
 125. Abraham G. Ovarian and adrenal contributions to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab*. 1974;39:340.
 126. Ross GT, Cargille CM, Lipsett MB, et al. Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Recent Prog Horm Res*. 1970;26:1–62.
 127. McCartney CR, Gingrich MB, Hu Y, Evans WS, Marshall JC. Hypothalamic regulation of cyclic ovulation: evidence that the increase in gonadotropin-releasing hormone pulse frequency during the follicular phase reflects the gradual loss of the restraining effects of progesterone. *J Clin Endocrinol Metab*. 2002;87:2194–2200.
 128. Shaw ND, Klingman KM, Srouji SS, Histed SN, Hall JE. Gonadotropin responses to estrogen-positive and -negative feedback are identical in African-American and Caucasian women. *J Clin Endocrinol Metab*. 2012;97. E106–109.
 129. McCartney CR, Blank SK, Marshall JC. Progesterone acutely increases LH pulse amplitude but does not acutely influence nocturnal LH pulse frequency slowing during the late follicular phase in women. *Am J Physiol Endocrinol Metab*. 2007;292. E900–606.
 130. Johnson D, Naqvi R. A positive feedback action of androgen on pituitary follicle stimulating hormone: induction of a cyclic phenomenon. *Endocrinology*. 1987;85:881.
 131. Melrose P, Gross R. Steroid effects on the secretory modalities of gonadotropin-releasing hormone release. *Endocrinology*. 1987;121:190.
 132. Soules M, Steiner R, Clifton D, Cohen N, Aksel S, Bremner W. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. *J Clin Endocrinol Metab*. 1984;58:378–383.
 133. McGee E, Hsueh A. Initial and cyclic recruitment of ovarian follicles. *Endrocr Rev*. 2000;21:200–214.
 134. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: A review of structure/function relationships. *Endocr Rev*. 1985;6:371–399.
 135. Richards J. Hormonal control of gene expression in the ovary. *Endocr Rev*. 1994;15:725.
 136. Li J, Kawamura K, Cheng Y, et al. Activation of dormant ovarian follicles to generate mature eggs. *Proc Natl Acad Sci U S A*. 2010;107:10280–10284.
 137. Ross C. Gonadotropins and preantral follicular maturation in women. *Fertil Steril*. 1974;25:52.
 138. Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat Genet*. 1997;15:201–204.
 139. Barnes RB, Namnoum AB, Rosenfield RL, Layman LC. The role of LH and FSH in ovarian androgen secretion and ovarian follicular development: Clinical studies in a patient with isolated FSH deficiency and multicystic ovaries: Case report. *Hum Reprod*. 2002;17:88–91.
 140. Meduri G, Touraine P, Beau I, et al. Delayed puberty and primary amenorrhea associated with a novel mutation of the human follicle-stimulating hormone receptor: clinical, histological, and molecular studies. *J Clin Endocrinol Metab*. 2003;88:3491–3498.
 141. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab*. 1999;84:2951–2956.
 142. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest*. 1998;101:2622–2629.
 143. Shiina H, Matsumoto T, Sato T, et al. Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci U S A*. 2006;103:224–229.
 144. Ehrmann DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*. 1995;16:322–353.
 145. Inkster S, Brodie A. Expression of aromatase cytochrome P-450 in premenopausal and postmenopausal human ovaries: an immunocytochemical study. *J Clin Endocrinol Metab*. 1991;73:717–726.
 146. McNatty KP, Makris A, Reinhold VN, De Grazia C, Osathanondh R, Ryan KJ. Metabolism of androstenedione by human ovarian tissues in vitro with particular reference to reductase and aromatase activity. *Steroids*. 1979;34:429–443.
 147. McNatty KP, Smith DM, Makris A, Osathanondh R, Ryan KJ. The microenvironment of the human antral follicle: Interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab*. 1979;49:851–860.
 148. Erickson GF, Hsueh AJ, Quigley ME, Rebar RW, Yen SS. Functional studies of aromatase activity in human granulosa cells from normal and polycystic ovaries. *J Clin Endocrinol Metab*. 1979;49:514–519.
 149. Tsang B, Armstrong D, Whitfield J. Steroid biosynthesis by isolated human ovarian follicular cells in vitro. *J Clin Endocrinol Metab*. 1980;51:1407–1411.
 150. Mason HD, Willis DS, Beard RW, Winston RM, Margara R, Franks S. Estradiol production by granulosa cells of normal and polycystic ovaries: relationship to menstrual cycle history and concentrations of gonadotropins and sex steroids in follicular fluid. *J Clin Endocrinol Metab*. 1994;79:1355–1360.
 151. Haning Jr RV, Hackett RJ, Flood CA, Loughlin JS, Zhao QY, Longcope C. Testosterone, a follicular regulator: key to anovulation. *J Clin Endocrinol Metab*. 1993;77:710–715.
 152. Kamitis V, Townson D, Friedman C, Danforth D. Recombinant human follicle-stimulating hormone stimulates multiple follicular growth, but minimal estrogen production in gonadotropin-releasing hormone antagonist-treated monkeys: examining the role of luteinizing hormone in follicular development and steroidogenesis. *J Clin Endocrinol Metab*. 1994;79:91–97.
 153. Zhou J, Kumar R, Matzuk MM, Bondy C. Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. *Mol Endocrinol*. 1997;11:1924–1933.
 154. McNatty KP, Hunter WM, MacNeilly AS, Sawers RS. Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human Graafian follicles throughout the menstrual cycle. *J Endocrinol*. 1975;64:555.
 155. McNatty KP, Makris A, De Grazia C, Osathanondh R, Ryan KJ. Steroidogenesis by recombined follicular cells from the human ovary in vitro. *J Clin Endocrinol Metab*. 1980;51:1286.
 156. Couse JF, Hewitt SC, Bunch DO, et al. Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science*. 1999;286:2328–2386.
 157. Lofrano-Porto A, Barra GB, Giacomini LA, et al. Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med*. 2007;357:897–904.
 158. Shoham Z, Balen A, Patel A, Jacobs HS. Results of ovulation induction using human menopausal gonadotropin or purified

- follicle-stimulating hormone in hypogonadotropic hypogonadism patients. *Fertil Steril*. 1991;56:1048–1053.
159. Schoot D, Bennink H, Mannaerts B, Lamberts S, Bouchard P, Fauser B. Human recombinant follicle-stimulating hormone induces growth of preovulatory follicles without concomitant increase in androgen and estrogen biosynthesis in a woman with isolated gonadotropin deficiency. *J Clin Endocrinol Metab*. 1992;74:1471–1473.
 160. Richards JS, Russell DL, Ochsner S, et al. Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. *Recent Prog Horm Res*. 2002;57:195–220.
 161. Radu A, Pichon C, Camparo P, et al. Expression of follicle-stimulating hormone receptor in tumor blood vessels. *N Engl J Med*. 2010;363:1621–1630.
 162. Jonassen J, Bose K, Richards J. Enhancement and desensitization of hormone-responsive adenylate cyclase in granulosa cells of preantral and antral ovarian follicles: Effects of estradiol and follicle-stimulating hormone. *Endocrinology*. 1982;111:74.
 163. Masciarelli S, Horner K, Liu C, et al. Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. *J Clin Invest*. 2004;114:196–205.
 164. Lindner HR, Tsafirri A, Lieberman ME, et al. Gonadotropin action on cultured Graafian follicles: Induction of maturation division of the mammalian oocyte and differentiation of the luteal cell. *Recent Prog Horm Res*. 1974;30:79.
 165. Moor RM, Dai Y, Lee C, Fulka Jr J. Oocyte maturation and embryonic failure. *Hum Reprod Update*. 1998;4:223–236.
 166. Tsafirri A, Cao X, Ashkenazi H, Motola S, Popliker M, Pomerantz SH. Resumption of oocyte meiosis in mammals: on models, meiosis activating sterols, steroids and EGF-like factors. *Mol Cell Endocrinol*. 2005;234:37–45.
 167. Chaffkin L, Luciano A, Peluso J. The role of progesterone in regulating human granulosa cell proliferation and differentiation in vitro. *J Clin Endocrinol Metab*. 1993;76:696–700.
 168. Schreiber J, Nakamura K, Truscella A, Erickson G. Progestins inhibit FSH-induced functional LH receptors in cultured rat granulosa cells. *Mol Cell Endocrinol*. 1982;25:113.
 169. Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev*. 1997;18:71–106.
 170. Couse JF, Bunch DO, Lindzey J, Schomberg DW, Korach KS. Prevention of the polycystic ovarian phenotype and characterization of ovulatory capacity in the estrogen receptor-alpha knockout mouse. *Endocrinology*. 1999;140:5855–5865.
 171. Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? [published erratum appears in *Endocr Rev*, 20(4), 459]. *Endocr Rev*. 1999;20:358–417.
 172. Cheng G, Weihua Z, Makinen S, et al. A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary. *Biol Reprod*. 2000;66:77–84.
 173. Gorsic LK, Dapas M, Legro RS, Hayes MG, Urbaneck M. Functional genetic variation in the anti-Mullerian hormone pathway in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2019;104:2855–2874.
 174. Catteau-Jonard S, Pigny P, Reyss AC, Decanter C, Poncelet E, Dewailly D. Changes in serum anti-Mullerian hormone level during low-dose recombinant follicular-stimulating hormone therapy for anovulation in polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2007;92:4138–4143.
 175. Grynberg M, Pierre A, Rey R, et al. Differential regulation of ovarian anti-mullerian hormone (AMH) by estradiol through alpha- and beta-estrogen receptors. *J Clin Endocrinol Metab*. 2012;97:E1649–1657.
 176. Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update*. 2014;20:370–385 [Erratum in: *Hum Reprod Update*, 2014, 20(5), 804].
 177. Burger HG, Groome NP, Robertson DM. Both inhibin A and B respond to exogenous follicle-stimulating hormone in the follicular phase of the human menstrual cycle. *J Clin Endocrinol Metab*. 1998;83:4167–4179.
 178. Welt CK, Smith ZA, Pauler DK, Hall JE. Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. *J Clin Endocrinol Metab*. 2001;86:2531–2537.
 179. Hirshfeld-Cytron J, Barnes RB, Ehrmann DA, Caruso A, Mortensen MM, Rosenfield RL. Characterization of functionally typical and atypical types of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2009;94:1587–1594.
 180. Pru JK, Tilly JL. Programmed cell death in the ovary: insights and future prospects using genetic technologies. *Mol Endocrinol*. 2001;15:845–853.
 181. Richards J, Hegin L, Caston L. Differentiation of rat ovarian thecal cells: evidence for functional luteinization. *Endocrinology*. 1986;118:1660–1668.
 182. Auletta F, Flint A. Mechanisms controlling corpus luteum function in sheep, cows, nonhuman primates, and women especially in relation to the time of luteolysis. *Endocr Rev*. 1988;9:88.
 183. Vande Wiele RL, Bogumil J, Dyrenfurth I, et al. Mechanisms regulating the menstrual cycle in women. *Recent Prog Horm Res*. 1970;1970(26):63.
 184. Patel SS, Beshay VE, Escobar JC, Suzuki T, Carr BR. Molecular mechanism for repression of 17alpha-hydroxylase expression and androstenedione production in granulosa cells. *J Clin Endocrinol Metab*. 2009;94:5163–5168.
 185. Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstet Gynecol*. 1996;87:13–17.
 186. Zhang K, Pollack S, Ghods A, et al. Onset of ovulation after menarche in girls: a longitudinal study. *J Clin Endocrinol Metab*. 2008;93:1186–1194.
 187. Lynch KE, Mumford SL, Schliep KC, et al. Assessment of anovulation in eumenorrheic women: comparison of ovulation detection algorithms. *Fertil Steril*. 2014;102:511–518. e2.
 188. Moghissi KS, Syner FN, Evans TN. A composite picture of the menstrual cycle. *Am J Obstet Gynecol*. 1972;114:405–418.
 189. Zhang B, Roth R. The insulin receptor-related receptor. *J Biol Chem*. 1992;267:18320–18328.
 190. Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev*. 2003;24:668–693.
 191. Massart F, Saggese G. Oestrogenic mycotoxin exposures and precocious pubertal development. *Int J Androl*. 2010;33:369–376.
 192. Jacobson-Dickman E, Lee MM. The influence of endocrine disruptors on pubertal timing. *Curr Opin Endocrinol Diabetes Obes*. 2009;16:25–30.
 193. Deboer MD, Steinman J, Li Y. Partial normalization of pubertal timing in female mice with DSS colitis treated with anti-TNF-alpha antibody. *J Gastroenterol*. 2012;47(6):647–654.
 194. Fischbein S. Intra-pair similarity in physical growth of monozygotic and of dizygotic twins during puberty. *Ann Hum Biol*. 1977;4:417–430.
 195. Garn SM, Bailey SM. Genetics and maturational processes. In: Falkner F, Tanner JM, eds. *Human Growth 1: Principles and Prenatal Growth*. NY: Plenum; 1978:307–330.
 196. Sharma JC. The genetic contribution to pubertal growth and development studied by longitudinal growth data on twins. *Ann Hum Biol*. 1983;10:163–171.
 197. Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M, Rose RJ. Common genetic influences on BMI and age at menarche. *Hum Biol*. 1995;67:739–753.
 198. Sun SS, Schubert CM, Chumlea WC, et al. National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics*. 2002;110:911–919.
 199. Chumlea WC, Schubert CM, Roche AF, et al. Age at menarche and racial comparisons in US girls. *Pediatrics*. 2003;111:110–113.
 200. Sedlmeyer IL, Hirschhorn JN, Palmert MR. Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab*. 2002;87:5581–5586.
 201. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab*. 2002;87:1613–1620.
 202. Eaves L, Silberg J, Foley D, et al. Genetic and environmental influences on the relative timing of pubertal change. *Twin Res*. 2004;7:471–481.
 203. Herman-Giddens ME, Kaplowitz PB, Wasserman R. Navigating the recent articles on girls' puberty in Pediatrics: what do we know and where do we go from here? *Pediatrics*. 2004;113:911–917.

204. Nathan BM, Hodges CA, Palmert MR. The use of mouse chromosome substitution strains to investigate the genetic regulation of pubertal timing. *Mol Cell Endocrinol.* 2006;254–255:103–108.
205. Ong KK, Elks CE, Li S, et al. Genetic variation in LIN28B is associated with the timing of puberty. *Nat Genet.* 2009;41:729–733.
206. Perry JR, Day F, Elks CE, et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. *Nature.* 2014;514:92–97.
207. Elks CE, Perry JR, Sulem P, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.* 2010;42:1077–1085.
208. Perry JR, Stolk L, Franceschini N, et al. Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat Genet.* 2009;41:648–650.
209. Tommiska J, Wehkalampi K, Vaaralahti K, Laitinen EM, Raivio T, Dunkel L. LIN28B in constitutional delay of growth and puberty. *J Clin Endocrinol Metab.* 2010;95:3063–3066.
210. Silveira-Neto AP, Leal LF, Emerman AB, et al. Absence of functional LIN28B mutations in a large cohort of patients with idiopathic central precocious puberty. *Horm Res Paediatr.* 2012;78:144–150.
211. Day FR, Thompson DJ, Helgason H, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet.* 2017;49:834–841.
212. Franco B, Guioli S, Pragliola A, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature.* 1991;353:529–536.
213. Seminara SB. Mechanisms of Disease: the first kiss—a crucial role for kisspeptin-1 and its receptor, G-protein-coupled receptor 54, in puberty and reproduction. *Nat Clin Pract Endocrinol Metab.* 2006;2:328–334.
214. Pitteloud N, Meysing A, Quinton R, et al. Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol.* 2006;254–255:60–69.
215. Pfaeffle RW, Savage JJ, Hunter CS, et al. Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *J Clin Endocrinol Metab.* 2007;92:1909–1919.
216. Sedlmeyer IL, Pearce CL, Trueman JA, et al. Determination of sequence variation and haplotype structure for the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes: investigation of role in pubertal timing. *J Clin Endocrinol Metab.* 2005;90:1091–1099.
217. Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev.* 2001;22:111–151.
218. Plant TM, Barker-Gibb ML. Neurobiological mechanisms of puberty in higher primates. *Hum Reprod Update.* 2004;10:67–77.
219. Dubois SL, Wolfe A, Radovick S, Boehm U, Levine JE. Estradiol restrains prepubertal gonadotropin secretion in female mice via activation of eralpha in kisspeptin neurons. *Endocrinology.* 2016;157:1546–1554.
220. Ojeda SR, Urbanski HF. Puberty in the rat. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction.* NY: Raven Press; 1994: 363–407.
221. Plant TM, Witchel SF. Puberty in non-human primates and humans. In: Challis JRC, de Kretser DM, Neill JD, et al., eds., *Knobil and Neill's Physiology of Reproduction.* 3 ed. NY: Elsevier; 2006: 2177–2230.
222. Seminara SB, Messenger S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med.* 2003;349:1614–1627.
223. Novaira HJ, Sonko ML, Hoffman G, et al. Disrupted kisspeptin signaling in GnRH neurons leads to hypogonadotropic hypogonadism. *Mol Endocrinol (Baltimore, Md).* 2014;28:225–238.
224. Morelli A, Marini M, Mancina R, et al. Sex steroids and leptin regulate the “first Kiss” (KiSS 1/G-protein-coupled receptor 54 system) in human gonadotropin-releasing-hormone-secreting neuroblasts. *J Sex Med.* 2008;5:1097–1113.
225. Semple RK, Achermann JC, Ellery J, et al. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2005;90:1849–1855.
226. Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M. Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. *Eur J Endocrinol.* 2005;153:845–852.
227. Navarro VM, Fernandez-Fernandez R, Castellano JM, et al. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *J Physiol.* 2004;561:379–386.
228. Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A.* 2005;102:2129–2134.
229. Bianco SD. A potential mechanism for the sexual dimorphism in the onset of puberty and incidence of idiopathic central precocious puberty in children: sex-specific kisspeptin as an integrator of puberty signals. *Front Endocrinol.* 2012;3:149.
230. Castellano JM, Navarro VM, Fernandez-Fernandez R, et al. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology.* 2005;146:3917–3925.
231. Navarro VM, Tena-Sempere M. Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nat Rev Endocrinol.* 2011;8:40–53.
232. Han SK, Gottsch ML, Lee KJ, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci.* 2005;25: 11349–11356.
233. Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB. Kisspeptin administration to women: A window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. *J Clin Endocrinol Metab.* 2012;97: E1458–1467.
234. Chan YM, Broder-Fingert S, Wong KM, Seminara SB. Kisspeptin/Gpr54-independent gonadotropin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. *J Neuroendocrinol.* 2009;21: 1015–1023.
235. Gianetti E, Tusset C, Noel SD, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab.* 2010;95:2857–2867.
236. d'Anglemont de Tassigny, X, Colledge WH. The role of kisspeptin signaling in reproduction. *Physiology (Bethesda).* 2010;25: 207–217.
237. de Croft S, Boehm U, Herbison AE. Neurokinin B activates arcuate kisspeptin neurons through multiple tachykinin receptors in the male mouse. *Endocrinology.* 2013;154:2750–2760.
238. Topaloglu AK, Reimann F, Guclu M, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet.* 2009;41:354–358.
239. Garcia JP, Guerriero KA, Keen KL, Kenealy BP, Seminara SB, Terasawa E. Kisspeptin and neurokinin B signaling network underlies the pubertal increase in GnRH release in female rhesus monkeys. *Endocrinology.* 2017;158:3269–3280.
240. Terasawa E, Garcia JP, Seminara SB, Keen KL. Role of Kisspeptin and Neurokinin B in Puberty in Female Non-Human Primates. *Front Endocrinol.* 2018;9:148.
241. Narayanaswamy S, Prague JK, Jayasena CN, et al. Investigating the KNDy Hypothesis in Humans by Coadministration of Kisspeptin, Neurokinin B, and Naltrexone in Men. *J Clin Endocrinol Metab.* 2016;101:3429–3436.
242. Sandoval-Guzman T, Rance NE. Central injection of senktide, an NK3 receptor agonist, or neuropeptide Y inhibits LH secretion and induces different patterns of Fos expression in the rat hypothalamus. *Brain Res.* 2004;1026:307–312.
243. Kung TT, Crawley Y, Jones H, et al. Tachykinin NK3-receptor deficiency does not inhibit pulmonary eosinophilia in allergic mice. *Pharmacol Res.* 2004;50:611–615.
244. Yang L, Zhang SX, Dong Q, Xiong ZB, Li X. Application of hormonal treatment in hypogonadotropic hypogonadism: more than ten years experience. *Intern Urol Nephrol.* 2012;44: 393–399.
245. Tusset C, Noel SD, Trarbach EB, et al. Mutational analysis of TAC3 and TACR3 genes in patients with idiopathic central pubertal disorders. *Arq Bras Endocrinol Metabol.* 2012;56:646–652.

246. Abreu AP, Dauber A, Macedo DB, et al. Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med*. 2013;368:2467–2475.
247. Ojeda SR, Dubay C, Lomniczi A, et al. Gene networks and the neuroendocrine regulation of puberty. *Mol Cell Endocrinol*. 2010;324:3–11.
248. Mitsushima D, Hei DL, Terasawa E. gamma-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci U S A*. 1994;91:395–399.
249. Ojeda SR, Lomniczi A, Mastronardi C, et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology*. 2006;147:1166–1174.
250. Ducret E, Anderson GM, Herbison AE. RFamide-related peptide-3, a mammalian gonadotropin-inhibitory hormone ortholog, regulates gonadotropin-releasing hormone neuron firing in the mouse. *Endocrinology*. 2009;150:2799–2804.
251. Clarkson J, Herbison AE. Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty. *Mol Cell Endocrinol*. 2006;254–255:32–38.
252. Ojeda SR, Terasawa E. Neuroendocrine regulation of puberty. In: Pfaff D, et al. eds. *Hormones, Brain and Behavior*. New York: Elsevier; 2002:589–659.
253. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol*. 2002;16:2872–2891.
254. Ojeda SR, Lomniczi A, Sandau US. Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. *J Neuroendocrinol*. 2008;20:732–742.
255. Mueller JK, Koch I, Lomniczi A, et al. Transcription of the human EAP1 gene is regulated by upstream components of a puberty-controlling Tumor Suppressor Gene network. *Mol Cell Endocrinol*. 2012;351:184–198.
256. Partsch CJ, Dreyer G, Gosch A, et al. Longitudinal evaluation of growth, puberty, and bone maturation in children with Williams syndrome. *J Pediatr*. 1999;134:82–89.
257. Messina A, Langlet F, Chachlaki K, et al. A microRNA switch regulates the rise in hypothalamic GnRH production before puberty. *Nat Neurosci*. 2016;19:835–844.
258. Ahmed K, LaPierre MP, Gasser E, et al. Loss of microRNA-7a2 induces hypogonadotropic hypogonadism and infertility. *J Clin Invest*. 2017;127:1061–1074.
259. Terasawa E, Noonan J, Nass T, Loose M. Posterior hypothalamic lesions advance the onset of puberty in the female rhesus monkey. *Endocrinology*. 1984;115:2241.
260. Mayer C, Acosta-Martinez M, Dubois SL, et al. Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. *Proc Natl Acad Sci U S A*. 2010;107:22693–22698.
261. Rance NE. Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. *Peptides*. 2009;30:111–122.
262. Dungan HM, Clifton DK, Steiner RA. Minireview: kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. *Endocrinology*. 2006;147:1154–1158.
263. Taziaux M, Staphorsius AS, Ghatei MA, Bloom SR, Swaab DF, Bakker J. Kisspeptin expression in the human infundibular nucleus in relation to sex, gender identity, and sexual orientation. *J Clin Endocrinol Metab*. 2016;101:2380–2389.
264. Pescovitz OH, Comite F, Cassorla F, et al. True precocious puberty complicating congenital adrenal hyperplasia: Treatment with a luteinizing hormone-releasing hormone analog. *J Clin Endocrinol Metab*. 1984;58:857.
265. Foster C, Comite F, Pescovitz O, Ross J, Loriaux D, Cutler CJ. Variable response to a long-acting agonist of luteinizing hormone-releasing hormone in girls with McCune-Albright syndrome. *J Clin Endocrinol Metab*. 1984;59:801.
266. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocr Rev*. 2015;36:E1–e150.
267. Doherty LF, Bromer JG, Zhou Y, Aldad TS, Taylor HS. In utero exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer. *Horm Cancer*. 2010;1:146–155.
268. Wilson ME, Tanner JM. Somatostatin analog treatment slows growth and the tempo of reproductive maturation in female rhesus monkeys. *J Clin Endocrinol Metab*. 1994;79:495–501.
269. Hiney JK, Srivastava V, Nyberg CL, Ojeda SR, Dees WL. Insulin-like growth factor I of peripheral origin acts centrally to accelerate the initiation of female puberty. *Endocrinology*. 1996;137:3717–3728.
270. Childs GV. Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. *Trends Endocrinol Metab*. 2000;11:168–175.
271. Marshall W. Interrelationships of skeletal maturation, sexual development and somatic growth in man. *Ann Human Biol*. 1974;1:29.
272. Simmons K, Greulich W. Menarcheal age and the height, weight, and skeletal age of girls age 7 to 17 years. *J Pediatr*. 1943;22:518.
273. Boyar RM, Finkelstein JW, David R, et al. Twenty-four hour patterns of plasma luteinizing hormone and follicle-stimulating hormone in sexual precocity. *N Engl J Med*. 1973;289:282–286.
274. Tanner J, Whitehouse R. A note on the bone age at which patients with true isolated growth hormone deficiency enter puberty. *J Clin Endocrinol Metab*. 1975;41:788.
275. Frisch R. Body fat, puberty, and fertility. *Biol Rev Camb Philos Soc*. 1984;59:161–188.
276. Rosenfield RL, Lipton RB, Drum ML. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. *Pediatrics*. 2009;123:84–88 [Erratum in: *Pediatrics*, 123(4), 1255].
277. Thankamony A, Ong KK, Ahmed ML, Ness AR, Holly JM, Dunger DB. Higher levels of IGF-I and adrenal androgens at age 8 years are associated with earlier age at menarche in girls. *J Clin Endocrinol Metab*. 2012;97: E786–790.
278. Rosenbaum M, Leibel RL. The role of leptin in human physiology [editorial; comment]. *N Engl J Med*. 1999;341:913–915.
279. Ahima RS, Kelly J, Elmquist JK, Flier JS. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia [see comments]. *Endocrinology*. 1999;140:4923–4931.
280. Tortoriello DV, McMinn JE, Chua SC. Increased expression of hypothalamic leptin receptor and adiponectin accompany resistance to dietary-induced obesity and infertility in female C57BL/6J mice. *Int J Obes*. 2007;31:395–402.
281. Elias CF. Leptin action in pubertal development: recent advances and unanswered questions. *Trends Endocrinol Metab*. 2012;23:9–15.
282. Mann DR, Johnson AO, Gimpel T, Castracane VD. Changes in circulating leptin, leptin receptor, and gonadal hormones from infancy until advanced age in humans. *J Clin Endocrinol Metab*. 2003;88:3339–3345.
283. Roemmich JN, Clark PA, Berr SS, et al. Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. *Am J Physiol*. 1998;275: E543–551.
284. Donato Jr J, Cravo RM, Frazao R, et al. Leptin's effect on puberty in mice is relayed by the ventral preammillary nucleus and does not require signaling in Kiss1 neurons. *J Clin Invest*. 2011;121:355–368.
285. Schwartz J, Cherny R. Intercellular communication within the anterior pituitary influencing the secretion of hypophysial hormones. *Endocr Rev*. 2011;13:453.
286. Evans JJ. Modulation of gonadotropin levels by peptides acting at the anterior pituitary gland. *Endocr Rev*. 1999;20:46–67.
287. Campbell RE, French-Mullen JM, Cowley MA, Smith MS, Grove KL. Hypothalamic circuitry of neuropeptide Y regulation of neuroendocrine function and food intake via the Y5 receptor subtype. *Neuroendocrinology*. 2001;74:106–119.
288. Lloyd B, Ravi P, Mendes N, Klibanski A, Misra M. Peptide YY levels across pubertal stages and associations with growth hormone. *J Clin Endocrinol Metab*. 2010;95:2957–2962.
289. Qiu X, Dao H, Wang M, et al. Insulin and leptin signaling interact in the mouse kiss1 neuron during the peripubertal period. *PLoS One*. 2015;10: e0121974.
290. Howard SR, Guasti L, Poliandri A, et al. Contributions of function-altering variants in genes implicated in pubertal timing and body mass for self-limited delayed puberty. *J Clin Endocrinol Metab*. 2018;103:649–659.

291. Manfredi-Lozano M, Roa J, Ruiz-Pino F, et al. Defining a novel leptin-melanocortin-kisspeptin pathway involved in the metabolic control of puberty. *Mol Metabol.* 2016;5:844–857.
292. Pomerants T, Tillmann V, Jurimae J, Jurimae T. Relationship between ghrelin and anthropometrical, body composition parameters and testosterone levels in boys at different stages of puberty. *J Endocrinol Invest.* 2006;29:962–967.
293. Fernandez-Fernandez R, Tena-Sempere M, Navarro VM, et al. Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: in vivo and in vitro studies. *Neuroendocrinology.* 2005;82:245–255.
294. Pugliese-Pires PN, Fortin JP, Arthur T, et al. Novel inactivating mutations in the GH secretagogue receptor gene in patients with constitutional delay of growth and puberty. *Eur J Endocrinol.* 2011;165:233–241.
295. Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab.* 2003;88:297–311.
296. Kluge M, Schussler P, Schmidt D, Uhr M, Steiger A. Ghrelin suppresses secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in women. *J Clin Endocrinol Metab.* 2012;97: E448–451.
297. Bruning JC, Gautam D, Burks DJ, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science.* 2000;289:2122–2125.
298. Reiter R. The pineal and its hormones in the control of reproduction in mammals. *Endocrinology.* 1980;1:109.
299. Brzezinski A. Melatonin in humans. *N Engl J Med.* 1997;336:186–195.
300. Rosenfield RL, Fang VS. The effects of prolonged physiologic estradiol therapy on the maturation of hypogonadal teenagers. *J Pediatr.* 1974;85:830–837.
301. Wray S, Hoffman G. Postnatal morphological changes in rat LHRH neurons correlated with sexual maturation. *Neuroendocrinology.* 1986;43:93–97.
302. Cottrell EC, Campbell RE, Han SK, Herbison AE. Postnatal remodeling of dendritic structure and spine density in gonadotropin-releasing hormone neurons. *Endocrinology.* 2006;147:3652–3661.
303. Plant TM. Gonadotropin-releasing hormone neuron remodeling: causal for puberty onset? *Trends Endocrinol Metab.* 2007;18:50–51.
304. Terasawa E. Postnatal remodeling of gonadotropin-releasing hormone I neurons: toward understanding the mechanism of the onset of puberty. *Endocrinology.* 2006;147:3650–3651.
305. Song WJ, Mondal P, Wolfe A, et al. Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell Metab.* 2014;19:667–681.
306. Bhattacharya M, Babwah AV. Kisspeptin: beyond the brain. *Endocrinology.* 2015;156:1218–1227.
307. Meriggiola M, Dahl K, Mather J, Bremner W. Follistatin decreases activin-stimulated FSH secretion with no effect on GnRH-stimulated FSH secretion in prepubertal male monkeys. *Endocrinology.* 1994;134:1967–1970.
308. Rosenfield RL, Bordini B. Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. *Brain Res.* 2010;1364:186–197.
309. Shaw ND, Histed SN, Srouji SS, Yang J, Lee H, Hall JE. Estrogen negative feedback on gonadotropin secretion: evidence for a direct pituitary effect in women. *J Clin Endocrinol Metab.* 2010;95:1955–1961.
310. Levine JE. Editorial: pulsatility in primates—a perspective from the placode. *Endocrinology.* 1999;140:1033–1035.
311. Wuttke W, Leonhardt S, Jarry H, Lopez P, Hirsch B. Involvement of catecholamines and amino acid neurotransmitters in the generation of GnRH pulses. In: Rosmanith W, Scherbaum W, eds. *New Developments in Biosciences 6: Neuroendocrinology of Sex Steroids, Basic Knowledge and Clinical Implications.* Berlin: de Gruyter; 1992:109–123.
312. Favit A, Wetsel W, Negro-Vilar A. Differential expression of g-aminobutyric acid receptors in immortalized luteinizing hormone-releasing hormone neurons. *Endocrinology.* 1993;133:1983–1989.
313. Dissen GA, Lomniczi A, Heger S, Neff TL, Ojeda SR. Hypothalamic EAP1 (enhanced at puberty 1) is required for menstrual cyclicity in nonhuman primates. *Endocrinology.* 2012;153:350–361.
314. Chappell PE, Lee J, Levine JE. Stimulation of gonadotropin-releasing hormone surges by estrogen. II. Role of cyclic adenosine 3', 5'-monophosphate. *Endocrinology.* 2000;141:1486–1492.
315. Radovick S, Ticknor CM, Nakayama Y, et al. Evidence for direct estrogen regulation of the human gonadotropin-releasing hormone gene. *J Clin Invest.* 1991;88:1649–1655.
316. Roy D, Angelini NL, Belsham DD. Estrogen directly represses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor- α (ER α)- and ER β -expressing GT1-7 GnRH neurons. *Endocrinology.* 1999;140:5045–5053.
317. Kupa JK, Jacobsen BM, Boen EA, et al. Direct binding of progesterone receptor to nonconsensus DNA sequences represses rat GnRH. *Mol Cell Endocrinol.* 1996;117:27–39.
318. Couzinet B, Young J, Kujas M, et al. The antigonadotropic activity of a 19-nor-progesterone derivative is exerted both at the hypothalamic and pituitary levels in women. *J Clin Endocrinol Metab.* 1999;84:4191–4196.
319. Wildt L, Hutchison JS, Marshall G, Pohl CR, Knobil E. On the site of action of progesterone in the blockade of the estradiol-induced gonadotropin discharge in the rhesus monkey. *Endocrinology.* 1981;109:1293–1294.
320. Garcia A, Herbon L, Barkan A, Papavasiliou S, Marshall J. Hyperprolactinemia inhibits gonadotropin-releasing hormone (GnRH) stimulation of the number of pituitary GnRH receptors. *Endocrinology.* 1985;117:954.
321. Milenkovic L, D'Angelo G, Kelly PA, Weiner RI. Inhibition of gonadotropin hormone-releasing hormone release by prolactin from GT1 neuronal cell lines through prolactin receptors. *Proc Natl Acad Sci U S A.* 1994;91:1244–1247.
322. Shaw ND, Gill S, Lavoie HB, Marsh EE, Hall JE. Persistence of sleep-associated decrease in GnRH pulse frequency in the absence of gonadal steroids. *J Clin Endocrinol Metab.* 2011;96:2590–2595.
323. Petraglia F, Sutton S, Vale W, Vale W, Plotsky P. Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophysial-portal circulation. *Endocrinology.* 1987;120:1083–1088.
324. Rivest S, Lee S, Attardi B, Rivier C. The chronic intracerebroventricular infusion of interleukin- β alters the activity of the hypothalamic-pituitary-gonadal axis of cycling rats. I. Effect on LHRH and gonadotropin biosynthesis and secretion. *Endocrinology.* 1993;133:2424–2430.
325. Vitale M, Chiochio S. Serotonin, a neurotransmitter involved in the regulation of luteinizing hormone release. *Endocr Rev.* 1993;14:480.
326. Conn P, Crowley WJ. Gonadotropin-releasing hormone and its analogues. *New Engl J Med.* 1991;324:93–103.
327. Lahlou N, Carel JC, Chaussain JL, Roger M. Pharmacokinetics and pharmacodynamics of GnRH agonists: clinical implications in pediatrics. *J Pediatr Endocrinol Metab.* 2000;13(Suppl 1):723–737.
328. Hirono M, Igarashi M, Matsumoto, S. Short- and auto-feedback mechanism of LH. *Endocrinology.* 1971;18:175.
329. Patriiti-Laborde N, Wolfson A, Odell W. Short loop feedback system for the control of follicle-stimulating hormone in the rabbit. *Endocrinology.* 1981;108:72.
330. Kanasaki H, Bedecarrats GY, Kam KY, Xu S, Kaiser UB. Gonadotropin-releasing hormone pulse frequency-dependent activation of extracellular signal-regulated kinase pathways in perfused LbetaT2 cells. *Endocrinology.* 2005;146:5503–5513.
331. McArdle C. Pituitary adenylate cyclase-activating polypeptide: A key player in reproduction? *Endocrinology.* 1994;135:815–816.
332. Drouin J, Labrie F. Interactions between 17 β -estradiol and progesterone in the control of luteinizing hormone and follicle-stimulating hormone release in rat anterior pituitary cells in culture. *Endocrinology.* 1981;108:52–57.
333. Turgeon JL, Waring DW. Androgen modulation of luteinizing hormone secretion by female rat gonadotropes. *Endocrinology.* 1999;140:1767–1774.
334. Hemrika DJ, Slaats EH, Kennedy JC, de Vries Robles-Korsen TJ, Schoemaker J. Pulsatile luteinizing hormone patterns in long term oral contraceptive users. *J Clin Endocrinol Metab.* 1993;77:420–426.

335. Couse JF, Yates MM, Walker VR, Korach KS. Characterization of the hypothalamic-pituitary-gonadal axis in estrogen receptor (ER) Null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. *Mol Endocrinol*. 2003;17:1039–1053.
336. Griffin LD, Mellon SH. Biosynthesis of the neurosteroid 3alpha-hydroxy-4-pregnen-20-one (3alphahp), a specific inhibitor of fsh release. *Endocrinology*. 2001;142:4617–4622.
337. Nicoletti I, Filippini P, Fedeli L, Santori PA, Santeusano F. Effect of estrogens and progesterone on gonadotropin and prolactin release in a patient with androgen insensitivity. *Obstet Gynecol*. 1981;58:527–532.
338. Wu S, Chen Y, Fajobi T, et al. Conditional knockout of the androgen receptor in gonadotropes reveals crucial roles for androgen in gonadotropin synthesis and surge in female mice. *Mol Endocrinol*. 2014;28:1670–1681.
339. Pastor CL, Griffin-Korf ML, Aloï JA, Evans WS, Marshall JC. Polycystic ovary syndrome: evidence for reduced sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. *J Clin Endocrinol Metab*. 1998;83:582–590.
340. Eagleson CA, Gingrich MB, Pastor CL, et al. Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. *J Clin Endocrinol Metab*. 2000;85:4047–4052.
341. Groome N, Illingworth P, O'Brien M, et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab*. 1996;81:1401–1405.
342. Hayes FJ, Hall JE, Boepple PA, Crowley Jr WF. Clinical review 96: Differential control of gonadotropin secretion in the human: endocrine role of inhibin. *J Clin Endocrinol Metab*. 1998;83:1835–1841.
343. Lumpkin M, Negro-Vilar A, Franchimont P, et al. Evidence for a hypothalamic site of action of inhibin to suppress FSH release. *Endocrinology*. 1981;108:1101.
344. Bilezikjian LM, Blount AL, Donaldson CJ, Vale WW. Pituitary actions of ligands of the TGF-beta family: activins and inhibins. *Reproduction*. 2006;132:207–215.
345. Nelson VL, Legro RS, Strauss 3rd JF, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Mol Endocrinol*. 1999;13:946–957.
346. Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidogenic enzyme messenger ribonucleic acids are overexpressed in thecal and granulosa cells from polycystic ovaries. *J Clin Endocrinol Metab*. 2001;86:1318–1323.
347. Sen A, Hammes SR. Granulosa cell-specific androgen receptors are critical regulators of ovarian development and function. *Mol Endocrinol*. 2010;24:1393–1403.
348. Rosenfield, R.L. Ovarian and adrenal function in polycystic ovary syndrome. *Endocrinol Metab Clin N Am*, 28, 265–293.
349. Taniguchi F, Couse JF, Rodriguez KF, Emmen JM, Poirier D, Korach KS. Estrogen receptor-alpha mediates an intraovarian negative feedback loop on thecal cell steroidogenesis via modulation of Cyp17a1 (cytochrome P450, steroid 17alpha-hydroxylase/17,20 lyase) expression. *FASEB J*. 2007;21:586–595.
350. Willis D, Mason H, Gilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. *J Clin Endocrinol Metab*. 1996;81:302–309.
351. Bergh C, Olsson JH, Hillensjo T. Effect of insulin-like growth factor I on steroidogenesis in cultured human granulosa cells. *Acta Endocrinol (Copenh)*. 1991;125:177–185.
352. Yoshimura Y, Washita M, Karube M, et al. Growth hormone stimulates follicular development by stimulating ovarian production of insulin-like growth factor-I. *Endocrinology*. 1994;135:887–894.
353. Mason HD, Martikainen H, Beard RW, Anyaoku V, Franks S. Direct gonadotrophic effect of growth hormone on oestradiol production by human granulosa cells in vitro. *J Endocrinol*. 1990;126:R1–R4.
354. Belfiore A, Malaguarnera R, Vella V, et al. Insulin receptor isoforms in physiology and disease: an updated view. *Endocr Rev*. 2017;38:379–431.
355. Wu S, Dival S, Nwaopara A, et al. Obesity induced infertility and hyperandrogenism are corrected by deletion of the insulin receptor in the ovarian theca cell. *Diabetes*. 2014;63:1270–1282.
356. McAllister JM, Modi B, Miller BA, et al. Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype. *Proc Natl Acad Sci U S A*. 2014;111. E1519–1527.
357. McAllister JM, Legro RS, Modi BP, Strauss 3rd JF. Functional genomics of PCOS: from GWAS to molecular mechanisms. *Trends Endocrinol Metab*. 2015;26:118–124.
358. Mayerhofer A, Hemmings Jr HC, Snyder GL, et al. Functional dopamine-1 receptors and DARPP-32 are expressed in human ovary and granulosa luteal cells in vitro. *J Clin Endocrinol Metab*. 1999;84:257–264.
359. Yamamoto N, Christenson LK, McAllister JM, Strauss 3rd JF. Growth differentiation factor-9 inhibits 3'5'-adenosine monophosphate-stimulated steroidogenesis in human granulosa and theca cells. *J Clin Endocrinol Metab*. 2002;87:2849–2856.
360. Agarwal SK, Vogel K, Weitsman SR, Magoffin DA. Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. *J Clin Endocrinol Metab*. 1999;84:1072–1076.
361. Feng P, Catt K, Knecht M. Transforming growth factor-beta stimulates meiotic maturation of the rat oocyte. *Endocrinology*. 1988;122:181.
362. Grossman MP, Nakajima ST, Fallat ME, Siow Y. Mullerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertil Steril*. 2008;89:1364–1370.
363. Aten RF, Polan ML, Bayless R, Behrman HR. A gonadotropin-releasing hormone (GnRH)-like protein in human ovaries: similarity to the GnRH-like ovarian protein of the rat. *J Clin Endocrinol Metab*. 1987;64:1288–1293.
364. Barnes RB, Scommegna A, Schreiber JR. Decreased ovarian response to human menopausal gonadotropin caused by subcutaneously administered gonadotropin-releasing hormone agonist. *Fertil Steril*. 1987;47:512.
365. Advis J, Richards J, Ojeda S. Hyperprolactinemia-induced precocious puberty: Studies on the intraovarian mechanism(s) by which PRL enhances ovarian responsiveness to gonadotropins in prepubertal rats. *J Clin Endocrinol Metab*. 1981;108:1333.
366. Demura R, Ono M, Demura H, Shizume K, Oouch H. Prolactin directly inhibits basal as well as estradiol-stimulated secretion of progesterone and 17beta-estradiol in the human ovary. *J Clin Endocrinol Metab*. 1982;54:1246.
367. Glickman SP, Rosenfield RL, Bergenstal RM, Helke J. Multiple androgenic abnormalities, including elevated free testosterone, in hyperprolactinemic women. *J Clin Endocrinol Metab*. 1982;55:251–257.
368. Rosenfield RL. Normal adrenarache. In: Rose BD, ed. *UpToDate*. Waltham, MA: UpToDate; 2018. www.uptodate.com/index.
369. Conley AJ, Bernstein RM, Nguyen AD. Adrenarache in nonhuman primates: the evidence for it and the need to redefine it. *J Endocrinol*. 2012;214:121–131.
370. Dumontet T, Sahut-Barnola I, Septier A, et al. PKA signaling drives reticularis differentiation and sexually dimorphic adrenal cortex renewal. *JCI Insight*. 2018;3.
371. Lerario AM, Finco I, LaPensee C, Hammer GD. Molecular mechanisms of stem/progenitor cell maintenance in the adrenal cortex. *Front Endocrinol (Lausanne)*. 2017;8:52.
372. Baquedano MS, Belgorosky A. Human adrenal cortex: epigenetics and postnatal functional zonation. *Horm Res Paediatr*. 2018;1–10.
373. Rege J, Nakamura Y, Satoh F, et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. *J Clin Endocrinol Metab*. 2013;98:1182–1188.
374. Turcu, A.F., Nanba, A.T., Auchus, R.J. (2018). The rise, fall, and resurrection of 11-oxygenated androgens in human physiology and disease. *Horm Res Paediatr* 1–8.
375. Palmert MR, Hayden DL, Mansfield MJ, et al. The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarache is a gradual process. *J Clin Endocrinol Metab*. 2001;86:4536–4542.
376. Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A. Relationship between the growth hormone/insulin-like growth factor-I axis, insulin sensitivity, and adrenal androgens in normal

- prepubertal and pubertal girls. *J Clin Endocrinol Metab.* 2003;88:1389–1393.
377. Blogowska A, Rzepka-Gorska I, Krzyzanowska-Swiniarska B. Body composition, dehydroepiandrosterone sulfate and leptin concentrations in girls approaching menarche. *J Pediatr Endocrinol Metab.* 2005;18:975–983.
 378. Majzoub JA, Topor LS. A new model for adrenarche: inhibition of 3beta-hydroxysteroid dehydrogenase type 2 by intra-adrenal cortisol. *Horm Res Paediatr.* 2018;1–9.
 379. Endoh A, Kristiansen S, Casson P, Buster J, Hornsby P. The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3β-hydroxysteroid dehydrogenase. *J Clin Endocrinol Metab.* 1996;81:3558–3565.
 380. l'Allemand D, Penhoat A, Lebrethon MC, et al. Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid levels and corticotropin steroidogenic responsiveness in cultured human adrenocortical cells. *J Clin Endocrinol Metab.* 1996;81:3892–3897.
 381. Baquedano MS, Berensztein E, Saraco N, et al. Expression of the IGF system in human adrenal tissues from early infancy to late puberty: implications for the development of adrenarche. *Pediatr Res.* 2005;58:451–458.
 382. Biason-Laubert A, Zachmann M, Schoenle EJ. Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. *Endocrinology.* 2000;141:1446–1454.
 383. Mantyselka A, Lindi V, Viitasalo A, et al. Associations of dehydroepiandrosterone sulfate with cardiometabolic risk factors in prepubertal children. *J Clin Endocrinol Metab.* 2018;103:2592–2600.
 384. Nordman H, Voutilainen R, Antikainen L, Jaaskelainen J. Prepubertal children born large for gestational age have lower serum DHEAS concentrations than those with a lower birth weight. *Pediatr Res.* 2017;82(2):285–289.
 385. Rittmaster R, Givner M. Effect of daily and alternate day low dose prednisone on serum cortisol and adrenal androgens in hirsute women. *J Clin Endocrinol Metab.* 1988;67:400–403.
 386. Cutler GJ, Davis S, Johnsonbaugh R, Loriaux L. Dissociation of cortisol and adrenal androgen secretion in patients with secondary adrenal insufficiency. *J Clin Endocrinol Metab.* 1979;49:604.
 387. Auchus RJ, Rainey WE. Adrenarche - physiology, biochemistry and human disease. *Clin Endocrinol.* 2004;60:288–296.
 388. Van Hulle S, Craen M, Callewaert B, et al. Delayed adrenarche may be an additional feature of immunoglobulin super family member 1 deficiency syndrome. *J Clin Res Pediatr Endocrinol.* 2016;8:86–91.
 389. Udhane SS, Fluck CE. Regulation of human (adrenal) androgen biosynthesis-New insights from novel throughput technology studies. *Biochem Pharmacol.* 2016;102:20–33.
 390. Path G, Bornstein SR, Ehrhart-Bornstein M, Scherbaum WA. Interleukin-6 and the interleukin-6 receptor in the human adrenal gland: expression and effects on steroidogenesis. *J Clin Endocrinol Metab.* 1997;82:2343–2349.
 391. Ehrhart-Bornstein M, Hinson J, Bornstein S, Scherbaum W, Vinson G. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. *Endocr Rev.* 1998;19:101–143.
 392. Martin DD, Schweizer R, Schwarze CP, Elmlinger MW, Ranke MB, Binder G. The early dehydroepiandrosterone sulfate rise of adrenarche and the delay of pubarche indicate primary ovarian failure in Turner syndrome. *J Clin Endocrinol Metab.* 2004;89:1164–1168.
 393. Cumming D, Rebar R, Hopper B, Yen S. Evidence for an influence of the ovary on circulating dehydroepiandrosterone sulfate levels. *J Clin Endocrinol Metab.* 1982;54:1069–1071.
 394. Noordam C, Dhir V, McNelis JC, et al. Inactivating PAPSS2 mutations in a patient with premature pubarche. *N Engl J Med.* 2009;360:2310–2318.
 395. Haning Jr RV, Austin CW, Carlson IH, Kuzma DL, Zweibel WJ. Role of dehydroepiandrosterone sulfate as a prehormone for ovarian steroidogenesis. *Obstet Gynecol.* 1985;65:199–205.
 396. Mantyselka A, Jaaskelainen J, Eloranta AM, et al. Associations of lifestyle factors with serum dehydroepiandrosterone sulphate and insulin-like growth factor-1 concentration in prepubertal children. *Clin Endocrinol.* 2018;88:234–242.
 397. Nguyen TV. Developmental effects of androgens in the human brain. *J Neuroendocrinol.* 2018;30.
 398. Paul S, Purdy R. Neuroactive steroids. *FASEB J.* 1992;6:2311–2322.
 399. Rege J, Nanba AT, Auchus RJ, et al. Adrenocorticotropin acutely regulates pregnenolone sulfate production by the human adrenal In vivo and In vitro. *J Clin Endocrinol Metab.* 2018;103:320–327.
 400. Schverer M, Lanfumey L, Baulieu EE, Froger N, Villey I. Neurosteroids: non-genomic pathways in neuroplasticity; involvement in neurological diseases. *Pharmacol Ther.* 2018;191.
 401. Grube M, Hagen P, Jedlitschky G. Neurosteroid transport in the brain: role of ABC and SLC transporters. *Front Pharmacol.* 2018;9:354.
 402. Campbell BC. Adrenarche and middle childhood. *Hum Nat.* 2009;22:327–349.
 403. Del Giudice, M. Sex, attachment, and the development of reproductive strategies. *Behav Brain Sci,* 32, 1–21; discussion 67.
 404. Herdt G, McClintock M. The magical age of 10. *Arch Sex Behav.* 2000;29:587–606.
 405. Apaja PM, Aatsinki JT, Rajaniemi HJ, Petaja-Repo UE. Expression of the mature luteinizing hormone receptor in rodent urogenital and adrenal tissues is developmentally regulated at a posttranslational level. *Endocrinology.* 2005;146:3224–3232.
 406. Krsmanovic LZ, Stojilkovic SS, Catt KJ. Pulsatile gonadotropin-releasing hormone release and its regulation. *Trends Endocrinol Metab.* 1996;7:56–59.
 407. Spiegel A, Shenker A, Weinstein L. Receptor-effector coupling by G proteins: implications for normal and abnormal signal transduction. *Endocr Rev.* 1992;13:536.
 408. Carvalho CR, Carvalheira JB, Lima MH, et al. Novel signal transduction pathway for luteinizing hormone and its interaction with insulin: activation of Janus kinase/signal transducer and activator of transcription and phosphoinositol 3-kinase/Akt pathways. *Endocrinology.* 2003;144:638–647.
 409. Adelman J, Mason A, Hayflick J, Seeburg P. Isolation of the gene and hypothalamic cDNA for the common precursor of gonadotropin-releasing hormone and prolactin release-inhibiting factor in human and rat. *Proc Natl Acad Sci (USA).* 1986;83:179.
 410. Redding TW, Schally AV, Arimura A, Matsuo H. Stimulation of release and synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in tissue cultures of rat pituitaries in response to natural and synthetic LH and FSH releasing hormone. *Endocrinology.* 1972;90:764–770.
 411. Kim HH, Mui KL, Nikrodhanond AA, Tamayo NC. Regulation of gonadotropin-releasing hormone in nonhypothalamic tissues. *Semin Reprod Med.* 2007;25:326–336.
 412. Kauffman AS, Rissman EF. A critical role for the evolutionarily conserved gonadotropin-releasing hormone II: mediation of energy status and female sexual behavior. *Endocrinology.* 2004;145:3639–3646.
 413. Millar R, Lowe S, Conklin D, et al. A novel mammalian receptor for the evolutionarily conserved type II GnRH. *Proc Natl Acad Sci U S A.* 2001;98:9636–9641.
 414. Comminos AN, Wall MB, Demetriou L, et al. Kisspeptin modulates sexual and emotional brain processing in humans. *J Clin Invest.* 2017;127:709–719.
 415. Phifer R, Midgley A, Spicer S. Immunohistologic and histologic evidence that follicle-stimulating hormone and luteinizing hormone are present in the same cell type in the human pars distalis. *J Clin Endocrinol Metab.* 1973;36:125.
 416. Hammond E, Griffin J, Odell W. A chorionic gonadotropin-secreting human pituitary cell. *J Clin Endocrinol Metab.* 1991;72:747–754.
 417. Combarnous Y. Molecular basis of the specificity of binding of glycoprotein hormones to their receptors. *Endocr Rev.* 1992;13:670–685.
 418. Dahl K, Stone M. FSH isoforms, radioimmunoassays, bioassays, and their significance. *J Androl.* 1992;13:11–12.
 419. Arey BJ, Stevis PE, Deecher DC, et al. Induction of promiscuous G protein coupling of the follicle-stimulating hormone (FSH) receptor: a novel mechanism for transducing pleiotropic actions of FSH isoforms. *Mol Endocrinol.* 1997;11:517–526.
 420. West CR, Carlson NE, Lee JS, et al. Acidic mix of FSH isoforms are better facilitators of ovarian follicular maturation and E2 production than the less acidic. *Endocrinology.* 2002;143:107–116.

421. Mi Y, Fiete D, Baenziger JU. Ablation of GalNAc-4-sulfotransferase-1 enhances reproduction by altering the carbohydrate structures of luteinizing hormone in mice. *J Clin Invest.* 2008;118:1815–1824.
422. Weiss J, Axelrod L, Whitcomb R, Harris P, Crowley W, Jameson J. Hypogonadism caused by a single amino acid substitution in the β subunit of luteinizing hormone. *N Engl J Med.* 1992;326:179–184.
423. Wide L, Eriksson K, Sluss PM, Hall JE. Serum half-life of pituitary gonadotropins is decreased by sulfonation and increased by sialylation in women. *J Clin Endocrinol Metab.* 2009;94:958–964.
424. Solano AR, Garcia-Vela A, Catt KJ, Dufau ML. Modulation of serum and pituitary luteinizing hormone bioactivity by androgen in the rat. *Endocrinology.* 1980;106:1941–1948.
425. Lucky AW, Rebar RW, Rosenfield RL, Roche-Bender N, Helke J. Reduction of the potency of luteinizing hormone by estrogen. *N Engl J Med.* 1979;300:1034–1036.
426. Dahl K, Biczak T, Hsueh A. Naturally occurring antihormones: Secretion of FSH antagonists by women treated with a GnRH analog. *Science.* 1988;239:72.
427. Tilly J, Aihara T, Nishimori K, et al. Expression of recombinant human follicle-stimulating hormone receptor: Species-specific ligand binding, signal transduction, and identification of multiple ovarian messenger ribonucleic acid transcripts. *Endocrinology.* 1992;131:799–806.
428. Taylor A, Khoury R, Crowley WJ. A comparison of 13 different immunometric assay kits for gonadotropins: implications for clinical investigation. *J Clin Endocrinol Metab.* 1994;79:240–247.
429. Raiti S, Foley Jr TP, Penny R, Blizzard RM. Measurement of the production rate of human luteinizing hormone using the urinary excretion technique. *Metab Clin Exp.* 1975;24:937–941.
430. Prentice L, Ryan R. LH and its subunits in human pituitary, serum and urine. *J Clin Endocrinol Metab.* 1975;40:303.
431. Yen S, Llerena O, Little B, Pearson OH. Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in man. *J Clin Endocrinol Metab.* 1968;28:1763–1767.
432. Yen S, Vela P, Rankin J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. *J Clin Endocrinol Metab.* 1970;30:435–442.
433. Veldhuis JD, Fraioli F, Rogol AD, Dufau ML. Metabolic clearance of biologically active luteinizing hormone in man. *J Clin Invest.* 1986;77:1122–1128.
434. Kohler P, Ross C, Odell W. Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J Clin Invest.* 1968;47:38.
435. Coble Jr YD, Kohler PO, Cargille CM, Ross GT. Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. *J Clin Invest.* 1969;48:359.
436. Rosenfield RL. Role of androgens in growth and development of the fetus, child, and adolescent. *Adv Pediatr.* 1972;19:171–213.
437. Baulieu EE, Corp'Échot C, Dray F, et al. An adrenal-secreted "androgen": dehydroisoandrosterone sulfate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates in man. *Recent Prog Horm Res.* 1965;21:411–500.
438. Sandberg E, Gurrpide E, Lieberman S. Quantitative studies on the metabolism of dehydroisoandrosterone sulfate. *Biochemistry.* 1964;3:1256–1267.
439. Longcope C, Pratt J. Blood production rates of estrogens in women with differing ratios of urinary estrogen conjugates. *Steroids.* 1977;29:483–492.
440. Strott C, Hoshimi T, Lipsett M. Plasma progesterone and 17-hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia. *J Clin Invest.* 1989;48:930–939.
441. Sinha Y. Prolactin variants. *Trends Endocrinol Metab.* 1992;3:100–106.
442. Frawley L. Role of the hypophyseal neurointermediate lobe in the dynamic release of prolactin. *Trends Endocrinol Metab.* 1994;5:107–112.
443. Pangas SA, Rademaker AW, Fishman DA, Woodruff TK. Localization of the activin signal transduction components in normal human ovarian follicles: implications for autocrine and paracrine signaling in the ovary. *J Clin Endocrinol Metab.* 2002;87:2644–2657.
444. Rosenfield RL, Lucky AW, Allen TD. The diagnosis and management of intersex. *Curr Probl Pediatr.* 1980;10:1–66.
445. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev.* 2011;32:81–151.
446. Miller WL. Disorders in the initial steps of steroid hormone synthesis. *J Steroid Biochem Mol Biol.* 2017;165:18–37.
447. Qin K, Rosenfield RL. Role of cytochrome P450c17 in polycystic ovary syndrome. *Mol Cell Endocrinol.* 1998;145:111–121.
448. Suzuki T, Miki Y, Nakata T, et al. Steroid sulfatase and estrogen sulfotransferase in normal human tissue and breast carcinoma. *J Steroid Biochem Mol Biol.* 2003;86:449–454.
449. Nelson VL, Qin KnK, Rosenfield RL, et al. The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2001;86:5925–5933.
450. Rosenfield RL. Relationship of androgens to female hirsutism and infertility. *J Reprod Med.* 1973;11:87–95.
451. Alonso LC, Rosenfield RL. Oestrogens and puberty. *Best Pract Res Clin Endocrinol Metab.* 2002;16:13–30.
452. Lin TJ, Billiar RB, Little B. Metabolic clearance of progesterone in the menstrual cycle. *J Clin Endocrinol Metab.* 1972;35:879–886.
453. Siiteri P, MacDonald P. Role of extraglandular estrogen in human endocrinology. In: Greep R, Astwood E, eds. *Handbook of Physiology.* American Physiology Society: Washington, DC; 1973:615.
454. Morimoto I, Edmiston A, Hawks D, Horton R. Studies of the origin of androstenediol and androstenediol glucuronide in young and elderly men. *J Clin Endocrinol Metab.* 1981;52:772.
455. Heinrichs WL, Tabei T, Kuwabara Y, et al. Differentiation and regulation of peripheral androgen metabolism in rats and rhesus monkeys. *Am J Obstet Gynecol.* 1979;135:974.
456. Mode A, Norstedt G, Eneroth H, Gustafsson J-A. Purification of liver feminizing factor from rat pituitaries and demonstration of its identity with growth hormone. *Endocrinology.* 1983;113:1250–1260.
457. Edman CD, MacDonald PC. Effect of obesity on conversion of plasma androstenedione to estrone in ovulatory and anovulatory young women. *Am J Obstet Gynecol.* 1978;130:456–461.
458. Kadlubar FF, Berkowitz GS, Delongchamp RR, et al. The CYP3A4*1B variant is related to the onset of puberty, a known risk factor for the development of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12:327–331.
459. Schroth W, Goetz MP, Hamann U, et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA.* 2009;302:1429–1436.
460. Harris RM, Wood DM, Bottomley L, et al. Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. *J Clin Endocrinol Metab.* 2004;89:1779–1787.
461. Giorgi E, Stein W. The transport of steroids into animal cells in culture. *Endocrinology.* 1981;108:688.
462. Martin KA, Chang RJ, Ehrmann DA, et al. Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. *J Clin Endocr Metab.* 2008;93:1105–1120.
463. Rosenfield RL. Studies of the relation of plasma androgen levels to androgen action in women. *J Steroid Biochem.* 1975;6:695–702.
464. Kahn SM, Hryb DJ, Nakhla AM, Romas NA, Rosner W. Sex hormone-binding globulin is synthesized in target cells. *J Endocrinol.* 2002;175:113–120.
465. Hammes A, Andreassen TK, Spoelgen R, et al. Role of endocytosis in cellular uptake of sex steroids [Comments in: *Cell.* 2005; 122(5), 647–9; *Cell.* 2006; 124(3), 455–456; author reply 456–7]. *Cell.* 2005;122:751–762.
466. Rosenfield RL, Moll GW. The role of proteins in the distribution of plasma androgens and estradiol. In: Molinatti G, Martini L, James V, eds. *Androgenization in Women.* New York: Raven Press; 1983:25–45.
467. Nestler J, Powers L, Matt D, et al. A direct effect of hyperinsulinemia on serum sex-hormone binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1991;72:83–89.

468. Simo R, Barbosa-Desongles A, Lecube A, Hernandez C, Selva DM. Potential role of tumor necrosis factor- α in downregulating sex hormone-binding globulin. *Diabetes*. 2012;61:372–382.
469. Nimrod A, Rosenfield RL, Otto P. Relationship of androgen action to androgen metabolism in isolated rat granulosa cells. *J Steroid Biochem*. 1980;13:1015–1019.
470. Wilson JD, Griffin JE, Russell DW. Steroid 5 α -reductase 2 deficiency. *Endocr Rev*. 1993;14:577–593.
471. Yang S, Fang Z, Gurates B, et al. Stromal PRs mediate induction of 17 β -hydroxysteroid dehydrogenase type 2 expression in human endometrial epithelium: a paracrine mechanism for inactivation of E2. *Mol Endocrinol*. 2001;15:2093–2105.
472. Hochberg RB. Biological esterification of steroids. *Endocr Rev*. 1998;19:331–348.
473. Baracat E, Haidar M, Lopez FJ, Pickar J, Dey M, Negro-Vilar A. Estrogen activity and novel tissue selectivity of delta8,9-dehydroestrone sulfate in postmenopausal women. *J Clin Endocrinol Metab*. 1999;84:2020–2027.
474. Katzenellenbogen BS, Montano MM, Ediger TR, et al. Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*. 2000;55:163–193; discussion 94–95.
475. O'Malley BW. A life-long search for the molecular pathways of steroid hormone action. *Mol Endocrinol*. 2005;19:1402–1411.
476. McDevitt MA, Glidewell-Kennedy C, Jimenez MA, et al. New insights into the classical and non-classical actions of estrogen: evidence from estrogen receptor knock-out and knock-in mice. *Mol Cell Endocrinol*. 2008;290:24–30.
477. Chen H, Hu B, Huang GH, Trainor AG, Abbott DH, Adams JS. Purification and characterization of a novel intracellular 17 β -estradiol binding protein in estrogen-resistant New World primate cells. *J Clin Endocrinol Metab*. 2003;88:501–504.
478. Cheung J, Smith DF. Molecular chaperone interactions with steroid receptors: an update. *Mol Endocrinol*. 2000;14:939–946.
479. Alarid ET, Bakopoulos N, Solodin N. Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. *Mol Endocrinol*. 1999;13:1522–1534.
480. Korenman S. Radio-ligand binding assay of specific estrogens using a soluble uterine macromolecule. *J Clin Endocrinol Metab*. 1968;28:127.
481. French FS, Lubahn DB, Brown TR, et al. Molecular basis of androgen insensitivity. *Rec Prog Horm Res*. 1990;46:1.
482. Connor CE, Norris JD, Broadwater G, et al. Circumventing tamoxifen resistance in breast cancers using antiestrogens that induce unique conformational changes in the estrogen receptor. *Cancer Res*. 2001;61:2917–2922.
483. Melamed M, Castano E, Notides AC, Sasson S. Molecular and kinetic basis for the mixed agonist-antagonist activity of estriol. *Mol Endocrinol*. 1997;11:1868–1878.
484. Kempainen JA, Langley E, Wong CI, Bobseine K, Kelce WR, Wilson EM. Distinguishing androgen receptor agonists and antagonists: distinct mechanisms of activation by medroxyprogesterone acetate and dihydrotestosterone. *Mol Endocrinol*. 1999;13:440–454.
485. McDonnell DP, Wardell SE. The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. *Curr Opin Pharmacol*. 2010;10:620–628.
486. Schmidt A, Kimmel DB, Bai C, et al. Discovery of the selective androgen receptor modulator MK-0773 using a rational development strategy based on differential transcriptional requirements for androgenic anabolism versus reproductive physiology. *J Biol Chem*. 2010;285:17054–17064.
487. Yang NN, Venugopalan M, Hardikar S, Glasebrook A. Identification of an estrogen response element activated by metabolites of 17 β -estradiol and raloxifene [see comments] [published erratum appears in *Science* 1997 Feb 28;275(5304):1249]. *Science*. 1996;273:1222–1225.
488. Dowsett M, Ashworth A. New biology of the oestrogen receptor. *Lancet*. 2003;362:260–262.
489. Smid-Koopman E, Blok LJ, Kuhne LC, et al. Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. *J Soc Gynecol Invest*. 2003;10:49–57.
490. McPhaul MJ, Young M. Complexities of androgen action. *J Am Acad Dermatol*. 2001;45: S87–94.
491. Lindberg MK, Moverare S, Skrtic S, et al. Estrogen receptor (ER)- β reduces ER α -regulated gene transcription, supporting a “ying yang” relationship between ER α and ER β in mice. *Mol Endocrinol*. 2003;17:203–208.
492. Smith EP, Boyd J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med*. 1994;331:1056–1061.
493. Nilsson S, Kuiper G, Gustafsson J-A. ER β : a novel estrogen receptor offers the potential for new drug development. *Trends Endocrinol Metab*. 1998;9:387–395.
494. Conneely OM, Lydon JP, De Mayo F, O'Malley BW. Reproductive functions of the progesterone receptor. *J Soc Gynecol Invest*. 2000;7: S25–32.
495. Barbulescu K, Geserick C, Schuttke I, Schleuning WD, Haendler B. New androgen response elements in the murine pem promoter mediate selective transactivation. *Mol Endocrinol*. 2001;15:1803–1816.
496. McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev*. 1999;20:321–324.
497. Laughlin GA, Dominguez CE, Yen SS. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. *J Clin Endocrinol Metab*. 1998;83:25–32.
498. Lu S, Jenster G, Epner DE. Androgen induction of cyclin-dependent kinase inhibitor p21 gene: role of androgen receptor and transcription factor Sp1 complex. *Mol Endocrinol*. 2000;14:753–760.
499. Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell*. 2001;104:719–730.
500. Nilsson O, Falk J, Ritzen EM, Baron J, Savendahl L. Raloxifene acts as an estrogen agonist on the rabbit growth plate. *Endocrinology*. 2003;144:1481–1485.
501. Cranney A, Adachi JD. Benefit-risk assessment of raloxifene in postmenopausal osteoporosis. *Drug Saf*. 2005;28:721–730.
502. Kelly MJ, Ronnekleiv OK. Membrane-initiated estrogen signaling in hypothalamic neurons. *Mol Cell Endocrinol*. 2008;290:14–23.
503. Zhao Z, Park C, McDevitt MA, et al. p21-Activated kinase mediates rapid estradiol-negative feedback actions in the reproductive axis. *Proc Natl Acad Sci U S A*. 2009;106:7221–7226.
504. Hammes SR, Levin ER. Extracellular steroid receptors: nature and actions. *Endocr Rev*. 2007;28:726–741.
505. Park CJ, Zhao Z, Glidewell-Kennedy C, et al. Genetic rescue of non-classical ER α signaling normalizes energy balance in obese ER α -null mutant mice. *J Clin Invest*. 2011;121:604–612.
506. Apostolakis EM, Garai J, Lohmann JE, Clark JH, O'Malley BW. Epidermal growth factor activates reproductive behavior independent of ovarian steroids in female rodents. *Mol Endocrinol*. 2000;14:1086–1098.
507. Mellon S. Neurosteroids: biochemistry, modes of action, and clinical relevance. *J Clin Endocrinol Metab*. 1994;78:1003–1008.
508. Baulieu EE. Neuroactive neurosteroids: dehydroepiandrosterone (DHEA) and DHEA sulphate. *Acta Paediatr Suppl*. 1999;88:78–80.
509. Zwain IH, Yen SS. Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology*. 1999;140:3843–3852.
510. Rhodes ME, Harney JP, Frye CA. Gonadal, adrenal, and neuroactive steroids' role in ictal activity. *Brain Res*. 2004;1000:8–18.
511. Ramirez VD, Zheng J. Membrane sex-steroid receptors in the brain. *Front Neuroendocrinol*. 1996;17:402–439.
512. Wetzel CH, Hermann B, Behl C, et al. Functional antagonism of gonadal steroids at the 5-hydroxytryptamine type 3 receptor. *Mol Endocrinol*. 1998;12:1441–1451.
513. McEwen BS. Clinical review 108: The molecular and neuroanatomical basis for estrogen effects in the central nervous system. *J Clin Endocrinol Metab*. 1999;84:1790–1797.
514. MacLusky N, McEwen B. Progesterone receptors in rat brain: Distribution and properties of cytoplasmic progesterone-binding sites. *Endocrinology*. 1980;106:192–202.
515. Attardi B. Facilitation and inhibition of the estrogen-induced luteinizing hormone surge in the rat by progesterone: Effects on cytoplasmic and nuclear estrogen receptors in the hypothalamic-preoptic area, pituitary, and uterus. *Endocrinology*. 1981;108:1487–1496.

516. Pacifici R. Cytokines, estrogen, and postmenopausal osteoporosis—the second decade [editorial; comment]. *Endocrinology*. 1998;139:2659–2661.
517. Gupta C, Goldman A. The arachidonic acid cascade is involved in the masculinizing action of testosterone on embryonic external genitalia in mice. *Proc Natl Acad Sci U S A*. 1989;83:4346–4349.
518. Maor G, Segev Y, Phillip M. Testosterone stimulates insulin-like growth factor-I and insulin-like growth factor-I-receptor gene expression in the mandibular condyle—a model of endochondral ossification. *Endocrinology*. 1999;140:1901–1910.
519. Ammini A, Vijayaraghavan M, Sabherwal U. Human female phenotypic development: role of fetal ovaries. *J Clin Endocrinol Metab*. 1994;79:604–608.
520. Kalloo N, Gearhart J, Barrack E. Sexually dimorphic expression of estrogen receptors, but not of androgen receptors in human fetal external genitalia. *J Clin Endocrinol Metab*. 1993;77:692–698.
521. Tewari K, Bonebrake RG, Asrat T, Shanberg AM. Ambiguous genitalia in infant exposed to tamoxifen in utero [letter]. *Lancet*. 1997;350:183.
522. Greco T, Duello T, Gorski J. Estrogen receptors, estradiol, and diethylstilbestrol in early development: the mouse as a model for the study of estrogen receptors and estrogen sensitivity in embryonic development of male and female reproductive tracts. *Endocr Rev*. 1993;14:59.
523. Woodruff J, Pickar J. Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. *Am J Obstet Gynecol*. 1994;170:1213–1223.
524. Futterweit W, Deligdisch L. Histopathological effects of exogenously administered testosterone in 19 female to male transsexuals. *J Clin Endocrinol Metab*. 1986;62:16–21.
525. Westhoff C. Clinical practice. Emergency contraception. *N Engl J Med*. 2003;349:1830–1835.
526. Rochwerger L, Buchwald M. Stimulation of the cystic fibrosis transmembrane regulator expression by estrogen in vivo. *Endocrinology*. 1993;133:921–930.
527. Paek SC, Merritt DF, Mallory SB. Pruritus vulvae in prepubertal children. *J Am Acad Dermatol*. 2001;44:795–802.
528. Wied C, Bibbo M. Evaluation of endocrinologic condition by exfoliative cytology. In: Cold J, ed. *Gynecologic Endocrinology*. 2nd ed. New York: Harper & Row; 1975.
529. Rosenfield RL, Fang VS, Dupon C, Kim MH, Refetoff S. The effects of low doses of depot estradiol and testosterone in teenagers with ovarian failure and Turner's syndrome. *J Clin Endocrinol Metab*. 1973;37:574–580.
530. Kuon RJ, Shi SQ, Maul H, et al. Pharmacologic actions of progestins to inhibit cervical ripening and prevent delivery depend on their properties, the route of administration, and the vehicle. *Am J Obstet Gynecol*. 2010;202(455). e1–9.
531. Armstrong J. Unintended consequences—the cost of preventing preterm births after FDA approval of a branded version of 17OHP. *N Engl J Med*. 2011;364:1689–1691.
532. Benson A, Heger A, Hayes J, Bailey R, Emans S. Appearance of the hymen in prepubertal girls. *Pediatrics*. 1992;89:387–394.
533. Gardner J. Descriptive study of genital variation in healthy, non-abused premenarcheal girls. *J Pediatr*. 1992;120:251–257.
534. Robbins S, Cotran R. The breast. In: Kumar V, ed. *Pathologic Basis of Disease*. Philadelphia: WB Saunders Company; 1979:1165–1191.
535. Rilemma J. Development of the mammary gland and lactation. *Trends Endocrinol Metab*. 1994;5:149–154.
536. Lyons W. Hormonal synergism in mammary growth. *Proc Roy Soc Lond [Biol]*. 1958;149:303.
537. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr Rev*. 1998;19:225–268.
538. Brisken C, O'Malley B. Hormone action in the mammary gland. *Cold Spring Harb Perspect Biol*. 2010;2010.
539. Butler LM, Potischman NA, Newman B, et al. Menstrual risk factors and early-onset breast cancer. *Cancer Causes Control*. 2000;11:451–458.
540. Poynter JN, Langholz B, Largent J, et al. Reproductive factors and risk of contralateral breast cancer by BRCA1 and BRCA2 mutation status: results from the WECARE study. *Cancer Causes Control*. 2010;21:839–846.
541. Ma Y, Katiyar P, Jones LP, et al. The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. *Mol Endocrinol*. 2006;20:14–34.
542. Deplewski D, Rosenfield RL. Role of hormones in pilosebaceous unit development. *Endocr Rev*. 2000;21:363–392.
543. Jave-Suarez LF, Langbein L, Winter H, Praetzel S, Rogers MA, Schweizer J. Androgen regulation of the human hair follicle: the type I hair keratin hHa7 is a direct target gene in trichocytes. *J Invest Dermatol*. 2004;122:555–564.
544. Walker WH. Is the “comb over” dying? A mouse model for male pattern baldness (androgenic alopecia). *Endocrinology*. 2010;151:1981–1983.
545. Ohnemus U, Uenalan M, Inzunza J, Gustafsson JA, Paus R. The hair follicle as an estrogen target and source. *Endocr Rev*. 2006;27:677–706.
546. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev*. 1989;10:68–91.
547. Callewaert F, Sinnesael M, Gielen E, Boonen S, Vanderschueren D. Skeletal sexual dimorphism: relative contribution of sex steroids, GH-IGF1, and mechanical loading. *J Endocrinol*. 2010;207:127–134.
548. Smith EP, Specker B, Korach KS. Recent experimental and clinical findings in the skeleton associated with loss of estrogen hormone or estrogen receptor activity. *J Steroid Biochem Mol Biol*. 2010;118:264–272.
549. Seeman E. The structural and biomechanical basis of the gain and loss of bone strength in women and men. *Endocrinol Metab Clin N Am*. 2003;32:25–38.
550. Mora S, Gilsanz V. Establishment of peak bone mass. *Endocrinol Metab Clin N Am*. 2003;32:39–63.
551. Migliaccio S, Newbold RR, Bullock BC, et al. Alterations of maternal estrogen levels during gestation affect the skeleton of female offspring. *Endocrinology*. 1996;137:2118–2125.
552. Eastell R. Role of oestrogen in the regulation of bone turnover at the menarche. *J Endocrinol*. 2005;185:223–234.
553. Cooke PS, Naaz A. Role of estrogens in adipocyte development and function. *Exp Biol Med (Maywood)*. 2004;229:1127–1135.
554. Woo JG, Dolan LM, Daniels SR, Goodman E, Martin LJ. Adolescent sex differences in adiponectin are conditional on pubertal development and adiposity. *Obes Res*. 2005;13:2095–2101.
555. Capllonch-Amer G, Llado I, Proenza AM, Garcia-Palmer FJ, Gianotti M. Opposite effects of 17-beta estradiol and testosterone on mitochondrial biogenesis and adiponectin synthesis in white adipocytes. *J Mol Endocrinol*. 2014;52:203–214.
556. Ek I, Arner P, Ryden M, et al. A unique defect in the regulation of visceral fat cell lipolysis in the polycystic ovary syndrome as an early link to insulin resistance. *Diabetes*. 2002;51:484–492.
557. Arner P. Effects of testosterone on fat cell lipolysis. Species differences and possible role in polycystic ovarian syndrome. *Biochimie*. 2005;87:39–43.
558. Chazenbalk G, Singh P, Irge D, Shah A, Abbott DH, Dumesic DA. Androgens inhibit adipogenesis during human adipose stem cell commitment to preadipocyte formation. *Steroids*. 2013;78:920–926.
559. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology*. 2003;144:5081–5088.
560. Du X, Rosenfield RL, Qin K. KLF15 is a transcriptional regulator of the human 17 β -hydroxysteroid dehydrogenase type 5 gene. A potential link between regulation of testosterone production and fat stores in women. *J Clin Endocrinol Metab*. 2009;94:2594–2601.
561. Veilleux A, Cote JA, Blouin K, et al. Glucocorticoid-induced androgen inactivation by aldo-keto reductase 1C2 promotes adipogenesis in human preadipocytes. *Am J Physiol Endocrinol Metab*. 2012;302:E941–E949.
562. Gupta V, Bhasin S, Guo W, et al. Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. *Mol Cell Endocrinol*. 2008;296:32–40.

563. O'Reilly MW, Kempegowda P, Walsh M, et al. AKR1C3-mediated adipose androgen generation drives lipotoxicity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2017;102:3327–3339.
564. Dicker A, Ryden M, Naslund E, et al. Effect of testosterone on lipolysis in human pre-adipocytes from different fat depots. *Diabetologia.* 2004;47:420–428.
565. Varlamov O, Bishop CV, Handu M, et al. Combined androgen excess and Western-style diet accelerates adipose tissue dysfunction in young adult, female nonhuman primates. *Hum Reprod.* 2017;32:1892–1902.
566. Newell-Fugate AE. The role of sex steroids in white adipose tissue adipocyte function. *Reproduction.* 2017;153:R133–R149.
567. Pedersen SB, Kristensen K, Hermann PA, Katzenellenbogen JA, Richelsen B. Estrogen controls lipolysis by up-regulating alpha2A-adrenergic receptors directly in human adipose tissue through the estrogen receptor alpha. Implications for the female fat distribution. *J Clin Endocrinol Metab.* 2004;89:1869–1878.
568. Law J, Bloor I, Budge H, Symonds ME. The influence of sex steroids on adipose tissue growth and function. *Horm Mol Biol Clin Invest.* 2014;19:13–24.
569. Liu P, Ji Y, Yuen T, et al. Blocking FSH induces thermogenic adipose tissue and reduces body fat. *Nature.* 2017;546:107–112.
570. Varlamov O, Chu MP, McGee WK, et al. Ovarian cycle-specific regulation of adipose tissue lipid storage by testosterone in female nonhuman primates. *Endocrinology.* 2013;154:4126–4135.
571. Klein KO, Rosenfield R, Santen RJ, et al. Estrogen replacement in Turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab.* 2018;103:1790–1803.
572. Jensen JT, Addis IB, Hennebold JD, Bogan RL. Ovarian lipid metabolism modulates circulating lipids in premenopausal women. *J Clin Endocrinol Metab.* 2017;102:3138–3145.
573. Smith GI, Reeds DN, Okunade AL, Patterson BW, Mittendorfer B. Systemic delivery of estradiol, but not testosterone or progesterone, alters very low density lipoprotein-triglyceride kinetics in postmenopausal women. *J Clin Endocrinol Metab.* 2014;99:E1306–1310.
574. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med.* 1991;325:1196–1204.
575. Santosa S, Bonnes SL, Jensen MD. Acute female hypogonadism alters adipose tissue fatty acid storage factors and chylomicronemia. *J Clin Endocrinol Metab.* 2016;101:2089–2098.
576. Perseghin G, Scifo P, Pagliato E, et al. Gender factors affect fatty acids-induced insulin resistance in nonobese humans: effects of oral steroidal contraception. *J Clin Endocrinol Metab.* 2001;86:3188–3196.
577. Herbst KL, Bhasin S. Testosterone action on skeletal muscle. *Curr Opin Clin Nutr Metab Care.* 2004;7:271–277.
578. Yarrow JF, McCoy SC, Borst SE. Intracrine and myotrophic roles of 5alpha-reductase and androgens: a review. *Med Sci Sports Exerc.* 2012;44:818–826.
579. Handelsman DJ, Hirschberg AL, Berman S. Circulating testosterone as the hormonal basis of sex differences in athletic performance. *Endocr Rev.* 2018;39:803–829.
580. Rosenthal SM. Approach to the patient: transgender youth: endocrine considerations. *J Clin Endocrinol Metab.* 2014;99:4379–4389.
581. Bailey JM, Vasey PL, Diamond LM, Breedlove SM, Vilain E, Epprecht M. Sexual orientation, controversy, and science. *Psychol Sci Public Interest.* 2016;17:45–101.
582. Arnold AP. A general theory of sexual differentiation. *J Neurosci Res.* 2017;95:291–300.
583. Dewing P, Shi T, Horvath S, Vilain E. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Brain Res Mol Brain Res.* 2003;118:82–90.
584. Carruth LL, Reisert I, Arnold AP. Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci.* 2002;5:933–934.
585. Arnold AP, Chen X. What does the “four core genotypes” mouse model tell us about sex differences in the brain and other tissues? *Front Neuroendocrinol.* 2002;30:1–9.
586. Gregg C, Zhang J, Butler JE, Haig D, Dulac C. Sex-specific parent-of-origin allelic expression in the mouse brain. *Science.* 2010;329:682–685.
587. Nugent BM, Wright CL, Shetty AC, et al. Brain feminization requires active repression of masculinization via DNA methylation. *Nat Neurosci.* 2015;18:690–697.
588. Schulz KM, Sisk CL. The organizing actions of adolescent gonadal steroid hormones on brain and behavioral development. *Neurosci Biobehav Rev.* 2016;70:148–158.
589. Piekarski DJ, Johnson CM, Boivin JR, et al. Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex? *Brain Res.* 2017;1654:123–144.
590. Koss WA, Frick KM. Sex differences in hippocampal function. *J Neurosci Res.* 2017;95:539–562.
591. Vige A, Gallou-Kabani C, Junien C. Sexual dimorphism in non-mendelian inheritance. *Pediatr Res.* 2008;63:340–347.
592. Sherwin BB. Estrogen and cognitive functioning in women. *Endocr Rev.* 2003;24:133–151. 2003.
593. McCarthy MM, Wright CL. Convergence of sex differences and the neuroimmune system in autism spectrum disorder. *Biol Psychiatry.* 2017;81:402–410.
594. Davis EC, Popper P, Gorski RA. The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. *Brain Res.* 1996;734:10–18.
595. Schulz KM, Zehr JL, Salas-Ramirez KY, Sisk CL. Testosterone programs adult social behavior before and during, but not after, adolescence. *Endocrinology.* 2009;150:3690–3698.
596. Lu SF, McKenna SE, Cologer-Clifford A, Nau EA, Simon NG. Androgen receptor in mouse brain: sex differences and similarities in autoregulation. *Endocrinology.* 1998;139:1594–1601.
597. Cooke BM, Tabibnia G, Breedlove SM. A brain sexual dimorphism controlled by adult circulating androgens. *Proc Natl Acad Sci U S A.* 1999;96:7538–7540.
598. Piekarski DJ, Boivin JR, Wilbrecht L. Ovarian hormones organize the maturation of inhibitory neurotransmission in the frontal cortex at puberty onset in female mice. *Curr Biol.* 2017;27:1735–1745. e3.
599. Swaab DF, Hofman MA. Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation. *Trends Neurosci.* 1995;18:264–270.
600. Hughes IA, Houk C, Ahmed SF, Lee PA. Consensus statement on management of intersex disorders. *Arch Dis Child.* 2006;91:554–563.
601. Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry.* 2007;62:847–855.
602. Herting MM, Sowell ER. Puberty and structural brain development in humans. *Front Neuroendocrinol.* 2017;44:122–137.
603. Heany SJ, van Honk J, Stein DJ, Brooks SJ. A quantitative and qualitative review of the effects of testosterone on the function and structure of the human social-emotional brain. *Metab Brain Dis.* 2016;31:157–167.
604. Joel D, Berman Z, Tavor I, et al. Sex beyond the genitalia: The human brain mosaic. *Proc Natl Acad Sci U S A.* 2015;112:15468–15473.
605. Auyeung B, Lombardo MV, Baron-Cohen S. Prenatal and postnatal hormone effects on the human brain and cognition. *Pflugers Arch.* 2013;465:557–571.
606. Seiger R, Hahn A, Hummer A, et al. Subcortical gray matter changes in transgender subjects after long-term cross-sex hormone administration. *Psychoneuroendocrinology.* 2016;74:371–379.
607. Gladue B, Clemens L. Flutamide inhibits testosterone-induced masculine sexual behavior in male and female rats. *Endocrinology.* 1980;106:1917–1922.
608. Poletti A, Negri-Cesi P, Rabuffetti M, Colciago A, Celotti F, Martini L. Transient expression of the 5alpha-reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. *Endocrinology.* 1988;139:2171–2178.
609. Abdelgadir S, Resko J, Ojeda S, Lephart E, McPhaul M, Roselli C. Androgens regulate aromatase cytochrome P450 messenger ribonucleic acid in rat brain. *Endocrinology.* 1994;135:395–401.
610. Beyer C, Green S, Hutchison J. Androgens influence sexual differentiation of embryonic mouse hypothalamic aromatase neurons in vitro. *Endocrinology.* 1994;135:1220–1226.
611. Roselli CE, Klosterman SA. Sexual differentiation of aromatase activity in the rat brain: effects of perinatal steroid exposure. *Endocrinology.* 1998;139:3193–3201.
612. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-

- related behaviors in female mice. *Endocrinology*. 1998;139:5070–5081.
613. Wright CL, McCarthy MM. Prostaglandin E2-induced masculinization of brain and behavior requires protein kinase A, AMPA/kainate, and metabotropic glutamate receptor signaling. *J Neurosci*. 2009;29:13274–13282.
 614. Bramble MS, Roach L, Lipson A, et al. Sex-specific effects of testosterone on the sexually dimorphic transcriptome and epigenome of embryonic neural stem/progenitor cells. *Sci Rep*. 2016;6:36916.
 615. Baron-Cohen S, Auyeung B, Norgaard-Pedersen B, et al. Elevated fetal steroidogenic activity in autism. *Mol Psychiatry*. 2015;20:369–376.
 616. Cooke BM, Woolley CS. Gonadal hormone modulation of dendrites in the mammalian CNS. *J Neurobiol*. 2005;64:34–46.
 617. Naftolin F, Garcia-Segura LM, Horvath TL, et al. Estrogen-induced hypothalamic synaptic plasticity and pituitary sensitization in the control of the estrogen-induced gonadotrophin surge. *Reprod Sci*. 2007;14:101–116.
 618. Shughrue P, Stumpf W, Elger W, Schulze P-E, Sar M. Progesterin receptor cells in the 8-day-old male and female mouse cerebral cortex: autoradiographic evidence for a sexual dimorphism in target cell number. *Endocrinology*. 1991;128:87–95.
 619. Mani S, Blaustein J, Allen J, Law S, O'Malley B, Clark J. Inhibition of rat sexual behavior by antisense oligonucleotides to the progesterone receptor. *Endocrinology*. 1994;135:1409–1414.
 620. Brotfain E, Gruenbaum SE, Boyko M, Kutz R, Zlotnik A, Klein M. Neuroprotection by estrogen and progesterone in traumatic brain injury and spinal cord injury. *Curr Neuropharmacol*. 2016;14:641–653.
 621. Schwamm LH. Progesterone for traumatic brain injury—resisting the sirens' song. *N Engl J Med*. 2014;371. 2522–2523.
 622. Tsuji M, Taguchi A, Ohshima M, Kasahara Y, Ikeda T. Progesterone and allopregnanolone exacerbate hypoxic-ischemic brain injury in immature rats. *Exp Neurol*. 2012;233:214–220.
 623. Levy J, Heller W. Gender differences in human neuropsychological function. In: Gerall AA, Moltz H, Ward I, eds. *Sexual Differentiation: A Lifespan Approach, Handbook of Behavioral Neurobiology*. New York: Plenum Press; 1992:245–274.
 624. Levine SC, Huttenlocher J, Taylor A, Langrock A. Early sex differences in spatial skill. *Dev Psychol*. 1999;35:940–949.
 625. Hyde JS, Mertz JE. Gender, culture, and mathematics performance. *Proc Natl Acad Sci U S A*. 2009;106:8801–8807.
 626. Schoentjes E, Deboutte D, Friedrich W. Child sexual behavior inventory: A Dutch-speaking normative sample. *Pediatrics*. 1999;104:885–893.
 627. Finkelstein JW, Susman EJ, Chinchilli VM, et al. Estrogen or testosterone increases self-reported aggressive behaviors in hypogonadal adolescents. *J Clin Endocrinol Metab*. 1997;82:2433–2438.
 628. Finkelstein JW, Susman EJ, Chinchilli VM, et al. Effects of estrogen or testosterone on self-reported sexual responses and behaviors in hypogonadal adolescents. *J Clin Endocrinol Metab*. 1998;83:2281–2285.
 629. Wilson JD. Androgens, androgen receptors, and male gender role behavior. *Horm Behav*. 2001;40:358–366.
 630. Burke SM, Manzouri AH, Savic I. Structural connections in the brain in relation to gender identity and sexual orientation. *Sci Rep*. 2017;7:17954.
 631. Manzouri A, Savic I. Cerebral sex dimorphism and sexual orientation. *Hum Brain Mapp*. 2018;39:1175–1186.
 632. Berglund H, Lindstrom P, Savic I. Brain response to putative pheromones in lesbian women. *Proc Natl Acad Sci U S A*. 2006;103:8269–8274.
 633. Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H. Genetic variation in a human odorant receptor alters odour perception. *Nature*. 2007;449:468–472.
 634. Stern K, McClintock MK. Regulation of ovulation by human pheromones [see comments]. *Nature*. 1998;392:177–179.
 635. Jacob S, McClintock MK. Psychological state and mood effects of steroidal chemosignals in women and men. *Horm Behav*. 2000;37:57–78.
 636. Yoon H, Enquist LW, Dulac C. Olfactory inputs to hypothalamic neurons controlling reproduction and fertility. *Cell*. 2005;123:669–682.
 637. Cohen L, Morgan J, Bozyk ME. Effects of the simultaneous administration of diethylstilbestrol and prednisolone on serum enzymes in Duchenne's muscular dystrophy. *J Med*. 1977;8:123–134.
 638. Chiaroni-Clarke RC, Munro JE, Ellis JA. Sex bias in paediatric autoimmune disease - Not just about sex hormones? *J Autoimmun*. 2016;69:12–23.
 639. Papanicolaou DA, Vgontzas AN. Interleukin-6: the endocrine cytokine [editorial; comment]. *J Clin Endocrinol Metab*. 2000;85:1331–1333.
 640. Dragin N, Bismuth J, Cizeron-Clairac G, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J Clin Invest*. 2016;126:1525–1537.
 641. Moreira-Filho CA, Bando SY, Bertonha FB, et al. Minipuberty and sexual dimorphism in the infant human thymus. *Sci Rep*. 2018;8:13169.
 642. Chow RW, Handelsman DJ, Ng MK. Minireview: rapid actions of sex steroids in the endothelium. *Endocrinology*. 2010;151:2411–2422.
 643. O'Donnell E, Goodman JM, Harvey PJ. Clinical review: Cardiovascular consequences of ovarian disruption: a focus on functional hypothalamic amenorrhea in physically active women. *J Clin Endocrinol Metab*. 2011;96:3638–3648.
 644. Rosing J, Middeldorp S, Curvers J, et al. Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study. *Lancet*. 1999;354:2036–2040.
 645. Lidegaard O, Lokkegaard E, Svendsen AL, Agger C. Hormonal contraception and risk of venous thromboembolism: national follow-up study. *BMJ*. 2009;339:b2890.
 646. Jick SS, Hernandez RK. Risk of non-fatal venous thromboembolism in women using oral contraceptives containing drospirenone compared with women using oral contraceptives containing levonorgestrel: case-control study using United States claims data. *BMJ*. 2011;342:d2151.
 647. Martin KA, Anderson RR, Chang RJ, et al. Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2018;103:1–25.
 648. Lidegaard O, Lokkegaard E, Jensen A, Skovlund CW, Keiding N. Thrombotic stroke and myocardial infarction with hormonal contraception. *N Engl J Med*. 2012;366:2257–2266.
 649. Wang X, Magkos F, Mittendorfer B. Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones. *J Clin Endocrinol Metab*. 2011;96:885–893.
 650. Forest M. Function of the ovary in the neonate and infant. *Eur J Obstet Gynecol Reprod Biol*. 1979;9:145–160.
 651. Kaplan SL, Edgar JC, Ford EG, et al. Size of testes, ovaries, uterus and breast buds by ultrasound in healthy full-term neonates ages 0-3 days. *Pediatr Radiol*. 2016;46:1837–1847.
 652. Solomon LM, Esterly NB. Neonatal dermatology. I. The newborn skin. *J Pediatr*. 1970;77:888–894.
 653. Greaves RF, Pitkin J, Ho CS, Baglin J, Hunt RW, Zacharin MR. Hormone modeling in preterm neonates: establishment of pituitary and steroid hormone reference intervals. *J Clin Endocrinol Metab*. 2015;100:1097–1103.
 654. Endocrine Sciences/LabCorp. *Expected Values and S.I. Unit Conversion Tables*. Calabasas Hills, CA: Esoterix Laboratory Services, Inc.; 2018. <https://www.endocrinesciences.com/sites/default/files/Endocrine%20Sciences%20Expected%20Values.pdf>.
 655. de Peretti E, Forest MG. Pitfalls in the etiological diagnosis of congenital adrenal hyperplasia in the early neonatal period. *Horm Res*. 1982;16:10–22.
 656. Mortensen M, Ehrmann DA, Littlejohn E, Rosenfield RL. Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. *J Clin Endocrinol Metab*. 2009;94:1579–1586.
 657. McKiernan JF, Hull D. Breast development in the newborn. *Arch Dis Child*. 1981;56:525–529.
 658. Kuiri-Hanninen T, Haanpaa M, Turpeinen U, et al. Postnatal ovarian activation has effects in estrogen target tissues in infant girls. *J Clin Endocrinol Metab*. 2013;98:4709–4716.
 659. Kuiri-Hanninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: minipuberty. *Horm Res Paediatr*. 2014;82:73–80.
 660. Hingre RV, Gross SJ, Hingre KS, Mayes DM, Richman RA. Adrenal steroidogenesis in very low birth weight preterm infants. *J Clin Endocrinol Metab*. 1994;78:266–270.
 661. Langlois D, Li JY, Saez JM. Development and function of the human fetal adrenal cortex. *J Pediatr Endocrinol Metab*. 2002;15 (Suppl 5):1311–1322.

662. Heckmann M, Hartmann MF, Kampschulte B, et al. Persistent high activity of the fetal adrenal cortex in preterm infants: is there a clinical significance? *J Pediatr Endocrinol Metab.* 2006;19:1303–1312.
663. Nykanen P, Heinonen K, Riepe FG, Sippell WG, Voutilainen R. Serum concentrations of adrenal steroids and their precursors as a measure of maturity of adrenocortical function in very premature newborns. *Horm Res Paediatr.* 2010;74:358–364.
664. Kamrath C, Hartmann MF, Boettcher C, Wudy SA. Reduced activity of 11beta-hydroxylase accounts for elevated 17alpha-hydroxyprogesterone in preterms. *J Pediatr.* 2014;165:280–284.
665. Sedin G, Bergquist C, Lindgren PG. Ovarian hyperstimulation syndrome in preterm infants. *Pediatr Res.* 1985;19:548–552.
666. Esen I, Demirel F. Images in clinical medicine. Preterm ovarian hyperstimulation. *N Engl J Med.* 2015;372:2336.
667. Vogiatzi MG, Pitt M, Oberfield S, Alter CA. Menstrual bleeding as a manifestation of mini-puberty of infancy in severe prematurity. *J Pediatr.* 2016;178:292–295.
668. Feuillan PP, Jones JV, Barnes K, Oerter-Klein K, Cutler Jr GB. Reproductive axis after discontinuation of gonadotropin-releasing hormone analog treatment of girls with precocious puberty: long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. *J Clin Endocrinol Metab.* 1999;84:44–49.
669. Lindhardt Johansen M, Hagen CP, Johannsen TH, et al. Anti-mullerian hormone and its clinical use in pediatrics with special emphasis on disorders of sex development. *Int J Endocrinol.* 2013;2013:198698.
670. Hagen CP, Aksglaede L, Sorensen K, et al. Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab.* 2010;95:5003–5010.
671. Rosenfield RL. Normal and almost normal variants of precocious puberty. Premature pubarche and premature thelarche revisited. *Horm Res.* 1994;41(Suppl 2):7–13.
672. Potau N, Ibanez L, Sentis M, Carrascosa A. Sexual dimorphism in the maturation of the pituitary-gonadal axis, assessed by GnRH agonist challenge. *Eur J Endocrinol.* 1999;141:27–34.
673. Brito VN, Batista MC, Borges MF, et al. Diagnostic value of fluorometric assays in the evaluation of precocious puberty. *J Clin Endocrinol Metab.* 1999;84:3539–3544.
674. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. *J Clin Endocrinol Metab.* 2007;92:1424–1429.
675. Maesaka H, Tachibana K, Adachi M, Okada T. Monthly urinary gonadotropin and ovarian hormone excretory patterns in normal girls and female patients with idiopathic precocious puberty. *Pediatr Res.* 1996;40:853–8560.
676. Bordini BD, Littlejohn EE, Rosenfield RL. LH dynamics in overweight girls with premature adrenarche and slowly progressive sexual precocity. *Int J Pediatr Endocrinol.* 2010;2010:724696.
677. Goodpasture J, Ghai K, Cara J, Rosenfield R. Potential of gonadotropin-releasing hormone agonists in the diagnosis of pubertal disorders in girls. *Clin Obstet Gynecol.* 1993;36:773–785.
678. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab.* 2006;91:4057–4063.
679. Depmann M, Faddy MJ, van der Schouw YT, et al. The relationship between variation in size of the primordial follicle pool and age at natural menopause. *J Clin Endocrinol Metab.* 2015;100. E845–851.
680. Ehara Y, Yen S, Siler T. Serum prolactin levels during puberty. *Am J Obstet Gynecol.* 1975;121:995.
681. Marshall W, Tanner J. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291.
682. Ross GT, Vande Wiele R. The ovary. In: Williams R, ed. *Textbook of Endocrinology.* 5th. ed. Philadelphia: WB Saunders Company; 1974.
683. Rosenfield RL, Bachrach LK, Chernausek SD, et al. Current age of onset of puberty. *Pediatrics.* 2000;106:622–623.
684. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. Relation of age at menarche to race, time period, and anthropometric dimensions: the Bogalusa Heart Study. *Pediatrics.* 2002;110:e43.
685. Demerath EW, Towne B, Chumlea WC, et al. Recent decline in age at menarche: the Fels Longitudinal Study. *Am J Hum Biol.* 2004;16:453–457.
686. Biro FM, Greenspan LC, Galvez MP, et al. Onset of breast development in a longitudinal cohort. *Pediatrics.* 2013;132:1019–1027.
687. Biro FM, Pajak A, Wolff MS, et al. Age of menarche in a longitudinal US cohort. *J Pediatr Adolesc Gynecol.* 2018;31:339–345.
688. Biro FM, Huang B, Crawford PB, et al. Pubertal correlates in black and white girls. *J Pediatr.* 2006;148:234–240.
689. Pereira A, Corvalan C, Uauy R, Klein KO, Mericq V. Ultrasensitive estrogen levels at 7 years of age predict earlier thelarche: evidence from girls of the growth and obesity Chilean cohort. *Eur J Endocrinol.* 2015;173:835–842.
690. Bordini BD, Littlejohn EE, Rosenfeld RL. Blunted sleep-related LH rise in healthy premenarcheal pubertal girls with elevated body mass index. *J Clin Endocrinol Metab.* 2009;94:1168–1175 [Comment in: *J Clin Endocrinol Metab.* 94, 1094–1096].
691. Ibañez L, Jimenez R, de Zegher F. Early puberty-menarche after precocious pubarche: relation to prenatal growth. *Pediatrics.* 2006;117:117–121.
692. Tanner JM, Davies PS. Clinical longitudinal standards for height and height velocity for North American children [see comments]. *J Pediatr.* 1985;107:317–329.
693. Bennett D, Ward M, Daniel WJ. The relationship of serum alkaline phosphatase concentrations to sex maturity ratings in adolescents. *J Pediatr.* 1976;88:633.
694. de Ridder CM, Thijssen JH, Bruning PF, Van den Brande JL, Zonderland ML, Erich WB. Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. *J Clin Endocrinol Metab.* 1992;75:442–446.
695. Treloar A, Boynton R, Benn B, Brown B. Variation of human menstrual cycle through reproductive life. *Int J Fertil.* 1967;12:77–84–126.
696. Rosenfield RL. Plasma free androgen patterns in hirsute women and their diagnostic implications. *Am J Med.* 1979;66:417–421.
697. Vekemans M, Delvoe P, L'Hermite M, Robyn C. Serum prolactin levels during the menstrual cycle. *J Clin Endocrinol Metab.* 1977;44:989.
698. Kolodny R, Jacobs L, Daughaday W. Mammary stimulation causes prolactin secretion in non-lactating women. *Nature.* 1972;238:284.
699. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network [see comments]. *Pediatrics.* 1997;99:505–512.
700. Maura N, Santen RJ, Colon-Otero G, et al. Estrogens and their genotoxic metabolites are increased in obese prepubertal girls. *J Clin Endocrinol Metab.* 2015;100:2322–2328.
701. Klein KO, Mericq V, Brown-Dawson JM, Larmore KA, Cabezas P, Cortinez A. Estrogen levels in girls with premature thelarche compared with normal prepubertal girls as determined by an ultrasensitive recombinant cell bioassay. *J Pediatr.* 1999;134:190–192.
702. Bizzarri C, Spadoni GL, Bottaro G, et al. The response to gonadotropin releasing hormone (GnRH) stimulation test does not predict the progression to true precocious puberty in girls with onset of premature thelarche in the first three years of life. *J Clin Endocrinol Metab.* 2014;99:433–439.
703. Kreiter M, Cara J, Rosenfield R. Modifying the outcome of complete precocious puberty. To treat or not to treat. In: Grave G, Cutler G, eds. *Sexual Precocity: Etiology, Diagnosis, and Management.* NY: Raven Press; 1993:109–120.
704. Roman R, Johnson MC, Codner E, Boric MA, aVila, A., Cassorla, F. Activating GNAS1 gene mutations in patients with premature thelarche. *J Pediatr.* 2004;145:218–222.
705. Lappalainen S, Utriainen P, Kuulasmaa T, Voutilainen R, Jaaskelainen J. Androgen receptor gene CAG repeat polymorphism and X-chromosome inactivation in children with premature adrenarche. *J Clin Endocrinol Metab.* 2008;93:1304–1309.
706. Rosenfield RL, Wroblewski K, Padmanabhan V, Littlejohn E, Mortensen M, Ehrmann DA. Antimullerian hormone levels are independently related to ovarian hyperandrogenism and polycystic ovaries. *Fertil Steril.* 2012;98:242–249.

707. Mantyselka A, Jaaskelainen J, Lindi V, et al. The presentation of adrenarche is sexually dimorphic and modified by body adiposity. *J Clin Endocrinol Metab.* 2014;99:3889–3894.
708. Marakaki C, Karapanou O, Gryparis A, Hochberg Z, Chrousos G, Papadimitriou A. Early adiposity rebound and premature adrenarche. *J Pediatr.* 2017;186:72–77.
709. Binder G, Schweizer R, Blumenstock G, Ferrand N. Adrenarche in Silver-Russell syndrome: timing and consequences. *J Clin Endocrinol Metab.* 2017;102:4100–4108.
710. Rosenfield RL, Rich BH, Lucky AW. Adrenarche as a cause of benign pseudopuberty in boys. *J Pediatr.* 1982;101:1005–1009.
711. Rappaport E, Forest M, Blayard F, Dewal-Beaupere G, Blizzard R, Migeon C. Plasma androgens and LH in scoliotic patients with premature pubarche. *J Clin Endocrinol Metab.* 1974;38:401.
712. Goodarzi MO, Carmina E, Azziz R. DHEA, DHEAS and PCOS. *J Steroid Biochem Mol Biol.* 2015;145:213–225.
713. Witchel SF, Lee PA, Suda-Hartman M, Hoffman EP. Hyperandrogenism and manifesting heterozygotes for 21-hydroxylase deficiency. *Biochem Mol Med.* 1997;62:151–158.
714. Nayak S, Lee PA, Witchel SF. Variants of the type II 3beta-hydroxysteroid dehydrogenase gene in children with premature pubic hair and hyperandrogenic adolescents. *Mol Genet Metab.* 1998;64:184–192.
715. Zhou R, Bird IM, Dumesic DA, Abbott DH. Adrenal hyperandrogenism is induced by fetal androgen excess in a rhesus monkey model of polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2005;90:6630–6637.
716. Buhl CS, Stodkilde-Jorgensen H, Videbech P, et al. Escitalopram ameliorates hypercortisolemia and insulin resistance in low birth weight men with limbic brain alterations. *J Clin Endocrinol Metab.* 2018;103:115–124.
717. Rosenfield RL. Premature adrenarche. In: Rose B, ed. *UpToDate*. Waltham, MA: UpToDate; 2018. www.uptodate.com/index.
718. Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *J Clin Endocrinol Metab.* 2008;93:723–728.
719. Sykiotis GP, Plummer L, Hughes VA, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A.* 2010;107:15140–15144.
720. Caronia LM, Martin C, Welt CK, et al. A genetic basis for functional hypothalamic amenorrhea. *N Engl J Med.* 2011;364:215–225.
721. Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L. Association of the timing of puberty with a chromosome 2 locus. *J Clin Endocrinol Metab.* 2008;93:4833–4839.
722. Cousminer DL, Leinonen JT, Sarin AP, et al. Targeted resequencing of the pericentromere of chromosome 2 linked to constitutional delay of growth and puberty. *PLoS One.* 2015;10:e0128524.
723. Howard SR, Guasti L, Ruiz-Babot G, et al. IGSF10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty. *EMBO Mol Med.* 2016;8:626–642.
724. Sun Y, Bak B, Schoenmakers N, et al. Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement. *Nat Genet.* 2012;44:1375–1381.
725. Joustra SD, Wehkalampi K, Oostdijk W, et al. IGSF1 variants in boys with familial delayed puberty. *Eur J Pediatr.* 2015;174:687–692.
726. Tomberg J, Sykiotis GP, Keefe K, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A.* 2011;108:11524–11529.
727. Pitteloud N, Acierno Jr JS, Meysing A, et al. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A.* 2006;103:6281–6286.
728. Xu C, Messina A, Somm E, et al. KLB, encoding beta-Klotho, is mutated in patients with congenital hypogonadotropic hypogonadism. *EMBO Mol Med.* 2017;9:1379–1397.
729. Zhu J, Choa RE, Guo MH, et al. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2015;100:E646–654.
730. Cassatella D, Howard SR, Acierno JS, et al. Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures. *Eur J Endocrinol.* 2018;178:377–388.
731. Chevrier L, Guimiot F, de Roux N. GnRH receptor mutations in isolated gonadotropic deficiency. *Mol Cell Endocrinol.* 2011;346:21–28.
732. Lin L, Conway GS, Hill NR, Dattani MT, Hindmarsh PC, Achermann JC. A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of growth and puberty with subsequent borderline oligospermia. *J Clin Endocrinol Metab.* 2006;91:5117–5121.
733. Vaaralampi K, Wehkalampi K, Tommiska J, Laitinen EM, Dunkel L, Raivio T. The role of gene defects underlying isolated hypogonadotropic hypogonadism in patients with constitutional delay of growth and puberty. *Fertil Steril.* 2011;95:2756–2758.
734. Chevalley T, Bonjour JP, Ferrari S, Rizzoli R. The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche. *J Clin Endocrinol Metab.* 2009;94:3424–3431.
735. Apter D, Viinikka L, Vihko R. Hormonal pattern of adolescent menstrual cycles. *J Clin Endocrinol Metab.* 1978;47:944–954.
736. Rosenfield RL. Clinical review: Adolescent anovulation: maturational mechanisms and implications. *J Clin Endocrinol Metab.* 2013;98:3572–3583.
737. Metcalf MG, Skidmore DS, Lowry GF, Mackenzie JA. Incidence of ovulation in the years after the menarche. *J Endocrinol.* 1983;97:213–219.
738. Sun BZ, Kangaroo T, Adams JM, et al. Healthy post-menarchal adolescent girls demonstrate multi-level reproductive axis immaturity. *J Clin Endocrinol Metab.* 2018;104:613–623.
739. Vollman RF. The menstrual cycle. *Major Prob Obstet Gynecol.* 1977;7:1–193.
740. Apter D, Vihko R. Serum pregnenolone, progesterone, 17-hydroxyprogesterone, testosterone, and 5alpha-dihydrotestosterone during female puberty. *J Clin Endocrinol Metab.* 1977;45:1039–1048.
741. American Academy of Pediatrics Committee on Adolescence; American College of Obstetricians and Gynecologists Committee on Adolescent Health Care, Diaz A, Laufer MR, Breech LL. Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics.* 2006;118:2245–2250.
742. Sieberg R, Nilsson CG, Stenman UH, Widholm O. Endocrinologic features of oligomenorrheic adolescent girls. *Fertil Steril.* 1986;46:852–857.
743. Venturoli S, Porcu E, Fabbri R, et al. Menstrual irregularities in adolescents: Hormonal pattern and ovarian morphology. *Horm Res.* 1986;24:269–279.
744. van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasings RA, Koppelaar C, Schoemaker J. Polycystic ovaries in adolescents and the relationship with menstrual cycle patterns, luteinizing hormone, androgens, and insulin. *Fertil Steril.* 2000;74:49–58.
745. Mortensen M, Rosenfield RL, Littlejohn E. Functional significance of polycystic-size ovaries in healthy adolescents. *J Clin Endocrinol Metab.* 2006;91:3786–3790.
746. Codner E, Villarreal C, Eyzaguirre FC, et al. Polycystic ovarian morphology in postmenarchal adolescents. *Fertil Steril.* 2011;95:702–706.
747. Lucky AW, Biro FM, Simbartl LA, Morrison JA, Sorg NW. Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study [see comments]. *J Pediatr.* 1997;130:30–39.
748. McClintock M. Menstrual synchrony and suppression. *Nature.* 1971;229:244.
749. Steinberg L, Morris AS. Adolescent development. *Annu Rev Psychol.* 2001;52:83–110.
750. Susman EJ, Finkelstein JW, Chinchilli VM, et al. The effect of sex hormone replacement therapy on behavior problems and moods in adolescents with delayed puberty. *J Pediatr.* 1998;133:521–525.
751. Dewald JF, Meijer AM, Oort FJ, Kerkhof GA, Bogels SM. The influence of sleep quality, sleep duration and sleepiness on school performance in children and adolescents: A meta-analytic review. *Sleep Med Rev.* 2010;14:179–189.
752. Feinberg I, Higgins LM, Khaw WY, Campbell IG. The adolescent decline of NREM delta, an indicator of brain maturation, is linked

- to age and sex but not to pubertal stage. *Am J Physiol Regul Integr Comp Physiol.* 2006;291: R1724–1729.
753. McHill AW, Klerman EB, Slater B, Kangaroo T, Mankowski PW, Shaw ND. The relationship between estrogen and the decline in delta power during adolescence. *Sleep.* 2017;40.
 754. Nada SE, Thompson RC, Padmanabhan V. Developmental programming: differential effects of prenatal testosterone excess on insulin target tissues. *Endocrinology.* 2010;151:5165–5173.
 755. Aono T, Miyake A, Kinugasa T, Kurachi K, Matsumoto K. Absence of positive feedback effect of oestrogen on LH release in patients with testicular feminization syndrome. *Acta Endocrinol (Copenh).* 1978;87:259–267.
 756. Khanna K, Sharma S, Gupta DK. Hydrometrocolpos etiology and management: past beckons the present. *Pediatr Surg Int.* 2018;34:249–261.
 757. Golan A, Langer R, Bukovsky I, Caspi E. Congenital anomalies of the müllerian system. *Fertil Steril.* 1989;51:747–754.
 758. Philibert P, Bignon-Laubert A, Rouzier R, et al. Identification and functional analysis of a new WNT4 gene mutation among 28 adolescent girls with primary amenorrhea and Müllerian duct abnormalities: A French collaborative study. *J Clin Endocrinol Metab.* 2008;93:895–900.
 759. Carel JC, Eugster EA, Rogol A, et al. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics.* 2009;123. e752–762.
 760. Brauner R, Adan L, Malandry F, Zantleifer D. Adult height in girls with idiopathic true precocious puberty. *J Clin Endocrinol Metab.* 1994;79:415–420.
 761. Junier M-P, Wolff A, Hoffman G, Ma Y, Ojeda S. Effect of hypothalamic lesions that induce precocious puberty on the morphological and functional maturation of the luteinizing hormone-releasing hormone neuronal system. *Endocrinology.* 1992;131:787–798.
 762. Cohen LE. Endocrine late effects of cancer treatment. *Endocrinol Metab Clin N Am.* 2005;34:769–789.
 763. Cacciari E, Zucchini S, Ambrosetto P, et al. Empty sella in children and adolescents with possible hypothalamic-pituitary disorders. *J Clin Endocrinol Metab.* 1994;78:767–771.
 764. Laue I, Comite F, Hench K, Loriaux D, Cutler CJ, Pescovitz O. Precocious puberty associated with neurofibromatosis and optic gliomas. *Am J Dis Child.* 1985;139:1097.
 765. Zacharin M. Precocious puberty in two children with neurofibromatosis type I in the absence of optic chiasmal glioma [see comments]. *J Pediatr.* 1997;130:155–157.
 766. Mahachoklertwattana P, Kaplan S, Grumbach M. The luteinizing hormone-releasing hormone-secreting hypothalamic hamartoma is a congenital malformation: natural history. *J Clin Endocrinol Metab.* 1993;77:118–124.
 767. Jung H, Carmel P, Schwartz MS, et al. Some hypothalamic hamartomas contain transforming growth factor alpha, a puberty-inducing growth factor, but not luteinizing hormone-releasing hormone neurons. *J Clin Endocrinol Metab.* 1999;84:4695–4701.
 768. Kitay J, Altschule M. *The Pineal Gland.* Cambridge, MA, Harvard Univ Press; 1964.
 769. Cohen R, Wurtman R, Axelrod J, et al. Some clinical, biochemical, and physiological actions of the pineal gland. *Ann Intern Med.* 1964;61:1144.
 770. Hibi I, Fujiwara K. Precocious puberty of cerebral origin: a cooperative study in Japan. *Prog Exp Tumor Res.* 2001;30:224–238.
 771. Starzyk J, Starzyk B, Bartnik-Mikuta A, Urbanowicz W, Dziatkowiak H. Gonadotropin releasing hormone-independent precocious puberty in a 5 year-old girl with suprasellar germ cell tumor secreting beta-hCG and alpha-fetoprotein. *J Pediatr Endocrinol Metab.* 2001;14:789–796.
 772. O'Marcaigh AS, Ledger GA, Roche PC, Parisi JE, Zimmerman D. Aromatase expression in human germinomas with possible biological effects. *J Clin Endocrinol Metab.* 1995;80:3763–3766.
 773. Baer K. Premature ovarian failure and precocious puberty. *Obstet Gynecol.* 1977;49:15s.
 774. Quigley C, Cowell C, Jimenez M, et al. Normal or early development of puberty despite gonadal damage in children treated for acute lymphoblastic leukemia. *N Engl J Med.* 1989;321: 143–151.
 775. Root AW, Moshang Jr T. Evolution of the hyperandrogenism-polycystic ovary syndrome from isosexual precocious puberty: report of two cases. *Am J Obstet Gynecol.* 1984;149:763–767.
 776. Lazar L, Meyerovitch J, de Vries L, Phillip M, Lebenthal Y. Treated and untreated women with idiopathic precocious puberty: long-term follow-up and reproductive outcome between the third and fifth decades. *Clin Endocrinol.* 2014;80:570–576.
 777. Teles MG, Bianco SD, Brito VN, et al. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med.* 2008;358:709–715.
 778. Silveira LG, Noel SD, Silveira-Neto AP, et al. Mutations of the KISS1 gene in disorders of puberty. *J Clin Endocrinol Metab.* 2010;95:2276–2280.
 779. Shin YL. An update on the genetic causes of central precocious puberty. *Ann Pediatr Endocrinol Metab.* 2016;21:66–69.
 780. Liu H, Kong X, Chen F. Mkrn3 functions as a novel ubiquitin E3 ligase to inhibit Nptx1 during puberty initiation. *Oncotarget.* 2017;8:85102–85109.
 781. Macedo DB, Abreu AP, Reis AC, et al. Central precocious puberty that appears to be sporadic caused by paternally inherited mutations in the imprinted gene makorin ring finger 3. *J Clin Endocrinol Metab.* 2014;99. E1097–1103.
 782. Settas N, Dacou-Voutetakis C, Karantza M, Kanakagantenbein C, Chrousos GP, Voutetakis A. Central precocious puberty in a girl and early puberty in her brother caused by a novel mutation in the MKRN3 gene. *J Clin Endocrinol Metab.* 2014;99. E647–651.
 783. Schreiner F, Gohlke B, Hamm M, Korsch E, Woelfle J. MKRN3 mutations in familial central precocious puberty. *Horm Res Paediatr.* 2014;82:122–126.
 784. de Vries L, Gat-Yablonski G, Dror N, Singer A, Phillip M. A novel MKRN3 missense mutation causing familial precocious puberty. *Hum Reprod (Oxford, England).* 2014;29:2838–2843.
 785. Bulcao Macedo D, Nahime Brito V, Latronico AC. New causes of central precocious puberty: the role of genetic factors. *Neuroendocrinology.* 2014;100:1–8.
 786. Neocleous V, Shammas C, Phelan MM, Nicolaou S, Phylactou LA, Skordis N. In silico analysis of a novel MKRN3 missense mutation in familial central precocious puberty. *Clin Endocrinol.* 2016;84:80–84.
 787. Lee HS, Jin HS, Shim YS, et al. Low Frequency of MKRN3 Mutations in Central Precocious Puberty Among Korean Girls. *Horm Metab Res.* 2016;48:118–122.
 788. Bessa DS, Macedo DB, Brito VN, et al. High frequency of MKRN3 mutations in male central precocious puberty previously classified as idiopathic. *Neuroendocrinology.* 2017;105:17–25.
 789. Dauber A, Cunha-Silva M, Macedo DB, et al. Paternally Inherited DLK1 Deletion associated with familial central precocious puberty. *J Clin Endocrinol Metab.* 2017;102:1557–1567.
 790. Falix FA, Aronson DC, Lamers WH, Gaemers IC. Possible roles of DLK1 in the Notch pathway during development and disease. *Biochim Biophys Acta.* 2012;1822:988–995.
 791. Grandone A, Capristo C, Cirillo G, et al. Molecular screening of MKRN3, DLK1, and KCNK9 genes in girls with idiopathic central precocious puberty. *Horm Res Paediatr.* 2017;88:194–200.
 792. Partsch CJ, Japing I, Siebert R, et al. Central precocious puberty in girls with Williams syndrome. *J Pediatr.* 2002;141:441–444.
 793. Hoffmann K, Heller R. Uniparental disomies 7 and 14. *Best Pract Res Clin Endocrinol Metab.* 2011;25:77–100.
 794. Grosso S, Balestri P, Anichini C, et al. Pubertal disorders in inv dup(15) syndrome. *Gynecol Endocrinol.* 2001;15:165–169.
 795. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med.* 2012;14:10–26.
 796. Saletti V, Canafoglia L, Cambiaso P, Russo S, Marchi M, Riva D. A CDKL5 mutated child with precocious puberty. *Am J Med Genet Part A.* 2009;149a:1046–1051.
 797. Blanco-Garcia M, Evain-Brion D, Roger M, Job M. Isolated menses in prepubertal girls. *Pediatrics.* 1985;76:43.
 798. Rosenfeld RG, Reitz RE, King AB, Hintz RL. Familial precocious puberty associated with isolated elevation of luteinizing hormone. *N Engl J Med.* 1980;303:859–862.
 799. Van Wyk J, Grumbach M. Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. *J Pediatr.* 1960;57:416.

800. Lindsay A, Voorhess M, MacGillivray M. Multicystic ovaries detected by sonography in children with hypothyroidism. *Am J Dis Child.* 1980;134:588.
801. Baranowski E, Hogler W. An unusual presentation of acquired hypothyroidism: the Van Wyk-Grumbach syndrome. *Eur J Endocrinol.* 2012;537–542.
802. Ryan GL, Feng X, d'Alva CB, et al. Evaluating the roles of follicle-stimulating hormone receptor polymorphisms in gonadal hyperstimulation associated with severe juvenile primary hypothyroidism. *J Clin Endocrinol Metab.* 2007;92: 2312–2317.
803. De Leener A, Montanelli L, Van Durme J, et al. Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. *J Clin Endocrinol Metab.* 2006;91: 555–562.
804. Cabrera SM, Dimeglio LA, Eugster EA. Incidence and characteristics of pseudoprecocious puberty because of severe primary hypothyroidism. *J Pediatr.* 2013;162:637–639.
805. Copmann T, Adams W. Relationship of polycystic ovary induction to prolactin secretion: Prevention of cyst formation by bromocriptine in the rat. *Endocrinology.* 1981;108:1095.
806. Shenker A, Weinstein L, Moran A, et al. Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein Gs. *J Pediatr.* 1993;123:509–518.
807. Frisch L, Copeland K, Boepple P. Recurrent ovarian cysts in childhood: Diagnosis of McCune-Albright syndrome by bone scan. *Pediatrics.* 1992;90:102–104.
808. Scanlon E, Burkett F, Sener S, et al. Breast carcinoma in an 11-year-old girl with Albright's syndrome. *Breast.* 1974;6:5.
809. Coles N, Comeau I, Munoz T, et al. Severe neonatal cholestasis as an early presentation of McCune-Albright syndrome. *J Clin Res Pediatr Endocrinol.* 2019;11:100–103.
810. Lumbroso S, Paris F, Sultan C. Activating Gsalpha mutations: analysis of 113 patients with signs of McCune-Albright syndrome—a European Collaborative Study. *J Clin Endocrinol Metab.* 2004;89: 2107–2113.
811. Kalter-Leibovici O, Dickerman Z, Zamir R, Weiss I, Kaufman H, Laron Z. Late onset 21-hydroxylase deficiency in a girl mimicking true sexual precocity. *J Pediatr Endocrinol.* 1989;3:121–124.
812. Uli N, Chin D, David R, et al. Menstrual bleeding in a female infant with congenital adrenal hyperplasia: altered maturation of the hypothalamic-pituitary-ovarian axis. *J Clin Endocrinol Metab.* 1997;82:3298–3302.
813. Boepple P. Case records of the Massachusetts General Hospital. *N Engl J Med.* 1989;321:1463.
814. Brauner R, Bashamboo A, Rouget S, et al. Clinical, biological and genetic analysis of prepubertal isolated ovarian cyst in 11 girls. *PLoS One.* 2010;5: e11282.
815. Young RH, Goodman A, Penson RT, Russell AH, Uppot RN, Tambouret RH. Case records of the Massachusetts General Hospital. Case 8-2010. A 22-year-old woman with hypercalcemia and a pelvic mass. *N Engl J Med.* 2010;362:1031–1040.
816. Jamieson S, Fuller PJ. Molecular pathogenesis of granulosa cell tumors of the ovary. *Endocr Rev.* 2012;33:109–144.
817. Gallion H, van Nagell JR, 3 Pt 1 JR, Donaldson E, Powell D. Ovarian dysgerminoma: Report of seven cases and review of the literature. *Am J Obstet Gynecol.* 1988;158:591–595.
818. Orselli RC, Bassler TJ. Theca granulosa cell tumor arising in adrenal. *Cancer.* 1973;31:474–477.
819. Mehenni H, Blouin JL, Radhakrishna U, et al. Peutz-Jeghers syndrome: confirmation of linkage to chromosome 19p13.3 and identification of a potential second locus, on 19q13.4. *Am J Hum Genet.* 1997;61:1327–1334.
820. Phornphutkul C, Okubo T, Wu K, et al. Aromatase p450 expression in a feminizing adrenal adenoma presenting as isosexual precocious puberty. *J Clin Endocrinol Metab.* 2001;86: 649–652.
821. Wilkin F, Gagne N, Paquette J, Oligny LL, Deal C. Pediatric adrenocortical tumors: molecular events leading to insulin-like growth factor II gene overexpression. *J Clin Endocrinol Metab.* 2000;85:2048–2056.
822. Fukami M, Shozu M, Soneda S, et al. Aromatase excess syndrome: identification of cryptic duplications and deletions leading to gain of function of CYP19A1 and assessment of phenotypic determinants. *J Clin Endocrinol Metab.* 2011;96: E1035–1043.
823. Saenz de Rodriguez CA, Bongiovanni AM, Conde de Borrego L. An epidemic of precocious development in Puerto Rican children. *J Pediatr.* 1985;107:393–396.
824. Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula [see comments]. *Lancet.* 1997;350:23–27.
825. Zava DT, Dollbaum CM, Blen M. Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med Soc Exp Biol Med.* 1998;217:369–378.
826. Henley DV, Lipson N, Korach KS, Bloch CA. Prepubertal gynecomastia linked to lavender and tea tree oils [Comment in *N Engl J Med*, 356, 2541–2544]. *N Engl J Med.* 2007;356: 479–485.
827. Linklater A, Hewitt JK. Premature thelarche in the setting of high lavender oil exposure. *J Paediatr Child Health.* 2015;51:235.
828. Kunz GJ, Klein KO, Clemons RD, Gottschalk ME, Jones KL. Virilization of young children after topical androgen use by their parents. *Pediatrics.* 2004;114:282–284.
829. Balducci R, Boscherini B, Mangiantini A, Morellini M, Toscanoni V. Isolated precocious pubarche: an approach. *J Clin Endocrinol Metab.* 1994;79:582–589.
830. Wartofsky L, Handelsman DJ. Standardization of hormonal assays for the 21st century. *J Clin Endocrinol Metab.* 2010;95: 5141–5143.
831. Trivin C, Couto-Silva AC, Sainte-Rose C, et al. Presentation and evolution of organic central precocious puberty according to the type of CNS lesion. *Clin Endocrinol.* 2006;65:239–245.
832. Bidlingmaier F, Butenandt O, Knorr D. Plasma gonadotropins and estrogens in girls with idiopathic precocious puberty. *Pediatr Res.* 1977;11:91.
833. Houk CP, Kunselman AR, Lee PA. Adequacy of a single unstimulated luteinizing hormone level to diagnose central precocious puberty in girls. *Pediatrics.* 2009;123: e1059–1063.
834. Harrington J, Palmert MR, Hamilton J. Use of local data to enhance uptake of published recommendations: an example from the diagnostic evaluation of precocious puberty. *Arch Dis Childhood.* 2014;99:15–20.
835. Houk CP, Kunselman AR, Lee PA. The diagnostic value of a brief GnRH analogue stimulation test in girls with central precocious puberty: a single 30-minute post-stimulation LH sample is adequate. *J Pediatr Endocrinol Metab.* 2008;21:1113–1118.
836. Sathasivam A, Garibaldi L, Shapiro S, Godbold J, Rapaport R. Leuprolide stimulation testing for the evaluation of early female sexual maturation. *Clin Endocrinol.* 2010;73:375–381.
837. Vestergaard ET, Schjorring ME, Kamperis K, et al. The follicle-stimulating hormone (FSH) and luteinizing hormone (LH) response to a gonadotropin-releasing hormone analogue test in healthy prepubertal girls aged 10 months to 6 years. *Eur J Endocrinol.* 2017;176:747–753.
838. Ibañez L, Potau N, Zampolli M, et al. Use of leuprolide acetate response patterns in the early diagnosis of pubertal disorders: comparison with the gonadotropin-releasing hormone test. *J Clin Endocrinol Metab.* 1994;78:30–35.
839. Rosenthal I, Refetoff S, Rich B, et al. Response to challenge with gonadotropin-releasing hormone agonist in a mother and her two sons with a constitutively activating mutation of the luteinizing hormone receptor—a Clinical Research Center study. *J Clin Endocrinol Metab.* 1996;81:3802–3806.
840. Rosenfield RL. Selection of children with precocious puberty for treatment with gonadotropin releasing hormone analogs. *J Pediatr.* 1994;124:989–991.
841. Carmina E, Dewailly D, Escobar-Morreale HF, et al. Non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency revisited: an update with a special focus on adolescent and adult women. *Hum Reprod, update.* 2017;23:580–599.
842. Shah RU, Lawrence C, Fickenscher KA, Shao L, Lowe LH. Imaging of pediatric pelvic neoplasms. *Radiol Clin North Am.* 2011;49:729–748. vi.
843. Balassy C, Navarro OM, Daneman A. Adrenal masses in children. *Radiol Clin North Am.* 2011;49:711–727. vi.
844. Salardi S, Orsini L, Cacciari E, et al. Pelvic ultrasonography in girls with precocious puberty, congenital adrenal hyperplasia, obesity, or hirsutism. *J Pediatr.* 1988;112:880.

845. Stratakis CA, Papageorgiou T, Premkumar A, et al. Ovarian lesions in Carney complex: clinical genetics and possible predisposition to malignancy. *J Clin Endocrinol Metab.* 2000;85:4359–4366.
846. Lonsdale RN, Roberts PF, Trowell JE. Autoimmune oophoritis associated with polycystic ovaries. *Histopathology.* 1991;19:77–81.
847. Pascale MM, Pugeat M, Roberts M, et al. Androgen suppressive effect of GnRH agonist in ovarian hyperthecosis and virilizing tumours. *Clin Endocrinol.* 1994;41:571–576.
848. Cantas-Orsdemir S, Garb JL, Allen HF. Prevalence of cranial MRI findings in girls with central precocious puberty: a systematic review and meta-analysis. *J Pediatr Endocrinol Metab.* 2018;31:701–710.
849. Grunt JA, Midyett LK, Simon SD, Lowe L. When should cranial magnetic resonance imaging be used in girls with early sexual development? *J Pediatr Endocrinol Metab.* 2004;17:775–780.
850. Latronico AC, Brito VN, Carel JC. Causes, diagnosis, and treatment of central precocious puberty. *Lancet Diabetes & Endocrinol.* 2016;4:265–274.
851. Lazar L, Padoa A, Phillip M. Growth pattern and final height after cessation of gonadotropin-suppressive therapy in girls with central sexual precocity. *J Clin Endocrinol Metab.* 2007;92:3483–3489.
852. Palmert MR, Malin HV, Boepple PA. Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. *J Clin Endocrinol Metab.* 1999;84:415–423.
853. Alvarez R, Grizzle W, Smith L, Miller D. Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver. *Am J Obstet Gynecol.* 1989;161:1653–1657.
854. Lazar L, Lebenthal Y, Yackobovitch-Gavan M, et al. Treated and untreated women with idiopathic precocious puberty: BMI evolution, metabolic outcome, and general health between third and fifth decades. *J Clin Endocrinol Metab.* 2015;100:1445–1451.
855. Fuld K, Chi C, Neely EK. A randomized trial of 1- and 3-month depot leuprolide doses in the treatment of central precocious puberty. *J Pediatr.* 2011;159:982–987. e1.
856. Fuqua JS. Treatment and outcomes of precocious puberty: an update. *J Clin Endocrinol Metab.* 2013;98:2198–2207.
857. Rahhal S, Clarke WL, Kletter GB, et al. Results of a second year of therapy with the 12-month histrelin implant for the treatment of central precocious puberty. *Int J Pediatr Endocrinol.* 2009;2009:812517.
858. Bertelloni S, Mucaria C, Baroncelli GI, Peroni D. Triptorelin depot for the treatment of children 2 years and older with central precocious puberty. *Expert Rev Clin Pharmacol.* 2018;11:659–667.
859. Klein KO, Baron J, Barnes KM, Pescovitz OH, Cutler Jr GB. Use of an ultrasensitive recombinant cell bioassay to determine estrogen levels in girls with precocious puberty treated with a luteinizing hormone-releasing hormone agonist. *J Clin Endocrinol Metab.* 1998;83:2387–2389.
860. Brito VN, Latronico AC, Arnhold IJ, Mendonca BB. A single luteinizing hormone determination 2 hours after depot leuprolide is useful for therapy monitoring of gonadotropin-dependent precocious puberty in girls. *J Clin Endocrinol Metab.* 2004;89:4338–4342.
861. Tanaka T, Niimi H, Matsuo N, et al. Results of long-term follow-up after treatment of central precocious puberty with leuprorelin acetate: evaluation of effectiveness of treatment and recovery of gonadal function. The TAP-144-SR Japanese Study Group on Central Precocious Puberty. *J Clin Endocrinol Metab.* 2005;90:1371–1376.
862. Fisher MM, Lemay D, Eugster EA. Resumption of puberty in girls and boys following removal of the histrelin implant. *J Pediatr.* 2014;164:912–6.e1.
863. Adan L, Souberbielle JC, Zucker JM. Adult height in 24 patients treated for growth hormone deficiency and early puberty. *J Clin Endocrinol Metab.* 1997;82:229–233.
864. Pasquino AM, Pucarelli I, Segni M, Matruncola M, Cerroni F, Cerrone F. Adult height in girls with central precocious puberty treated with gonadotropin-releasing hormone analogues and growth hormone [published erratum appears in *J Clin Endocrinol Metab.* 84(6), 1978]. *J Clin Endocrinol Metab.* 1999;84:449–452.
865. Carel JC, Roger M, Ispas S, et al. Final height after long-term treatment with triptorelin slow release for central precocious puberty: importance of statural growth after interruption of treatment. French study group of Decapeptyl in Precocious Puberty. *J Clin Endocrinol Metab.* 1999;84:1973–1978.
866. Silverman LA, Neely EK, Kletter GB, et al. Long-term continuous suppression with once-yearly histrelin subcutaneous implants for the treatment of central precocious puberty: a final report of a phase 3 Multicenter Trial. *J Clin Endocrinol Metab.* 2015;100:2354–2363.
867. Letterie GS, Stevenson D, Shah A. Recurrent anaphylaxis to a depot form of GnRH analogue. *Obstet Gynecol.* 1991;78:943–946.
868. Wojniesz S, Callens N, Sutterlin S, et al. Cognitive, Emotional, and Psychosocial Functioning of Girls Treated with Pharmacological Puberty Blockage for Idiopathic Central Precocious Puberty. *Front Psychol.* 2016;7:1053.
869. Magiakou MA, Manousaki D, Papadaki M, et al. The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. *J Clin Endocrinol Metab.* 2010;95:109–117.
870. Rosenfield RL. Essentials of growth diagnosis. *Endocrinol Metab Clin N Am.* 1996;25:743–758.
871. Papadimitriou A, Beri D, Tsiaila A, Fretzayas A, Psychou F, Nicolaidou P. Early growth acceleration in girls with idiopathic precocious puberty. *J Pediatr.* 2006;149:43–46.
872. Lazar L, Kauli R, Pertzalan A, Phillip M. Gonadotropin-suppressive therapy in girls with early and fast puberty affects the pace of puberty but not total pubertal growth or final height. *J Clin Endocrinol Metab.* 2002;87:2090–2094.
873. Richman RA, Underwood LE, French FS, Van Wyk JJ. Adverse effects of large doses of medroxyprogesterone (MPA) in idiopathic isosexual precocity. *J Pediatr.* 1971;79:963.
874. Lopez LM, Grimes DA, Schulz KF, Curtis KM. Steroidal contraceptives: effect on bone fractures in women. *Cochrane Database Syst Rev.* 2006; CD006033.
875. Eugster EA, Rubin SD, Reiter EO, Plourde P, Jou HC, Pescovitz OH. Tamoxifen treatment for precocious puberty in McCune-Albright syndrome: a multicenter trial. *J Pediatr.* 2003;143:60–66.
876. Wit JM, Hero M, Nunez SB. Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol.* 2012;8:135–147.
877. Neyman A, Eugster EA. Treatment of girls and boys with McCune-Albright syndrome with precocious puberty – Update 2017. *Pediatr Endocrinol Rev.* 2017;15:136–141.
878. Corica D, Aversa T, Pepe G, De Luca F, Wasniewska M. Peculiarities of precocious puberty in boys and girls with McCune-Albright syndrome. *Front Endocrinol.* 2018;9:337.
879. Holland F, Kirsch S, Selby R. Gonadotropin-independent precocious puberty ("testotoxicosis"): influence of maturational status on response to ketoconazole. *J Clin Endocrinol Metab.* 1987;64:328–333.
880. Laven JS, Lumbroso S, Sultan C, Fauser BC. Dynamics of ovarian function in an adult woman with McCune-Albright syndrome. *J Clin Endocrinol Metab.* 2001;86:2625–2630.
881. Leet AI, Collins MT. Current approach to fibrous dysplasia of bone and McCune-Albright syndrome. *J Child Orthop.* 2007;1:3–17.
882. Rosenfield RL, Barnes RB. Menstrual disorders in adolescence. *Endocrinol Metab Clin N Am.* 1993;22:491–505.
883. Razdan AK, Rosenfield RL, Kim MH. Endocrinologic characteristics of partial ovarian failure. *J Clin Endocrinol Metab.* 1976;43:449–452.
884. Winslow KL, Toner JP, Brzyski RG, Oehninger SC, Acosta AA, Muasher SJ. The gonadotropin-releasing hormone agonist stimulation test—a sensitive predictor of performance in the flare-up in vitro fertilization cycle. *Fertil Steril.* 1991;56:711–717.
885. Knauff EA, Eijkemans MJ, Lambalk CB, et al. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. *J Clin Endocrinol Metab.* 2009;94:786–792.
886. Hendriks AE, Laven JS, Valkenburg O, et al. Fertility and ovarian function in high-dose estrogen-treated tall women. *J Clin Endocrinol Metab.* 2011;96:1098–1105.
887. Bidet M, Bachelot A, Bissauge E, et al. Resumption of ovarian function and pregnancies in 358 patients with premature ovarian failure. *J Clin Endocrinol Metab.* 2011;96:3864–3872.
888. Mazzanti L, Cacciari E, Bergamaschi R, et al. Pelvic ultrasonography in patients with Turner syndrome: age-related findings in different karyotypes. *J Pediatr.* 1997;131:135–140.

889. Birgit B, Julius H, Carsten R, et al. Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab.* 2009;94:74–80.
890. De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet.* 2011;376:911–921.
891. Cunniff C, Jones K, Benirschke K. Ovarian dysgenesis in individuals with chromosomal abnormalities. *Hum Genet.* 1990;86:552–556.
892. Hansen J, Boyar R, Shapiro L. Gonadal function in trisomy 21. *Horm Res.* 1982;12:345.
893. Bovicelli L, Orsini LF, Rizzo N, Montacuti V, Bacchetta M. Reproduction in Down syndrome. *Obstet Gynecol.* 1982;59:13S–17S.
894. Pelletier J, Bruening W, Kashtan C, et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell.* 1991;67:437–447.
895. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, Baltimore D. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. *Genes Dev.* 1996;10:2411–2422.
896. Chrzanowska KH, Szarras-Czapnik M, Gajdulewicz M, et al. High prevalence of primary ovarian insufficiency in girls and young women with Nijmegen breakage syndrome: evidence from a longitudinal study. *J Clin Endocrinol Metab.* 2010;95:3133–3140.
897. Beysen D, De Paeppe A, De Baere E. FOXL2 mutations and genomic rearrangements in BPES. *Hum Mutat.* 2009;30:158–169.
898. Kaufman FR, Devgan S. Classical galactosemia: a review. *Endocrinologist.* 1995;5:189–197.
899. Lieman H, Santoro N. Premature ovarian failure: a modern approach to diagnosis and treatment. *Endocrinologist.* 1997;7:314–321.
900. McDonough PG. Selected enquiries into the causation of premature ovarian failure. *Hum Fertil (Camb).* 2003;6:130–136.
901. Chand AL, Harrison CA, Shelling AN. Inhibin and premature ovarian failure. *Hum Reprod, Update.* 2010;16:39–50.
902. Karlberg S, Tiitinen A, Alftan H, Lipsanen-Nyman M. Premature ovarian insufficiency and early depletion of the ovarian reserve in the monogenic Mullerian aplasia disorder. *Hum Reprod (Oxford, England).* 2018;33:1254–1261.
903. Domenice S, Machado AZ, Ferreira FM, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. *Birth Defects Res Part C.* 2016;108:309–320.
904. Smirin-Yosef P, Zuckerman-Levin N, Tzur S, et al. A biallelic mutation in the homologous recombination repair gene SPIDR1 is associated with human gonadal dysgenesis. *J Clin Endocrinol Metab.* 2017;102:681–688.
905. Layman LC. Editorial: BMP15—the first true ovarian determinant gene on the X-chromosome? *J Clin Endocrinol Metab.* 2006;91:1673–1676.
906. Wallace WH. Oncofertility and preservation of reproductive capacity in children and young adults. *Cancer.* 2011;117:2301–2310.
907. Chemaitilly W, Mertens AC, Mitby P, et al. Acute ovarian failure in the childhood cancer survivor study. *J Clin Endocrinol Metab.* 2006;91:1723–1728.
908. Sklar CA, Mertens AC, Mitby P, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. *J Natl Cancer Inst.* 2006;98:890–896.
909. Ginsberg JP. New advances in fertility preservation for pediatric cancer patients. *Curr Opin Pediatr.* 2011;23:9–13.
910. Antal Z, Sklar CA. Gonadal function and fertility among survivors of childhood cancer. *Endo Metab Clin North Am.* 2015;44(4):739–749.
911. Taylan E, Oktay KH. Current state and controversies in fertility preservation in women with breast cancer. *World J Clin Oncol.* 2017;8:241–248.
912. Oktay K, Sonmez M, Oktem O, Fox K, Emons G, Bang H. Absence of conclusive evidence for the safety and efficacy of gonadotropin-releasing hormone analogue treatment in protecting against chemotherapy-induced gonadal injury. *Oncologist.* 2007;12:1055–1066.
913. Anazodo A, Ataman-Millhouse L, Jayasinghe Y, Woodruff TK. Oncofertility—An emerging discipline rather than a special consideration. *Pediatr Blood Cancer.* 2018;65:e27297.
914. Chemaitilly W, Li Z, Krasin MJ, et al. Premature ovarian insufficiency in childhood cancer survivors: are report from the St. Jude Lifetime Cohort. *J Clin Endocrinol Metab.* 2017;102(7):2242–2250.
915. Green DM, Nolan VG, Goodman PJ, et al. The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure: a report from the Childhood Cancer Survivor Study. *Pediatr Blood Cancer.* 2014;61:53–67.
916. Sarafoglou K, Boulad F, Gillio A, Sklar C. Gonadal function after bone marrow transplantation for acute leukemia during childhood [see comments]. *J Pediatr.* 1997;130:210–216.
917. Toledo SP, Brunner HG, Kraaij R, et al. An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46, XX female. *J Clin Endocrinol Metab.* 1996;81:3850–3854.
918. Layman LC, McDonough PG. Mutations of follicle stimulating hormone-beta and its receptor in human and mouse: genotype/phenotype. *Mol Cell Endocrinol.* 2000;161:9–17.
919. Salvi R, Pralong FP. Molecular characterization and phenotypic expression of mutations in genes for gonadotropins and their receptors in humans. *Front Horm Res.* 2010;39:1–12.
920. Namnoum AB, Merriam GR, Moses AM, Levine MA. Reproductive dysfunction in women with Albright's hereditary osteodystrophy. *J Clin Endocrinol Metab.* 1998;83:824–829.
921. Woodruff TK. Preserving fertility during cancer treatment. *Nat Med.* 2009;15:1124–1125.
922. Nelson LM. Clinical practice. Primary ovarian insufficiency. *N Engl J Med.* 2009;360:606–614.
923. Tsigkou A, Marzotti S, Borges L, et al. High serum inhibin concentration discriminates autoimmune oophoritis from other forms of primary ovarian insufficiency. *J Clin Endocrinol Metab.* 2008;93:1263–1269.
924. Lucky AW, Rebar RW, Blizzard RM, Goren EM. Pubertal progression in the presence of elevated serum gonadotropins in girls with multiple endocrine deficiencies. *J Clin Endocrinol Metab.* 1977;45:673–678.
925. Kuwahara A, Kamada M, Irahara M, Naka O, Yamashita T, Aono T. Autoantibody against testosterone in a woman with hypergonadotropic hypogonadism. *J Clin Endocrinol Metab.* 1998;83:14–16.
926. Sahakitrungruang T, Huang N, Tee MK, et al. Clinical, genetic, and enzymatic characterization of P450 oxidoreductase deficiency in four patients. *J Clin Endocrinol Metab.* 2009;94:4992–5000.
927. Tanae A, Katsumata N, Sato N, Horikawa R, Tanaka T. Genetic and endocrinological evaluations of three 46,XX patients with congenital lipoid adrenal hyperplasia previously reported as having presented spontaneous puberty. *Endocr J.* 2000;47:629–634.
928. Bernard V, Kherra S, Francou B, et al. Familial multiplicity of estrogen insensitivity associated with a loss-of-function ESR1 mutation. *J Clin Endocrinol Metab.* 2017;102:93–99.
929. Quaynor SD, Stradtman Jr EW, Kim HG, et al. Delayed puberty and estrogen resistance in a woman with estrogen receptor alpha variant. *N Engl J Med.* 2013;369:164–171.
930. Layman LC. Genetics of human hypogonadotropic hypogonadism. *Am J Med Genet.* 1999;89:240–248.
931. Eldar-Geva T, Hirsch HJ, Benarroch F, Rubinstein O, Gross-Tsur V. Hypogonadism in females with Prader-Willi syndrome from infancy to adulthood: variable combinations of a primary gonadal defect and hypothalamic dysfunction. *Eur J Endocrinol.* 2010;162:377–384.
932. Pitteloud N, Quinton R, Pearce S, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest.* 2007;117:457–463.
933. Cole LW, Sidis Y, Zhang C, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab.* 2008;93:3551–3559.
934. Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, et al. CHD7 mutations in patients initially diagnosed with Kallmann syndrome—the clinical overlap with CHARGE syndrome. *Clin Genet.* 2009;5:65–71.
935. Beranova M, Oliveira LM, Bedecarrats GY, et al. Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2001;86:1580–1588.
936. de Roux N, Young J, Brailley-Tabard S, Misrahi M, Milgrom E, Schaison G. The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of

- hypogonadism in affected kindred. *J Clin Endocrinol Metab.* 1999;84:567–572.
937. Seminara SB, Beranova M, Oliveira LMB, Martin KA, Crowley WFJ, Hall JE. Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *J Clin Endocrinol Metab.* 2000;85:556–562.
938. Kelberman D, Rizzotti K, Lovell-Badge R, Robinson IC, Dattani MT. Genetic regulation of pituitary gland development in human and mouse. *Endocr Rev.* 2009;30:790–829.
939. Prince KL, Walvoord EC, Rhodes SJ. The role of homeodomain transcription factors in heritable pituitary disease. *Nat Rev Endocrinol.* 2011;7:727–737.
940. Romero CJ, Nesi-Franca S, Radovick S. The molecular basis of hypopituitarism. *Trends Endocrinol Metab.* 2009;20:506–516.
941. Fischer-Posovszky P, von Schnurbein J, Moepps B, et al. A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. *J Clin Endocrinol Metab.* 2010;95:2836–2840.
942. Romero CJ, Pine-Twaddell E, Radovick S. Novel mutations associated with combined pituitary hormone deficiency. *J Mol Endocrinol.* 2011;46:R93–R102.
943. Israel D, Chua Jr S. Leptin receptor modulation of adiposity and fertility. *Trends Endocrinol Metab.* 2010;21:10–16.
944. Nizard J, Dommergue M, Clement K. Pregnancy in a woman with a leptin-receptor mutation. *N Engl J Med.* 2012;366:1064–1065.
945. Layman WS, Hurd EA, Martin DM. Reproductive dysfunction and decreased GnRH neurogenesis in a mouse model of CHARGE syndrome. *Hum Mol Genet.* 2011;20:3138–3150.
946. Lomniczi A, Garcia-Rudaz C, Ramakrishnan R, et al. A single-nucleotide polymorphism in the EAP1 gene is associated with amenorrhea/oligomenorrhea in nonhuman primates. *Endocrinology.* 2012;153:339–349.
947. Beate K, Joseph N, Nicolas de R, Wolfram K. Genetics of isolated hypogonadotropic hypogonadism: role of GnRH receptor and other genes. *Int J Endocrinol.* 2012;2012:147893.
948. Bouligand J, Ghervan C, Tello JA, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med.* 2009;360:2742–2748.
949. Merke DP, Tajima T, Baron J, Cutler Jr GB. Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the DAX1 gene. *N Engl J Med.* 1999;340:1248–1252.
950. de Zegher F, Jaeken J. Endocrinology of the carbohydrate-deficient glycoprotein syndrome type 1 from birth through adolescence. *Pediatr Res.* 1995;37:395.
951. Achard C, Courtillot C, Lahuna O, et al. Normal spermatogenesis in a man with mutant luteinizing hormone. *N Engl J Med.* 2009;361:1856–1863.
952. Kelberman D, Turton JP, Woods KS, et al. Molecular analysis of novel PROP1 mutations associated with combined pituitary hormone deficiency (CPHD). *Clin Endocrinol.* 2009;70:96–103.
953. Parks JS, Brown MR, Hurley DL, Phelps CJ, Wajnrajch MP. Heritable disorders of pituitary development. *J Clin Endocrinol Metab.* 1999;84:4362–4370.
954. Wolf NI, Vanderver A, van Spaendonck RM, et al. Clinical spectrum of 4H leukodystrophy caused by POLR3A and POLR3B mutations. *Neurology.* 2014;83:1898–1905.
955. Margolin DH, Kousi M, Chan YM, et al. Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N Engl J Med.* 2013;368:1992–2003.
956. Topaloglu AK, Lomniczi A, Kretschmar D, et al. Loss-of-function mutations in PNPLA6 encoding neuropathy target esterase underlie pubertal failure and neurological deficits in Gordon Holmes syndrome. *J Clin Endocrinol Metab.* 2014;99:E2067–E2075.
957. Tata B, Huijbregts L, Jacquier S, et al. Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. *PLoS Biol.* 2014;12:e1001952.
958. Aligianis IA, Johnson CA, Gissen P, et al. Mutations of the catalytic subunit of RAB3GAP cause Warburg Micro syndrome. *Nat Genet.* 2005;37:221–223.
959. Bem D, Yoshimura S, Nunes-Bastos R, et al. Loss-of-function mutations in RAB18 cause Warburg micro syndrome. *Am J Hum Genet.* 2011;88:499–507.
960. Barkan A, Kelch R, Marshall J. Isolated gonadotrope failure in the polyglandular autoimmune syndrome. *N Engl J Med.* 1985;312:1535.
961. Komatsu M, Kondo T, Yamauchi K, et al. Antipituitary antibodies in patients with the primary empty sella syndrome. *J Clin Endocrinol Metab.* 1988;67:633.
962. Hendricks SA, Lippe BM, Kaplan SA, Bentson JR. Hypothalamic atrophy with progressive hypopituitarism in an adolescent girl. *J Clin Endocrinol Metab.* 1981;52:562.
963. Rappaport R, Brauner R, Czernichow P, et al. Effect of hypothalamic and pituitary irradiation on pubertal development in children with cranial tumors. *J Clin Endocrinol Metab.* 1982;54:1164–1168.
964. Rose SR, Schreiber RE, Kearney NS, et al. Hypothalamic dysfunction after chemotherapy. *J Pediatr Endocrinol Metab.* 2004;17:55–66.
965. Van den Berghe G, de Zegher F, Bouillon R. Clinical review 95: Acute and prolonged critical illness as different neuroendocrine paradigms. *J Clin Endocrinol Metab.* 1998;83:1827–1834.
966. Green DM, Nolan VG, Kawashima T, et al. Decreased fertility among female childhood cancer survivors who received 22-27 Gy hypothalamic/pituitary irradiation: a report from the Childhood Cancer Survivor Study. *Fertil Steril.* 2011;95:1922–1927. 7. e1.
967. Molitch M. Gonadotroph-cell pituitary adenomas. *N Engl J Med.* 1991;324:626–627.
968. Fairburn CG, Harrison PJ. Eating disorders. *Lancet.* 2003;2003(361):407–416.
969. American Academy of Pediatrics Policy Statement. Identifying and treating eating disorders. *Pediatrics.* 2003;111:204–211.
970. Yager J, Andersen AE. Clinical practice. Anorexia nervosa. *N Engl J Med.* 2005;353:1481–1488.
971. Le Grange D, Doyle PM, Swanson SA, Ludwig K, Glunz C, Kreipe RE. Calculation of expected body weight in adolescents with eating disorders. *Pediatrics.* 2012;129(2). e438–446.
972. Pugliese M, Lifshitz F, Grad C, Fort P, Marks-Katz M. Fear of obesity. A cause of short stature and delayed puberty. *N Engl J Med.* 1983;309:513.
973. Golden N, Kreitzer P, Jacobson M, et al. Disturbances in growth hormone secretion and action in adolescents with anorexia nervosa. *J Pediatr.* 1994;125:655–660.
974. Newman M, Halmi K. The endocrinology of anorexia nervosa and bulimia nervosa. *Endocrinol Metab Clin N Am.* 1988;17:195.
975. Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: mechanisms and management. *Nat Rev Endocrinol.* 2017;13:174–186.
976. Frisch R, McArthur J. Menstrual cycles as a determinant of minimum weight for height necessary for their maintenance or onset. *Science.* 1974;185:949.
977. Misra M, Prabhakaran R, Miller KK, et al. Role of cortisol in menstrual recovery in adolescent girls with anorexia nervosa. *Pediatr Res.* 2006;59:598–603.
978. Boyar RM, Katz J, Finkelstein JW, et al. Anorexia nervosa: Immaturity of the 24-hour luteinizing hormone secretory pattern. *N Engl J Med.* 1974;291:861.
979. Beumont PJ, George GC, Pimstone BL, Vinik AI. Body weight and the pituitary response to hypothalamic releasing hormones in patients with anorexia nervosa. *J Clin Endocrinol Metab.* 1976;43:487.
980. Marshall J, Fraser T. Amenorrhoea in anorexia nervosa: Assessment and treatment with clomiphene citrate. *Br Med J.* 1971;4:590.
981. Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med.* 2004;351:987–997.
982. Chrousos G, Gold P. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA.* 1992;267:1244–1252.
983. Winterer J, Gwirtsman HE, George DT, Kaye WH, Loriaux DL, Cutler Jr GB. Adrenocorticotropin-stimulated adrenal androgen secretion in anorexia nervosa: impaired secretion at low weight

- with normalization after long-term weight recovery. *J Clin Endocrinol Metab.* 1985;61:693.
984. Mecklenburg RS, Loriaux DL, Thompson RH, Andersen AE, Lipsett MB. Hypothalamic dysfunction in patients with anorexia nervosa. *Medicine.* 1974;53:147.
 985. Petrides J, Mueller G, Kalogeras K, Chrousos G, Gold P, Deuster P. Exercise-induced activation of the hypothalamic-pituitary-adrenal axis: Marked differences in the sensitivity to glucocorticoid suppression. *J Clin Endocrinol Metab.* 1994;79:377–383.
 986. Molitch M. Pathologic hyperprolactinemia. *Endocrinol Metab Clin N Am.* 1992;21:877–901.
 987. Colao A, Loche S, Cappa M, et al. Prolactinomas in children and adolescents. Clinical presentation and long-term follow-up. *J Clin Endocrinol Metab.* 1998;83:2777–2780.
 988. Blanco-Favela F, Quintal Ma G, Chavez-Rueda AK, et al. Anti-prolactin autoantibodies in paediatric systemic lupus erythematosus patients. *Lupus.* 2001;10:803–808.
 989. Schlechte JA. The macroprolactin problem. *J Clin Endocrinol Metab.* 2002;87:5408–5409.
 990. Newey PJ, Gorvin CM, Cleland SJ, et al. Mutant prolactin receptor and familial hyperprolactinemia (Comment in *N Engl J Med.* 2014;370:9768). *N Engl J Med.* 2013;369:2012–2020.
 991. Klibanski A. Clinical practice. Prolactinomas. *N Engl J Med.* 2010;362:1219–1226.
 992. Mendelson J, Mello N, Teoh S, Ellingboe J, Cochlin J. Cocaine effects on pulsatile secretion of anterior pituitary, gonadal, and adrenal hormones. *J Clin Endocrinol Metab.* 1989;69:1256.
 993. Tallo D, Malarkey W. Physiologic concentrations of dopamine fail to suppress prolactin secretion in patients with idiopathic hyperprolactinemia or prolactinomas. *Am J Obstet Gynecol.* 1985;151:651–655.
 994. Lippi G, Plebani M. Macroprolactin: searching for a needle in a haystack? *Clin Chem Lab Med.* 2016;54:519–522.
 995. Sauder S, Frager M, Case C, Kelch R, Marshall J. Abnormal patterns of pulsatile luteinizing hormone secretion in women with hyperprolactinemia and amenorrhea: Responses to bromocriptine. *J Clin Endocrinol Metab.* 1984;59:941.
 996. Gordon CM, Ackerman KE, Berga SL, et al. Functional Hypothalamic Amenorrhea: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2017;102:1413–1439.
 997. Braverman PK, Breech L. American Academy of Pediatrics. Clinical report—gynecologic examination for adolescents in the pediatric office setting. *Pediatrics.* 2010;126:583–590.
 998. Sane K, Pescovitz O. The clitoral index: A determination of clitoral size in normal girls and in girls with abnormal sexual development. *J Pediatr.* 1992;120:264–266.
 999. Conte F, Grumbach M, Ito Y, Fisher C, Simpson E. A syndrome of female pseudohermaphroditism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J Clin Endocrinol Metab.* 1994;78:1287–1292.
 1000. Hagen CP, Main KM, Kjaergaard S, Juul A. FSH, LH, inhibin B and estradiol levels in Turner syndrome depend on age and karyotype: longitudinal study of 70 Turner girls with or without spontaneous puberty. *Hum Reprod.* 2010;25:134–141.
 1001. Braslavsky D, Grinspon RP, Ballerini MG, et al. Hypogonadotropic hypogonadism in infants with congenital hypopituitarism: a challenge to diagnose at an early stage. *Horm Res Paediatr.* 2015;84:289–297.
 1002. Greeley SA, Littlejohn E, Husain AN, Waggoner D, Gundeti M, Rosenfield RL. The effect of the testis on the ovary: structure-function relationships in a neonate with a unilateral ovotestis (ovotesticular disorder of sex development). *Horm Res Paediatr.* 2017;87:205–212.
 1003. Rosenfield RL. Menstrual disorders and hyperandrogenism in adolescence. In: Radovick S, MacGillivray MH, eds. *Pediatric Endocrinology a Practical Clinical Guide.* Totowa: N. J., Humana Press, Inc.; 2003:451–478.
 1004. Chan YM, Lippincott MF, Kusa TO, Seminara SB. Divergent responses to kisspeptin in children with delayed puberty. *JCI insight.* 2018;3.
 1005. Rebar R, Connolly H. Clinical features of young women with hypergonadotropic amenorrhea. *Fertil Steril.* 1990;53:804–810.
 1006. Petakov MS, Damjanovic SS, Nikolic-Durovic MM, et al. Pituitary adenomas secreting large amounts of prolactin may give false low values in immunoradiometric assays. The hook effect. *J Endocrinol Invest.* 1998;21:184–188.
 1007. Kovacs K, Horvath E. Tumors of the pituitary gland. In: Hartmann W, Sobin L, eds. *Atlas of Tumor Pathology.* Washington, DC: Armed Forces Inst of Pathology; 1983: 205–209.
 1008. Schroeder I, Johnson J, Malarkey W. Cerebrospinal fluid prolactin: A reflection of abnormal prolactin secretion in patients with pituitary tumors. *J Clin Endocrinol Metab.* 1976; 43:1255.
 1009. Germain N, Galusca B, Le Roux CW, et al. Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. *Am J Clin Nutr.* 2007;85:967–971.
 1010. Gardner D. Pathogenesis of anorexia nervosa. *Lancet.* 1993;341: 1631–1634.
 1011. Molitch ME, Thorner MO, Wilson C. Management of prolactinomas. *J Clin Endocrinol Metab.* 1997;82:996–1000.
 1012. Colao A, Di Sarno A, Landi ML, et al. Macroprolactinoma shrinkage during cabergoline treatment is greater in naive patients than in patients pretreated with other dopamine agonists: a prospective study in 110 patients. *J Clin Endocrinol Metab.* 2000;85: 2247–2252.
 1013. Melmed S, Casanueva FF, Hoffman AR, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96:273–288.
 1014. Lock J, le Grange D, Agras WS, Dare C. *Treatment Manual for Anorexia Nervosa.* New York: Guilford; 2001.
 1015. Misra M, Miller KK, Tsai P, et al. Elevated peptide YY levels in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab.* 2006;91:1027–1033.
 1016. Katz R, Mazer C, Litt I. Anorexia nervosa by proxy. *J Pediatr.* 1985;107:247.
 1017. Rothchild E, Owens R. Adolescent girls who lack functioning ovaries. *J Am Acad Child Psychiatry.* 1972;11:88.
 1018. Bondy CA, for the Turner Syndrome Consensus Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner syndrome study group. *J Clin Endocrinol Metab.* 2007;92:10–25.
 1019. Rosenfeld RG, Frane J, Attie KM, et al. Six-year results of a randomized prospective trial of human growth hormone and oxandrolone in Turner syndrome. *J Pediatr.* 1992;121:49–55.
 1020. Rosenfield RL, Devine N, Hunold JJ, Mauras N, Moshang Jr T, Root AW. Salutary effects of combining early very low-dose systemic estradiol with growth hormone therapy in girls with Turner syndrome. *J Clin Endocrinol Metab.* 2005;90:6424–6430.
 1021. Ross JL, Quigley CA, Cao D, et al. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. *N Engl J Med.* 2011;364:1230–1242.
 1022. Klein KO, Rosenfield RL, Santen RJ, et al. Estrogen replacement in Turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab.* 2018;103:1790–1803.
 1023. Rosenfield R, Kiess W, Keizer-Schrama S. Physiologic induction of puberty in Turner syndrome with very low-dose estradiol. In: Gravholt C, Bondy C, eds. *Wellness for Girls and Women With Turner Syndrome.* Amsterdam: Elsevier Science; 2006:71–79.
 1024. Taboada M, Santen R, Lima J, et al. Pharmacokinetics and pharmacodynamics of oral and transdermal 17beta estradiol in girls with Turner syndrome. *J Clin Endocrinol Metab.* 2011;96: 3502–3510.
 1025. Laliberte F, Dea K, Duh MS, Kahler KH, Rolli M, Lefebvre P. Does the route of administration for estrogen hormone therapy impact the risk of venous thromboembolism? Estradiol transdermal system versus oral estrogen-only hormone therapy. *Menopause.* 2011;18:1052–1059.
 1026. Renoux C, Dell'Aniello S, Suissa S. Hormone replacement therapy and the risk of venous thromboembolism: a population-based study. *J Thrombos Haemostas.* 2010;8:979–986. 2010.
 1027. Gordon CM, Pitts SA. Approach to the adolescent requesting contraception. *J Clin Endocrinol Metab.* 2012;97:9–15.
 1028. Zuckerman-Levin N, Frolova-Bishara T, Militianu D, Levin M, Aharon-Peretz J, Hochberg Z. Androgen replacement therapy in Turner syndrome: a pilot study. *J Clin Endocrinol Metab.* 2009;94:4820–4827.

1029. Stanhope R, Pringle P, Brook C, Adams J, Jacobs N. Induction of puberty by pulsatile gonadotropin releasing hormone. *Lancet*. 1987;2:552.
1030. Decourt C, Robert V, Anger K, et al. A synthetic kisspeptin analog that triggers ovulation and advances puberty. *Sci Rep*. 2016;6:26908.
1031. Sykiotis GP, Hoang XH, Avbelj M, et al. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. *J Clin Endocrinol Metab*. 2010;95:3019–3027.
1032. Devroey P, Camus M, Palermo G, et al. Placental production of estradiol and progesterone after oocyte donation in patients with primary ovarian failure. *Am J Obstet Gynecol*. 1990;162:66–70.
1033. Lado-Abeal J, Rodriguez-Arnao J, Newell-Price JDC, et al. Menstrual abnormalities in women with Cushing's disease are correlated with hypercortisolemia rather than raised circulating androgen levels. *J Clin Endocrinol Metab*. 1998;83:3083–3088.
1034. Kawamura K, Cheng Y, Suzuki N, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci U S A*. 2013;110:17474–17479.
1035. Zou K, Yuan Z, Yang Z, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat Cell Biol*. 2009;11:631–636.
1036. Gravholt CH, Andersen NH, Conway GS, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol*. 2017;177:G1–G70.
1037. Oktay K, Bedoschi G, Berkowitz K, et al. Fertility preservation in women with Turner syndrome: a comprehensive review and practical guidelines. *J Pediatr Adolesc Gynecol*. 2016;29:409–416.
1038. Perkins RB, Hall JE, Martin KA. Neuroendocrine abnormalities in hypothalamic amenorrhea: spectrum, stability, and response to neurotransmitter modulation. *J Clin Endocrinol Metab*. 1999;84:1905–1911.
1039. Shaw R. Differential response to LHRH following oestrogen therapy in women with amenorrhoea. *Br Obstet Gynaecol*. 1979;86:69–75.
1040. Weiss C, Nachtigall L, Ganguly M. Induction of an LH surge with estradiol benzoate. *Obstet Gynecol*. 1976;47:415–418.
1041. Misra M, Miller KK, Kuo K, et al. Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. *Am J Physiol Endocrinol Metab*. 2005;289: E347–356.
1042. Constantini N, Warren M. Menstrual dysfunction in swimmers: A distinct entity. *J Clin Endocrinol Metab*. 1995;80:2740.
1043. Centeno ML, Sanchez RL, Cameron JL, Bethea CL. Hypothalamic expression of serotonin 1A, 2A and 2C receptor and GAD67 mRNA in female cynomolgus monkeys with different sensitivity to stress. *Brain Res*. 2007;1142:1–12.
1044. Feicht CB, Johnson TS, Martin BJ, Sparkes KE, Wagner Jr WW. Secondary amenorrhoea in athletes. *Lancet*. 1978;2:1145–1146.
1045. Tofler IR, Stryer BK, Micheli LJ, Herman LR. Physical and emotional problems of elite female gymnasts [see comments]. *N Engl J Med*. 1996;335:281–283.
1046. Warren M. Amenorrhea in endurance runners. *J Clin Endocrinol Metab*. 1992;75:1393–1397.
1047. Drinkwater B, Bruemner B, Chestnut CI. Menstrual history as a determinant of current bone density in young athletes. *JAMA*. 1990;263:545–548.
1048. Frisch R, Snow R, Johnson L, Gerard B, Barbieri R, Rosen B. Magnetic resonance imaging of overall and regional body fat, estrogen metabolism, and ovulation of athletes compared to controls. *J Clin Endocrinol Metab*. 1993;77:471–477.
1049. Weltman E, Stern R, Doershuk C, Moir R, Palmer K, Jaffe A. Weight and menstrual function in patients with eating disorders and cystic fibrosis. *Pediatrics*. 1990;85:282–287.
1050. Frisch R, Wyshak C, Vincent I. Delayed menarche and amenorrhea in ballet dancers. *N Engl J Med*. 1980;303:17.
1051. Rakoff A. Psychogenic factors in anovulatory women. *Fertil Steril*. 1962;13:1.
1052. Herzog AG. Menstrual disorders in women with epilepsy. *Neurology*. 2006;66: S23–28.
1053. Yen SSC. Chronic anovulation due to CNS-hypothalamic-pituitary dysfunction. In: Yen Y, Jaffe R, eds. *Reproductive Endocrinology*. Philadelphia: WB Saunders Company; 1978.
1054. Carson S, Buster J. Ectopic pregnancy. *N Engl J Med*. 1993;16:1174.
1055. Rochester D, Jain A, Polotsky AJ, et al. Partial recovery of luteal function after bariatric surgery in obese women. *Fertil Steril*. 2009;92:1410–1415.
1056. Netzer NC, Eliasson AH, Strohl KP. Women with sleep apnea have lower levels of sex hormones. *Sleep Breath*. 2003;7:25–29.
1057. Tsutsui K, Son YL, Kiyohara M, Miyata I. Discovery of GnIH and its role in hypothyroidism-induced delayed puberty. *Endocrinology*. 2018;159:62–68.
1058. Maruo T, Nayashi M, Matsuo H, Yamamoto T, Okada H, Mochizuki M. The role of thyroid hormone as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of cultured porcine granulosa cells. *Endocrinology*. 1987;121:1233.
1059. Asch RH, Smith CG, Siler-Khodr TM, Pauerstein CJ. Effects of Δ 5-tetrahydrocannabinol during the follicular phase of the rhesus monkey (Mocaca mulatto). *J Clin Endocrinol Metab*. 1981;52:50–55.
1060. Dees WL, Dissen GA, Hiney JK, Lara F, Ojeda SR. Alcohol ingestion inhibits the increased secretion of puberty-related hormones in the developing female rhesus monkey. *Endocrinology*. 2000;141:1325–1331.
1061. Chen EC, Samuels MH, Luther MF, et al. Cocaine impairs follicular phase pulsatile gonadotropin secretion in rhesus monkeys. *J Soc Gynecol Invest*. 1998;5:311–316.
1062. Battaglia DF, Beaver AB, Harris TG, Tanhehco E, Viguie C, Karsch FJ. Endotoxin disrupts the estradiol-induced luteinizing hormone surge: interference with estradiol signal reading, not surge release. *Endocrinology*. 1999;140:2471–2479.
1063. Oerter K, Kampf G, Munson P, Nienhuis A, Cassorla F, Manasco P. Multiple hormone deficiencies in children with hemochromatosis. *J Clin Endocrinol Metab*. 1993;76:357–361.
1064. South S, Asplin C, Carlsen E, et al. Alterations in luteinizing hormone secretory activity in women with insulin-dependent diabetes mellitus and secondary amenorrhoea. *J Clin Endocrinol Metab*. 1993;76:1048–1053.
1065. Lim V, Kathpalia S, Henriquez C. Endocrine abnormalities associated with chronic renal failure. *Med Clin N Am*. 1978;62:1341.
1066. Boyar RM, Kapen S, Weitzman ED, Hellman L. Pituitary microadenoma and hyperprolactinemia. *N Engl J Med*. 1976;294:263.
1067. Shearman R. Secondary amenorrhoea after oral contraceptives—treatment and follow-up. *Contraception*. 1974;11:123.
1068. van der Steeg N, Bennink H. Bromocriptine for induction of ovulation in normoprolactinaemic post-pill anovulation. *Lancet*. 1977;1:502.
1069. Ortiz A, Hirol M, Stanczyk FZ, Goebelsmann U, Mishell Jr DR. Serum medroxy-progesterone acetate (MPA) concentrations and ovarian function following intramuscular injection of depo-MPA. *J Clin Endocrinol Metab*. 1977;44:32.
1070. Bolognese R, Piver S, Feldman J. Galactorrhea and abnormal menses associated with a long-acting progesterone. *JAMA*. 1967;199:100.
1071. Hirshberg B, Conn PM, Uwaifo GI, Blauer KL, Clark BD, Nieman LK. Ectopic luteinizing hormone secretion and anovulation. *N Engl J Med*. 2003;348:312–317.
1072. Valimaki MJ, Tiitinen A, Alfthan H, et al. Ovarian hyperstimulation caused by gonadotroph adenoma secreting follicle-stimulating hormone in 28-year-old woman. *J Clin Endocrinol Metab*. 1999;84:4204–4208.
1073. Piaditis G, Angellou A, Kontogeorgos G, et al. Ectopic bioactive luteinizing hormone secretion by a pancreatic endocrine tumor, manifested as luteinized granulosa-thecal cell tumor of the ovaries. *J Clin Endocrinol Metab*. 2005;90:2097–2103.
1074. Zimmer CA, Ehrmann DA, Rosenfield RL. Potential diagnostic utility of intermittent administration of short-acting GnRH agonist administration in gonadotropin deficiency. *Fertil Steril*. 2010;94:2697–2702.
1075. Misra M, Klibanski A. Bone health in anorexia nervosa. *Curr Opin Endocrinol Diabetes Obes*. 2011;18:376–382.
1076. Munro MG, Critchley HOD, Fraser IS, FIGO Menstrual Disorders Committee. The two FIGO systems for normal and abnormal uterine bleeding symptoms and classification of causes of abnormal uterine bleeding in the reproductive years: 2018 revisions. *Int J Gynaecol Obstet*. 2018;143:393–408.

1077. Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremner WJ. Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. *J Clin Endocrinol Metab.* 1989;69:804–812.
1078. Daya S. Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects. *Am J Obstet Gynecol.* 1989;161:1009–1011.
1079. Stouffer R, Hodgen C, Ottobre A, Christina C. Follicular fluid treatment during the follicular versus luteal phase of the menstrual cycle: Effects on corpus luteum function. *J Clin Endocrinol Metab.* 1984;58:1027.
1080. Hinney B, Henze C, Kuhn W, Wuttke W. The corpus luteum insufficiency: a multifactorial disease. *J Clin Endocrinol Metab.* 1996;81:565–570.
1081. Seppala M, Nirvonen E, Ranta T. Hyperprolactinaemia and luteal insufficiency. *Lancet.* 1976;1:229.
1082. McNeely MJ, Soules MR. The diagnosis of luteal phase deficiency: a critical review. *Fertil Steril.* 1988;50:1–15.
1083. Azziz R, Carmina E, Dewailly D, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril.* 2009;91:456–488.
1084. O'Brien SH. Evaluation and management of heavy menstrual bleeding in adolescents: the role of the hematologist. *Hematology Am Soc Hematol Educ Program* 2018;2018:390–398.
1085. Haamid F, Sass AE, Dietrich JE. Heavy menstrual bleeding in adolescents. *J Pediatr Adolesc Gynecol.* 2017;30:335–340.
1086. Kennedy S. Primary dysmenorrhoea. *Lancet.* 1997;349:1116.
1087. Ammeman S, Shafer M-A, Snyder D. Ectopic pregnancy in adolescents: a clinical review for pediatricians. *J Pediatr.* 1990;117:677–684.
1088. Bachmann GA, Rosen R, Pinn VW, et al. Vulvodynia: a state-of-the-art consensus on definitions, diagnosis and management. *J Reprod Med.* 2006;51:447–456.
1089. Olive DL. Gonadotropin-releasing hormone agonists for endometriosis. *N Engl J Med.* 2008;359:1136–1142.
1090. Bulun SE. Endometriosis. *N Engl J Med.* 2009;360:268–279.
1091. Rosenwaks Z, Jones GS, Henzl MR, Dubin NH, Ghodgaonkar RB, Hoffman S. Naproxin sodium, aspirin, and placebo in primary dysmenorrhea. *Am J Obstet Gynecol.* 1981;140:592.
1092. DeVane G. Editorial: Premenstrual syndrome. *J Clin Endocrinol Metab.* 1991;72:250.
1093. Grady-Weliky TA. Clinical practice. Premenstrual dysphoric disorder. *N Engl J Med.* 2003;348:433–438.
1094. Schachter SC. Hormonal considerations in women with seizures. *Arch Neurol.* 1988;45:1267–1270.
1095. Dalton K. Cyclical criminal acts in premenstrual syndrome. *Lancet.* 1980;2:1070.
1096. Schmidt PJ, Nieman LK, Danaceau MA, Adams LF, Rubinow DR. Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome [see comments]. *N Engl J Med.* 1998;338:209–216.
1097. Roca CA, Schmidt PJ, Altemus M, et al. Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. *J Clin Endocrinol Metab.* 2003;88:3057–3063.
1098. Wang M, Seippel L, Purdy RH, Backstrom T. Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnan-20-one. *J Clin Endocrinol Metab.* 1996;81:1076–1082.
1099. Rutanen EM, Teppo AM, Stenman UH, Tiitinen A, Fyhrquist F, Ylikorkala O. Recurrent fever associated with progesterone action and persistently elevated serum levels of immunoreactive tumor necrosis factor- α and interleukin-6. *J Clin Endocrinol Metab.* 1993;76:1594–1598.
1100. Cannon JG, Angel JB, Abad LW, et al. Interleukin-1 beta, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. *J Clin Immunol.* 1997;17:253–261.
1101. Rosenfield RL. Polycystic ovary syndrome in adolescents. In: Rose BD, ed. *UpToDate*. 2018. Waltham, MA, www.uptodate.com/index.
1102. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol.* 1935;29:181–191.
1103. Ibanez L, Oberfield SE, Witchel S, et al. An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. *Horm Res Paediatr.* 2017;88:371–395.
1104. Suikkari A-M, MacLachlan V, Montalto J, Calderon I, Healy D, McLachlan R. Ultrasonographic appearance of polycystic ovaries is associated with exaggerated ovarian androgen and oestradiol responses to gonadotropin-releasing hormone agonist in women undergoing assisted reproduction treatment. *Hum Reprod.* 1995;10:513–519.
1105. Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril.* 1988;69:682–690.
1106. Lucky AW, McGuire J, Rosenfield RL, Lucky PA, Rich BH. Plasma androgens in women with acne vulgaris. *J Invest Dermatol.* 1983;81:70–74.
1107. Witchel SF, Oberfield S, Rosenfield RL, et al. The diagnosis of polycystic ovary syndrome during adolescence. *Horm Res Paediatr.* 2015;83:376–389.
1108. Rosenfield RL, Ehrmann DA, Littlejohn E. Adolescent polycystic ovary syndrome due to functional ovarian hyperandrogenism persists into adulthood. *J Clin Endocrinol Metab.* 2015;100:1537–1543.
1109. Ybarra M, Franco RR, Cominato L, Sampaio RB, Sucena da Rocha SM, Damiani D. Polycystic ovary syndrome among obese adolescents. *Gynecol Endocrinol.* 2018;34:45–48.
1110. Dumesic DA, Akopians AL, Madrigal VK, et al. Hyperandrogenism accompanies increased intra-abdominal fat storage in normal weight polycystic ovary syndrome women. *J Clin Endocrinol Metab.* 2016;101:4178–4188.
1111. Li L, Feng Q, Ye M, He Y, Yao A, Shi K. Metabolic effect of obesity on polycystic ovary syndrome in adolescents: a meta-analysis. *J Obstet Gynaecol.* 2017;37:1036–1047.
1112. de Sousa G, Schluter B, Buschatz D, et al. A comparison of polysomnographic variables between obese adolescents with polycystic ovarian syndrome and healthy, normal-weight and obese adolescents. *Sleep Breath.* 2010;14:33–38.
1113. Nandalike K, Agarwal C, Strauss T, et al. Sleep and cardiometabolic function in obese adolescent girls with polycystic ovary syndrome. *Sleep Med.* 2012;13:1307–1312.
1114. Michaliszyn SF, Lee S, Tfayli H, Arslanian S. Polycystic ovary syndrome and nonalcoholic fatty liver in obese adolescents: association with metabolic risk profile. *Fertil Steril.* 2013;100(6).
1115. Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. *J Pediatr.* 2001;138:38–44.
1116. Kim JY, Tfayli H, Michaliszyn SF, Lee S, Arslanian S. Distinguishing characteristics of metabolically healthy versus metabolically unhealthy obese adolescent girls with polycystic ovary syndrome. *Fertil Steril.* 2016;105:1603–1611.
1117. Palmert MR, Gordon CM, Kartashov AI, Legro RS, Emans SJ, Dunaif A. Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2002;87:1017–1023.
1118. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care.* 1999;22:141–146.
1119. Rossi B, Sukalich S, Droz J, et al. Prevalence of metabolic syndrome and related characteristics in obese adolescents with and without polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2008;93:4780–4786.
1120. Hart R, Doherty DA, Mori T, et al. Extent of metabolic risk in adolescent girls with features of polycystic ovary syndrome. *Fertil Steril.* 2011;95:2347–2353. 53. e1.
1121. Gourgari E, Lodish M, Shamburek R, et al. Lipoprotein particles in adolescents and young women with PCOS provide insights into their cardiovascular risk. *J Clin Endocrinol Metab.* 2015;100:4291–4298.
1122. Rosenfield RL, Mortensen M, Wroblewski K, Littlejohn E, Ehrmann DA. Determination of the source of androgen excess

- in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. *Hum Reprod.* 2011;26:3138–3146.
1123. Cipolletta D. Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. *Immunology.* 2014;142:517–525.
1124. Kim JY, Tfayli H, Michalyszyn SF, Arslanian S. Impaired lipolysis, diminished fat oxidation, and metabolic inflexibility in obese girls with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2018;103:546–554.
1125. Gourgari E, Lodish M, Keil M, et al. Bilateral adrenal hyperplasia as a possible mechanism for hyperandrogenism in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2016;101:3353–3360.
1126. Maas KH, Chuan S, Harrison E, Cook-Andersen H, Duleba AJ, Chang RJ. Androgen responses to adrenocorticotropic hormone infusion among individual women with polycystic ovary syndrome. *Fertil Steril.* 2016;106:1252–1257.
1127. Corbould A. Effects of androgens on insulin action in women: is androgen excess a component of female metabolic syndrome? *Diabetes Metab Res Rev.* 2008;24:520–532.
1128. Gooren LJ, Giltay EJ, Bunck MC. Long-term treatment of transsexuals with cross-sex hormones: extensive personal experience. *J Clin Endocrinol Metab.* 2008;93:19–25.
1129. Huang G, Tang E, Aakil A, et al. Testosterone dose-response relationships with cardiovascular risk markers in androgen-deficient women: a randomized, placebo-controlled trial. *J Clin Endocrinol Metab.* 2014;99: E1287–1293.
1130. True CA, Takahashi DL, Burns SE, et al. Chronic combined hyperandrogenemia and western-style diet in young female rhesus macaques causes greater metabolic impairments compared to either treatment alone. *Hum Reprod.* 2017;32:1880–1891.
1131. Bishop CV, Mishler EC, Takahashi DL, et al. Chronic hyperandrogenemia in the presence and absence of a western-style diet impairs ovarian and uterine structure/function in young adult rhesus monkeys. *Hum Reprod.* 2018;33:128–139.
1132. Dallel M, Sarray S, Douma Z, et al. Differential association of DENND1A genetic variants with polycystic ovary syndrome in Tunisian but not Bahraini Arab women. *Gene.* 2018;647:79–84.
1133. Barnes RB, Rosenfield RL, Ehrmann DA, et al. Ovarian hyperandrogenism as a result of congenital adrenal virilizing disorders: Evidence for perinatal masculinization of neuroendocrine function in women. *J Clin Endocrinol Metab.* 1994;79:1328–1333.
1134. Barnes RB, Rosenfield RL, Burstein S, Ehrmann DA. Pituitary-ovarian responses to nafarelin testing in the polycystic ovary syndrome. *N Engl J Med.* 1989;320:559–565.
1135. Toscano V, Balducci R, Bianchi P, Mangiantini A, Sciarra F. Ovarian 17-ketosteroid reductase deficiency as a possible cause of polycystic ovarian disease. *J Clin Endocrinol Metab.* 1990;71:288–292.
1136. Kanova N, Bicikova M. Hyperandrogenic states in pregnancy. *Physiol Res.* 2011;60:243–252.
1137. Meldrum DR, Frumar AM, Shamonki IM, Benirschke K, Judd HL. Ovarian and adrenal steroidogenesis in a virilized patient with gonadotropin-resistant ovaries and hilus cell hyperplasia. *Obstet Gynecol.* 1980;56:216–221.
1138. Carbanaru G, Prasad P, Scoccia B, et al. The hormonal phenotype of Nonclassic 3 beta-hydroxysteroid dehydrogenase (HSD3B) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited HSD3B2 deficiency. *J Clin Endocrinol Metab.* 2004;89:783–794.
1139. Charmandari E, Kino T, Ichijo T, Chrousos GP. Generalized glucocorticoid resistance: clinical aspects, molecular mechanisms, and implications of a rare genetic disorder. *J Clin Endocrinol Metab.* 2008;93:1563–1572.
1140. Qin K, Rosenfield RL. Mutations of the hexose-6-phosphate dehydrogenase gene rarely cause hyperandrogenemic polycystic ovary syndrome. *Steroids.* 2011;76:135–139. 2011.
1141. Lawson AJ, Walker EA, Lavery GG, et al. Cortisone-reductase deficiency associated with heterozygous mutations in 11beta-hydroxysteroid dehydrogenase type 1. *Proc Natl Acad Sci U S A.* 2011;108:4111–4116.
1142. Oostdijk W, Idkowiak J, Mueller JW, et al. PAPSS2 deficiency causes androgen excess via impaired DHEA sulfation - in vitro and in vivo studies in a family harboring two novel PAPSS2 mutations. *J Clin Endocrinol Metab.* 2015. jc20143556.
1143. Futterweit W, Krieger DT. Pituitary tumors associated with hyperprolactinemia and polycystic ovary disease. *Fertil Steril.* 1979;31:608–613.
1144. Filho RB, Domingues L, Naves L, Ferraz E, Alves A, Casulari LA. Polycystic ovary syndrome and hyperprolactinemia are distinct entities. *Gynecol Endocrinol.* 2007;23:267–272.
1145. Speiser PW, Susin M, Sasano H, Bohrer S, Markowitz J. Ovarian hyperthecosis in the setting of portal hypertension. *J Clin Endocrinol Metab.* 2000;85:873–877.
1146. Bas S, Guran T, Atay Z, et al. Premature pubarche, hyperinsulinemia and hypothyroxinemia: novel manifestations of congenital portosystemic shunts (Abernethy malformation) in children. *Horm Res Paediatr.* 2015;83:282–287.
1147. Tanaka YO, Tsunoda H, Kitagawa Y, Ueno T, Yoshikawa H, Saida Y. Functioning ovarian tumors: direct and indirect findings at MR imaging. *Radiographics.* 2004;24(Suppl 1):S147–S166.
1148. Rosenfield RL, Cohen RM, Talerman A. Lipid cell tumor of the ovary in reference to adult-onset congenital adrenal hyperplasia and polycystic ovary syndrome. *J Reprod Med.* 1987;32:363–369.
1149. Mandel FP, Voet RL, Weiland AJ, Judd HL. Steroid secretion by masculinizing and “feminizing” hilus cell tumors. *J Clin Endocrinol Metab.* 1981;52:779–784.
1150. Givens JR, Andersen RN, Wisner WL, Donelson AJ, Coleman SA. A testosterone-secreting, gonadotropin-responsive pure thecoma and polycystic ovarian disease. *J Clin Endocrinol Metab.* 1975;41:845–853.
1151. Dunaif A, Scully RE, Andersen RN, Chapin DS, Crowley Jr WF. The effects of continuous androgen secretion on the hypothalamic-pituitary axis in woman: evidence from a luteinized thecoma of the ovary. *J Clin Endocrinol Metab.* 1984;59:389–393.
1152. Fragoso MC, Latronico AC, Carvalho FM, et al. Activating mutation of the stimulatory G protein (gsp) as a putative cause of ovarian and testicular human stromal Leydig cell tumors. *J Clin Endocrinol Metab.* 1998;83:2074–2078.
1153. Klotz RK, Muller-Holzner E, Fessler S, et al. Leydig-cell-tumor of the ovary that responded to GnRH-analogue administration - case report and review of the literature. *Exp Clin Endocrinol Diabetes.* 2010;118:291–297.
1154. Basaria S. Androgen abuse in athletes: detection and consequences. *J Clin Endocrinol Metab.* 2010;95:1533–1543.
1155. Fiers T, Wu F, Moghetti P, Vanderschueren D, Lapauw B, Kaufman JM. Reassessing free-testosterone calculation by liquid chromatography-tandem mass spectrometry direct equilibrium dialysis. *J Clin Endocrinol Metab.* 2018;103:2167–2174.
1156. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2013;98:4565–4592.
1157. Pall M, Azziz R, Beires J, Pignatelli D. The phenotype of hirsute women: a comparison of polycystic ovary syndrome and 21-hydroxylase-deficient nonclassic adrenal hyperplasia. *Fertil Steril.* 2010;94:684–689.
1158. Prassopoulos V, Laspas F, Vlachou F, Efthimiadou R, Gogou L, Andreou J. Leydig cell tumour of the ovary localised with positron emission tomography/computed tomography. *Gynecol Endocrinol.* 2011;27:837–839.
1159. Buggs C, Rosenfield RL. Polycystic ovary syndrome in adolescence. *Endocrinol Metab Clin N Am.* 2005;34:677–705.
1160. Rosenfield RL. Clinical practice. Hirsutism [Comment in: *N Engl J Med.* 2006 Apr 6;354(14):1533-5; author reply 1533-5]. *N Engl J Med.* 2005;353:2578–2588.
1161. Eichenfield LF, Krakowski AC, Piggott C, et al. Evidence-based recommendations for the diagnosis and treatment of pediatric acne. *Pediatrics.* 2013;131(Suppl 3). S163–186.
1162. Oelkers W, Foidart JM, Dombrovicz N, Welter A, Heithecker R. Effects of a new oral contraceptive containing an antiminerocorticoid progestogen, drospirenone, on the renin-aldosterone system, body weight, blood pressure, glucose tolerance, and lipid metabolism. *J Clin Endocrinol Metab.* 1995;80:1816–1821.
1163. Gambineri A, Patton L, Vaccina A, et al. Treatment with flutamide, metformin, and their combination added to a hypocaloric

- diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab.* 2006;91:3970–3980.
1164. Moretti C, Guccione L, Di Giacinto P, et al. Combined oral contraception and bicalutamide in polycystic ovary syndrome and severe hirsutism: a double-blind randomized controlled trial. *J Clin Endocrinol Metab.* 2018;103:824–838.
1165. Koukourakis MI, Kakouratos C, Kalamida D, et al. Comparison of the effect of the antiandrogen apalutamide (ARN-509) versus bicalutamide on the androgen receptor pathway in prostate cancer cell lines. *Anticancer Drugs.* 2018;29:323–333.
1166. Leibel NI, Baumann EE, Kocherginsky M, Rosenfield RL. Relationship of adolescent polycystic ovary syndrome to parental metabolic syndrome. *J Clin Endocrinol Metab.* 2006;91:1275–1283.
1167. Berni TR, Morgan CL, Berni ER, Rees DA. Polycystic ovary syndrome is associated with adverse mental health and neurodevelopmental outcomes. *J Clin Endocrinol Metab.* 2018;103:2116–2125.
1168. Styne DM, Arslanian SA, Connor EL, et al. Pediatric obesity—assessment, treatment, and prevention: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102:709–757.
1169. Gardner CD, Trepanowski JF, Del Gobbo LC, et al. Effect of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion: the DIETFITS randomized clinical trial. *JAMA.* 2018;319:667–779.
1170. Ornstein RM, Copperman NM, Jacobson MS. Effect of weight loss on menstrual function in adolescents with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol.* 2011;24:161–165.
1171. Wong JM, Gallagher M, Gooding H, et al. A randomized pilot study of dietary treatments for polycystic ovary syndrome in adolescents. *Pediatr Obes.* 2016;11:210–220.
1172. Escobar-Morreale HF, Santacruz E, Luque-Ramirez M, Botella Carretero JJ. Prevalence of ‘obesity-associated gonadal dysfunction’ in severely obese men and women and its resolution after bariatric surgery: a systematic review and meta-analysis. *Hum Reprod, update.* 2017;23:390–408.
1173. Ehrmann D, Cavaghan M, Imperial J, Sturis J, Rosenfield R, Polonsky K. Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 1997;82:524–530.
1174. Bridger T, MacDonald S, Baltzer F, Rodd C. Randomized placebo-controlled trial of metformin for adolescents with polycystic ovary syndrome. *Arch Pediatr Adolesc Med.* 2006;160:241–246.
1175. Hoeger K, Davidson K, Kochman L, Cherry T, Kopin L, Guzik DS. The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metab.* 2008;93:4299–4306.
1176. Ladson G, Dodson WC, Sweet SD, et al. The effects of metformin with lifestyle therapy in polycystic ovary syndrome: a randomized double-blind study. *Fertil Steril.* 2011;95(1059–66). e1–7.
1177. Al Khalifah RA, Florez ID, Dennis B, Thebane L, Bassillious E. Metformin or oral contraceptives for adolescents with polycystic ovarian syndrome: a meta-analysis. *Pediatrics.* 2016;137. e20154089.
1178. Bredella MA, McManus S, Misra M. Impact of metformin monotherapy versus metformin with oestrogen-progesterone on lipids in adolescent girls with polycystic ovarian syndrome. *Clin Endocrinol.* 2013;79:199–203.
1179. Glintborg D, Sidelmann JJ, Altinok ML, Mumm H, Andersen M. Increased thrombin generation in women with polycystic ovary syndrome: A pilot study on the effect of metformin and oral contraceptives. *Metab Clin Exp.* 2015;64:1272–1278.

17 Turner Syndrome

Philippe Backeljauw, Steven D. Chernausek, Claus Højbjerg Gravholt, Paul Kruszka

CHAPTER OUTLINE

HISTORICAL BACKGROUND	627
GENETICS	627
Chromosomal Origins	627
Epidemiology	628
Turner Karyotypes	628
X Chromosome Genes and Turner Syndrome	628
X Chromosome Genomic Imprinting	631
Diagnostic Tests	631
Indications for Karyotype Testing	632
Differential Diagnosis	632
Prenatal Diagnosis	633
CLINICAL CHARACTERISTICS AND COMORBIDITIES	633
Lymphedema	633
Growth Failure, Short Stature, and Skeletal Anomalies	634
Ovarian Insufficiency	636
Gonadoblastoma	637
Cardiovascular System Anomalies	638
Renal Anomalies	642
Otologic Disorders	643
Autoimmunity	643
Disorders of the Gastrointestinal System	643
Abnormalities of Carbohydrate Metabolism	644
Neurocognition and Psychological Features	644
MEDICAL MANAGEMENT	645
Initial Evaluation	645
Cardiovascular Assessment and Monitoring	646
Patients with Y Chromosome	647
Growth-Promoting Therapies	648
Puberty, Pubertal Induction, and Feminization	649
TRANSITION TO ADULT CARE	650
Outpatient Care and Treatment	651
References	652

HISTORICAL BACKGROUND

Turner syndrome (TS) is defined as a disorder caused by complete or partial absence of the second sex chromosome, with or without cell line mosaicism. This further needs to be associated with one or more characteristic physical features in a phenotypic female, such as short stature or primary ovarian insufficiency. The eponym comes from a study published in 1938 by Henry Turner, who described seven women with short stature, sexual infantilism, neck webbing, a low posterior hairline, and an increased carrying angle or cubitus valgus¹ (Fig. 17.1). Several years earlier (in 1930), the clinical geneticist Otto Ullrich had independently described an 8-year-old girl with short stature, lymphedema of the hands and feet, neck webbing, a high arched palate, low-set external ears, and some other characteristics that are now recognized features of TS.² Ullrich subsequently recognized that his patient and those of Turner

probably had the same condition.³ He further called attention to the work of Bonnevie, who described neck distention and malformations of the ears, face, and limb buds in mice, secondary to dissection of subcutaneous fetal tissues by fluid. Ullrich suggested that fetal lymphatic obstruction may cause neck webbing and other features of TS and proposed the eponym *Bonnevie-Ullrich* to describe this constellation of anomalies. Ullrich's contributions gave rise to the eponym *Ullrich-Turner syndrome* more commonly used in Europe.

Endocrine and pathology studies performed already in the 1940s revealed the presence of primary ovarian failure in TS women, further associated with elevated gonadotropins, reduced estrogen concentrations, and "streak" ovaries, consisting of connective tissue depleted of germ cells. These early studies also described an increased incidence of hypertension and aortic disease in young TS individuals.⁴ The first link between TS and a sex chromosome anomaly was provided in 1954 by Polani and colleagues, who reported on three women with TS and coarctation of the aorta, while also being sex chromatin negative.⁵ Soon thereafter, advances in cytogenetic identification of specific chromosomes revealed that TS was associated with the presence of a single X chromosome (X monosomy).⁶ These observations signified a major shift in our appreciation of the role of the human sex chromosomes in sex determination, as reviewed by Opitz and Pallister.⁷ They also described the significant heterogeneity of patients grouped under the umbrella of gonadal dysgenesis. *Dysgenesis* and *agenesis* represent inaccurate descriptions for TS individuals' ovarian histopathology, because fetal ovarian development appears normal in TS, and the ovarian tissue degeneration occurs mainly around the time of birth. Although eponyms have their disadvantages, the designation *Turner* (or *Ullrich-Turner*) *syndrome* is also more specific than *gonadal dysgenesis*.

GENETICS

Chromosomal Origins

In 1959 TS was linked to monosomy X in a 14-year-old girl by using a karyotype of colchicine-arrested metaphase bone marrow cells.⁸ In a subsequent study of 307 individuals with gonadal dysgenesis and sex chromosome anomalies, the short arm of the X chromosome was identified as the critical region responsible for TS.⁹

Today, 6 decades after the first karyotyping of TS, the molecular mechanisms that lead to monosomy X and TS are still not completely understood. Based on other aneuploidies, especially the trisomies, such as Down syndrome, the most popular hypothesis for the molecular basis of TS is an error in meiosis. A maternal X is present in 70% to 80% of individuals with TS,¹⁰ suggesting a male predisposition to meiotic errors. Fluorescent in situ hybridization (FISH) studies in sperm support a male predisposition to meiotic errors in the sex chromosomes as autosomal disomy occurs in 0.1% and sex chromosome disomy occurs in 0.27%.¹¹ If indeed TS results from an error in meiosis, unlike for trisomy investigations, the stage of meiosis that contributes to monosomy X cannot be determined. For example, in Down syndrome, nonsegregation is known to occur in meiosis II, when two chromosome 21s are homologous.

Besides an error in meiosis, the other possibility explaining monosomy X is a postzygotic nonsegregation in mitosis.

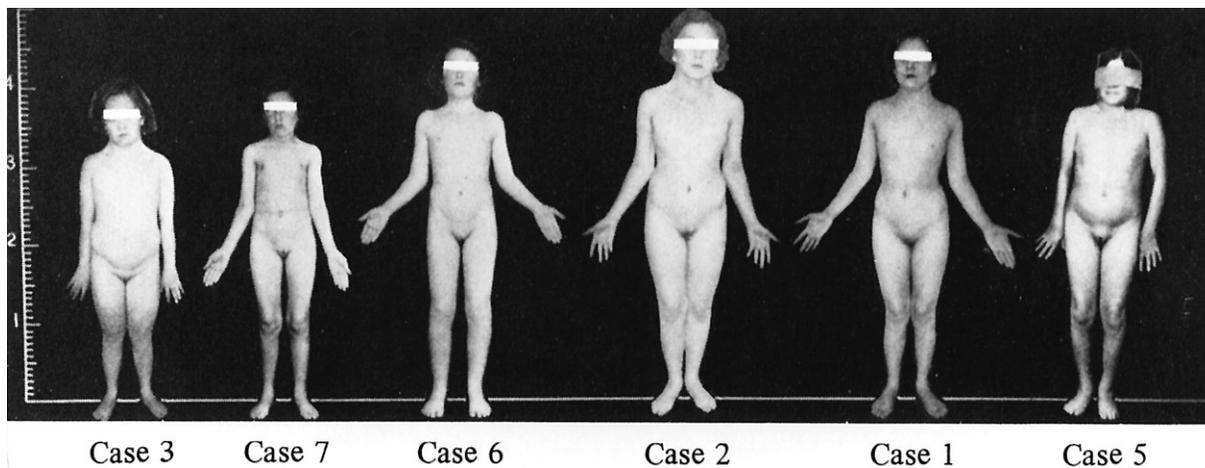


Fig. 17.1 Patients described by Dr. Henry Turner. Note the height marker at the left indicating the short stature, although with large variation in absolute height among these women. Note also lack of obesity among these women evaluated in the 1930s. (From Turner, H. H. (1938). A syndrome of infantilism, congenital webbed neck and cubitus valgus. *Endocrinology*, 23, 566.)

Postzygotic nonsegregation is an attractive hypothesis considering that approximately half of individuals with TS are mosaic. However, there are no empirical data in humans to support this hypothesis. However, there is evidence in the mouse model that the Y chromosome is predisposed to nondisjunction during early cleavage division, and more research in humans should provide further answers.¹²

In addition to nonsegregation in meiosis and mitosis, there are a number of structural anomalies of the X chromosome, including isochromosome X [46,X,i(Xq)] and partial deletion of Xp and Xq. The X isochromosome is the most common isochromosome in humans, and it is likely caused by chromosome breakage and recombination in the proximal Xp, resulting in an isodicentric X i(Xq) chromosome.¹³

Epidemiology

TS is a common finding in newborn females with a birth incidence of 1 in 2500.¹⁴ However, TS is diagnosed more frequently prenatally. Using the Danish Cytogenetic Central Register, Gravholt et al. compared the prenatal prevalence of TS to its postnatal prevalence.¹⁵ A postnatal prevalence of 32/100,000 was found compared with 176/100,000 in diagnoses by amniocentesis and 392/100,000 by chorionic villus sampling (CVS).¹⁵ Wharton and Hook, in their study of the New York state registry, also found a higher prevalence in prenatal diagnoses: 22.2/100,000 and 85/100,000 in postnatal age and prenatally by amniocentesis, respectively.¹⁶

Turner Karyotypes

Most karyotype determinations are made from mitotic peripheral lymphocytes that have been arrested in metaphase or prometaphase. The limitation of the peripheral blood karyotype is that only blood is tested and no other tissues, thus mosaicism cannot be tested reliably. A second tissue, such as skin fibroblasts, or buccal mucosa cells, or bladder epithelial cells, should be evaluated when a peripheral blood karyotype is normal and TS is still suspected.¹⁷ A large Danish study has set the fractions of various peripheral blood karyotypes and found that in 781 individuals with TS: 45% were found to have 45,X; 11% had a karyotype that included an isochromosome, such as 46X,i(Xq) or 45,X/46X,i(Xq); and the remaining 44% were mostly mosaic, such as 45,X/46,XX.¹⁸ Supporting further a high rate of

mosaicism in TS, a review of 532 live born girls and women with TS found that approximately half were mosaic.¹⁹

From a clinical and genetic counseling perspective, it is important to consider why and when a karyotype is obtained, as the timing of karyotype analysis often correlates with the phenotype severity in TS. Many prenatal karyotypes diagnosing TS have been done for advanced maternal age and compared with postnatal diagnoses, these individuals have decreased cardiac anomalies and decreased incidence of phenotypic features, such as neck webbing and low hairline, while characterized by a higher incidence of mosaicism.²⁰ For the purpose of genetic counseling of 45,X/46,XY mosaics, it is important to highlight that in a study of 92 cases of prenatally diagnosed 45,X/46,XY mosaic cases, 95% had normal male genitalia.²¹

A significant finding on a karyotype is the presence of Y chromosome material, which has implications in clinical management. A Y chromosome detected by karyotype is a risk for gonadoblastoma.¹⁷ In a study of 114 females with TS, 14 (12.2%) had Y material found by polymerase chain reaction (PCR), of which seven had detectable Y chromosome material on karyotype.²² Interestingly, of the 10 individuals in this study who underwent ovariectomy, only one (10%) had a gonadoblastoma. In another study of 171 individuals with TS, 14 (8%) were found to have Y chromosome material by Y chromosome repeat markers, and four of the 12 individuals who had a gonadectomy had gonadoblastoma detected.²³ In a more recent study of 130 individuals with TS, three individuals were found to have Y chromosome material by karyotype and six by reverse transcription-PCR.²⁴ Of these nine individuals with Y chromosome material, all underwent gonadectomy and one was found to have a gonadoblastoma. Although the aforementioned studies are heterogeneous in their methods, the risk of gonadoblastoma based on the best evidence available is roughly 10% if Y chromosome material is present. Current recommendation is for gonadectomy to be performed in TS individuals with Y chromosome material found on a standard karyotype analysis (Fig. 17.2).¹⁷

X Chromosome Genes and Turner Syndrome

Genotype-Phenotype Associations

Over 160 million years ago, the X and Y chromosomes were homologous, until the Y chromosome acquired the sex

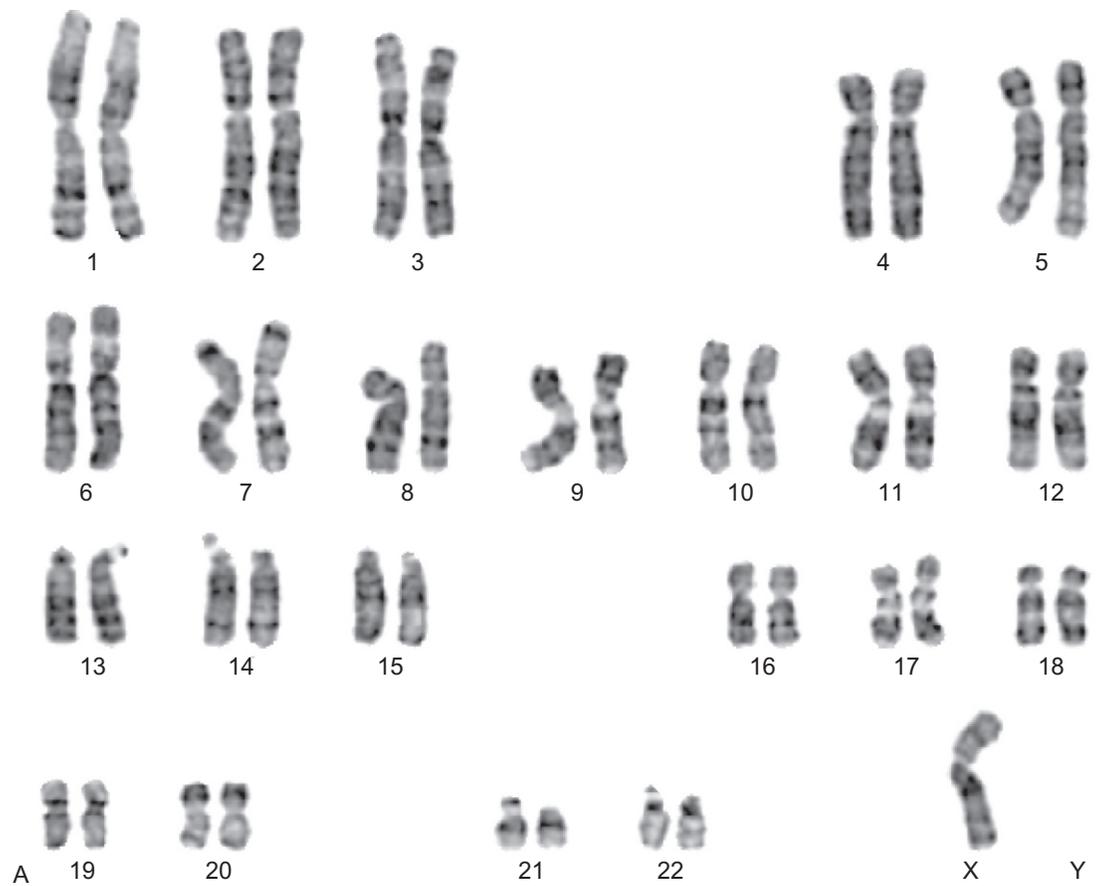


Fig. 17.2 A, Karyotype of an individual with Turner syndrome where there is absence of a second sex chromosome (45,X).

Continued

determining gene *SRY* and slowly lost many of its genes because of isolation from recombination by inversion.²⁵ A dosage compensation system evolved to equalize expression of the X chromosome genes on the 46,XX female and 46,XY male.²⁵ The compensation mechanism is X chromosome inactivation (XCI), which is achieved by the ribonucleic acid (RNA) coding gene *XIST* expressed on the inactive X chromosome; *XIST* then coats the inactive X chromosome making it inactive.²⁶ However, 20% to 30% of X-linked genes escape X inactivation and are expressed on the inactive X chromosome.²⁷ Pseudoautosomal region 1 (PAR1) located at the distal tip of the short arm of the X chromosome (Xp) and the short arm of chromosome Y (Yp) contains 24 genes that escape this X inactivation. The pseudoautosomal region (PAR) is divided into two regions, PAR1 at the terminus of the short arm of X and Y, and PAR2 on the long arm. The PARs pair and recombine during meiosis. There are four genes on PAR2 on both the X and Y chromosomes, and these genes, interestingly, do not escape X inactivation on the X chromosome.^{25,28} There are further 17 X-Y ancestral homologs, of which also 14 escape X inactivation. However, the remaining majority of X-linked genes that escape XCI do not have homologs on the Y chromosome.²⁹ Genes that escape XCI are haploinsufficient in TS and have historically been candidates for genotype-phenotype investigations. However, few X genes have been correlated with the TS phenotype. The short stature homeobox containing gene on the X chromosome (*SHOX*) is the most cited example (Fig. 17.3).

SHOX is located at Xp22 on the pseudoautosomal region of the X chromosome. Both males and females have two copies of *SHOX*. *SHOX*, a transcription factor, has been implicated in short stature and the skeletal anomalies associated with TS.³⁰ In a study of over 1600 prepubertal non-TS children with short stature, deletions or pathogenic variants in *SHOX* were found in approximately 4% of individuals.³¹ *SHOX* heterozygous variants, most commonly a deletion, may cause isolated short stature or other anomalies, such as mesomelia or the Madelung wrist deformity, observed in Leri-Weill dyschondrosteosis. The concept of haploinsufficiency of a gene, such as *SHOX* located in the PAR, is an attractive explanation for part of the TS phenotype. However, there is currently little evidence of any other monogenic variants on the PARs associated with the TS phenotype.

In addition to short stature, infertility and primary ovarian insufficiency are phenotypes for which a genetic etiology has been sought. Mapping partial X chromosome deletions in women, with and without TS, has provided loci associated with primary ovarian insufficiency (POI). Starting with the short arm of the X chromosome (Xp), TS phenotypes including POI have been mapped to Xp11.2-Xp22.1.³² In non-TS women, there is one gene located in this locus that has been associated with POI. Bone morphogenetic protein 15 (*BMP15*), located on Xp11.2, is an oocyte growth factor that stimulates folliculogenesis and granulosa cell (GC) growth, and has been implicated in POI. Di Pasquale et al. presented

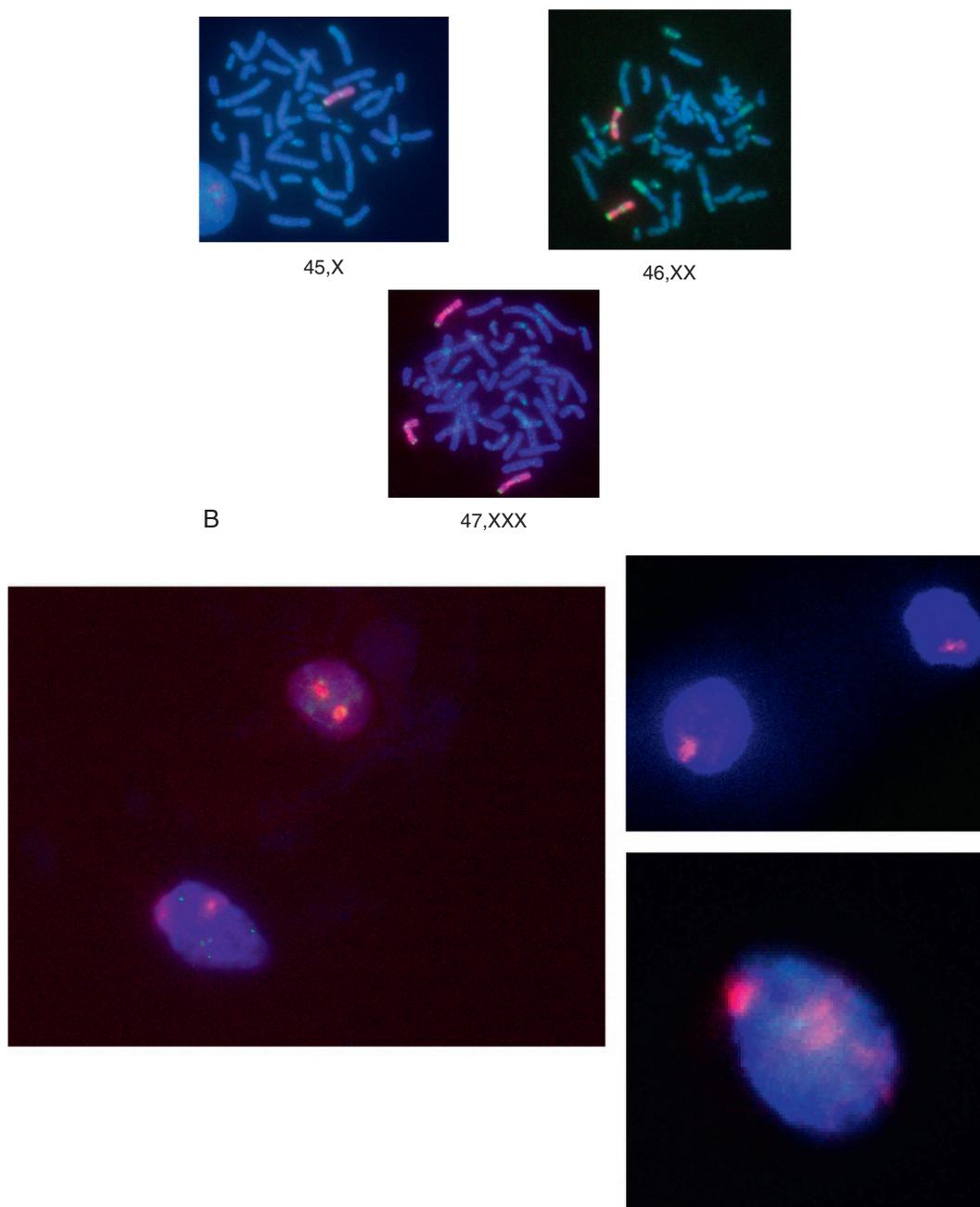


Fig. 17.2, cont'd B, Fluorescence in situ hybridization (FISH) with two-color XY specific probes. Chromosomes are in metaphase and counterstained with DAPI (*blue*). Note that there are three different X chromosome numbers: 45,X; 46,XXX; and 47,XXX. The X chromosomes are colored in red; the small amount of green is the shared regions of the X and Y chromosomes, or the pseudoautosomal regions. (Provided by Dr. Amalia Dutra, NHGRI.)

two sisters with a heterozygous missense variant in *BMP15* (p. Y235C) and functional studies suggested a dominant negative mechanism.³³ In a more recent study of 300 women with POI, six variants were found in *BMP15* with three of the variants leading to marked decrease in protein production.³⁴ Interestingly, there is a report of a small 554kb duplication involving the genes *BMP15* and *SHROOM4* in a woman with TS and spontaneous menarche, adding more evidence to *BMP15*'s association with POI.³⁵

On the long arm of the X chromosome (Xq), a number of X chromosome-autosome translocations in non-TS women have

identified two additional loci or critical regions associated with POI: Xq13-Xq21 (critical region I) and Xq23-Xq27 (critical region II).^{36,37} In these critical regions, a small number of women without TS have shown associations with genetic variants and POI. In an X-autosome translocation [t(X;11)(q24;q13)] found in both a mother and daughter with POI, disruption in the gene progesterone receptor membrane component-1 (*PGRMC1*) was found, based on reduced gene expression levels.³⁸ *PGRMC1* mediates antiapoptotic action of progesterone and several cytochrome P450 reactions in ovarian cells. Mansouri et al. screened 67 females with POI and found one

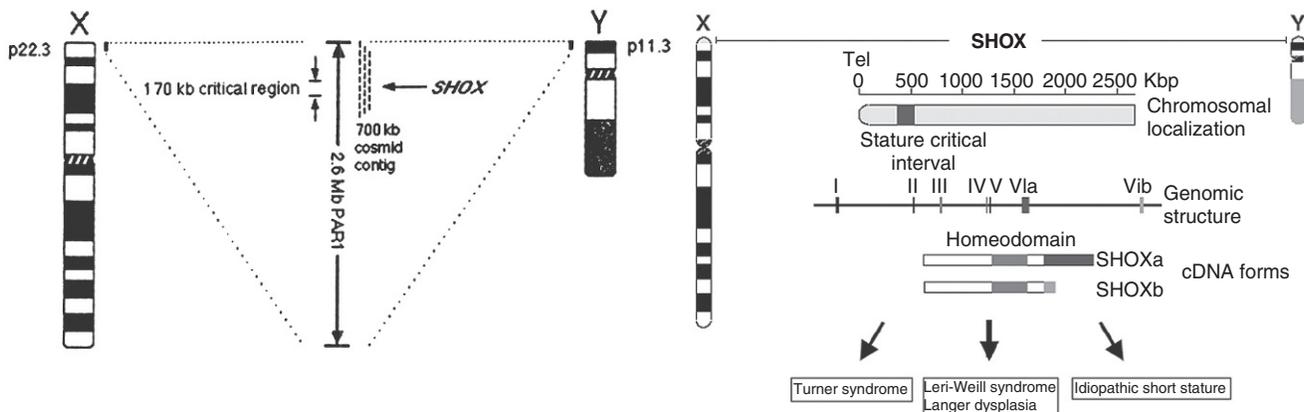


Fig. 17.3 X and Y chromosome ideograms showing the terminal pseudoautosomal regions (PAR) at Xp22.3 and Yp11.3 where the *SHOX* gene has been mapped (A&C), and X chromosome regions historically associated with aspects of phenotype. (From Zinn, A. R. (1997). Growing interest in Turner Syndrome. *Nat Genet*, 16, 3. With permission from Macmillan Publisher Ltd.)

person with a p.H165R variant that was shown to interfere with cytochrome P450 7A1 (*CYP7A1*) binding to *PGRMC1*.³⁸ In a large database of over 43,000 presumed healthy individuals, this particular variant was found in 0.2%.³⁹ Located in Xq21, a report of five daughters with POI to a consanguineous couple identified a homozygous variant in premature ovarian failure protein 1B (*POF1B*).⁴⁰ This variant was also shown to affect nonmuscle myosin binding.⁴⁰ One prior report in a woman with POI demonstrated a translocation involving chromosome 1 and the X chromosome [t(X;1)(q21;p34)] with a breakpoint in the third intron of *POF1B*.⁴¹ *POF1B* is of interest as it escapes X inactivation and is located in POI1. Also located in critical region I, dachshund homolog 2 (*DACH2*) was first identified by fine-mapping the breakpoint of an autosome-X chromosome translocation in a patient with POI.⁴² Two missense variants in *DACH2* have been associated with women with POI. However, there is no functional studies' evidence to support *DACH2*'s role in POI.

Although not in the two critical loci noted earlier, two missense variants in androgen receptor gene (*AR*) on Xq12 have been associated with POI in Indian women with POI,⁴³ but the study did not evaluate these variants with functional studies. The androgen receptor is of potential interest as deficiency of *AR* in the mouse model results in POI.⁴⁴

A gene on the X chromosome worthy of special mention is fragile X mental retardation (*FMR1*) on Xq27.3. A premutation, 50–200 CGG repeats in the 5' untranslated CGG repeat, of *FMR1* on Xq27.3 leads to methylation-coupled silencing of *FMR1* and absence of the fragile X mental retardation protein (*FMRP*), causing the classical Fragile X syndrome.⁴⁵ Fragile X syndrome is a common X-linked cause of cognitive impairment in males when the CGG repeat is greater than 200.⁴⁵ Although haploinsufficiency of *FMR1* is unlikely to cause POI in TS, a premutation is a common cause of POI, explaining 5% of sporadic POI cases and 10% to 15% of familial cases.⁴⁶ The mechanism of POI in *FMR1* premutation is thought to be an increase in messenger RNA (mRNA).⁴⁷

Short stature and POI are the most studied phenotypes in TS, and cognitive impairment will only be discussed briefly. The gene *XIST* has been associated with cognitive impairment in TS. Cognitive impairment is infrequently associated with TS and has mostly been observed in individuals with mosaic (short) ring chromosome [45,X/46,X,r(X)]. Cognitive impairment was first described in nine of 21 individuals with such a ring chromosome.⁴⁸ The initial hypothesis for cognitive impairment was thought to be absence of the *XIST* gene on the ring

chromosome, resulting in the failure of the X-inactivation of the ring chromosome.⁴⁹ However, Leppig noted that in nine individuals with an r(X), eight had *XIST* genes present, questioning the original "absence of *XIST*" hypothesis.⁴⁸

It must be emphasized that genotype-phenotype relationships in TS remain largely unknown. New approaches, such as methylation- and RNA expression studies are beginning to provide new answers. In a lymphocyte methylation and RNA expression study comparing TS women and 46,XX and 46,XY controls, women with TS were found to have genome wide hypomethylation altered expression in genes on both the autosomes and sex chromosomes compared with controls.⁵⁰ Other studies have identified X chromosome microRNA that escapes X-inactivation and may play an important role in disease differences between males and females.⁵¹

X Chromosome Genomic Imprinting

Imprinting studies for TS have shown mixed results. Skuse et al. proposed that individuals with 45,X who inherited a paternal X chromosome had a higher social cognition, meaning a significant difference in verbal and higher-order executive function skills.⁵² This study was based on 80 individuals with TS, of whom 55 had inherited a maternal X. In a smaller study of 39 participants with TS, Bishop et al. found that immediate and delayed recall was in the normal range for girls with TS.⁵³ A study that looked at brain morphology in 40 girls with 45,X (23 girls with a maternal X and 17 with a paternal X) found differences in brain morphology based on X chromosome origin: those who inherited a paternal X had a thicker cortex in the temporal regions, and those who inherited a maternal X had enlargement of gray matter in superior frontal regions.⁵⁴ Lepage et al. also noted that girls with a paternal X had a lower full-scale IQ than those that had inherited a maternal X. In contrast to these studies, a study of 50 girls with TS showed no association between parental X and IQ scores, as determined by the Wechsler Intelligence Scale for Children.⁵⁵ Finally, for the most recognized phenotype of short stature, there is no evidence of X chromosome imprinting based on a small study of 25 individuals with TS.⁵⁶

Diagnostic Tests

There are multiple testing options available for TS diagnosis, both prenatally and postnatally. As noted earlier, the first

diagnosis was made in 1959 by karyotype testing and this method continues to be the standard of care.^{8,17,57} A karyotype can be made from any metaphase or prometaphase cell that is actively dividing, including peripheral blood lymphocytes, amniocytes, and fibroblasts. Chromosomal microarray has the same level of accuracy as a karyotype, but has the advantage of more precise chromosomal breakpoints in the case of partial deletions and translocations.⁵⁸ Although not a first-line test for TS, next generation sequencing can accurately diagnose TS and has the advantage of being able to simultaneously diagnose single gene variant conditions such as Noonan syndrome.⁵⁹ FISH may be used to diagnose TS and has the advantage of being faster than karyotype testing, and can be performed on nondividing cells, such as those derived from buccal swabs.

Indications for Karyotype Testing

There is a distinct biphasic pattern in the diagnosis of Turner syndrome with a substantial proportion being diagnosed around the time of birth, and another large group being diagnosed around 12 years of age (Fig. 17.4). When there is a clinical suspicion of TS, the American College of Medical Genetics recommends a 20-cell karyotype⁵⁷ that should be able to detect mosaicism greater than 11%.⁶⁰ For a prenatal 45,X karyotype diagnosis, TS should be confirmed with a postnatal karyotype¹⁷.

Differential Diagnosis

When TS is suspected prenatally or postnatally, other genetic etiologies should be considered. In the prenatal period, TS may be diagnosed with noninvasive prenatal testing (NIPT) or CVS. Confined placental mosaicism, when the placenta is 45,X and the fetus is euploid, is in the differential diagnosis and should be resolved with amniocentesis. In addition to NIPT and CVS, prenatal ultrasound findings may suggest TS and other syndromes (Fig. 17.5). The following prenatal ultrasound findings may be found in other genetic syndromes: hygroma colli, fetal hydrops, coarctation of the aorta, and increased nuchal translucency.⁶¹ In first trimester ultrasound of 185 singleton pregnancies with cystic hygroma colli, the most frequent chromosomal abnormality was TS ($n = 49$; 26.5%), followed by trisomy 21 ($n = 32$; 17.3%), and trisomy 18 ($n = 27$; 14.6%).⁶² In this same study, 15 euploid fetuses were tested for Noonan syndrome and

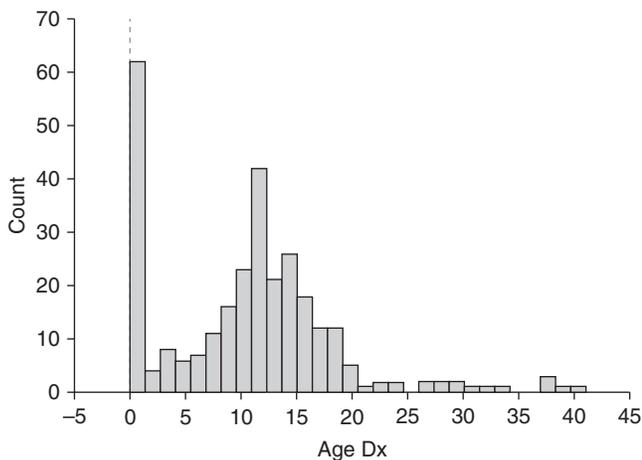


Fig. 17.4 Age of Turner diagnosis. The histogram shows the age of diagnosis for 292 patients evaluated in the National Institutes of Health Turner natural history protocol between 2005 and 2010. The median age of diagnosis was 11 years.

six (40%) were found to have it.⁶² In 65 cases of hydrops fetalis, six (9.2%) were diagnosed with TS, three (4.6%) with trisomy 21, and two cases with Noonan syndrome (3%).⁶³

Postnatally, TS may be suspected in childhood because of short stature, or during the pubertal age because of (primary) amenorrhea. Short stature affects 3% of children and there are numerous genetic etiologies.³¹ Common etiologies on the differential diagnosis include Noonan syndrome and haploinsufficiency for the *SHOX* gene. Although Noonan syndrome has been labeled “male TS” by some in the past,^{64,65}

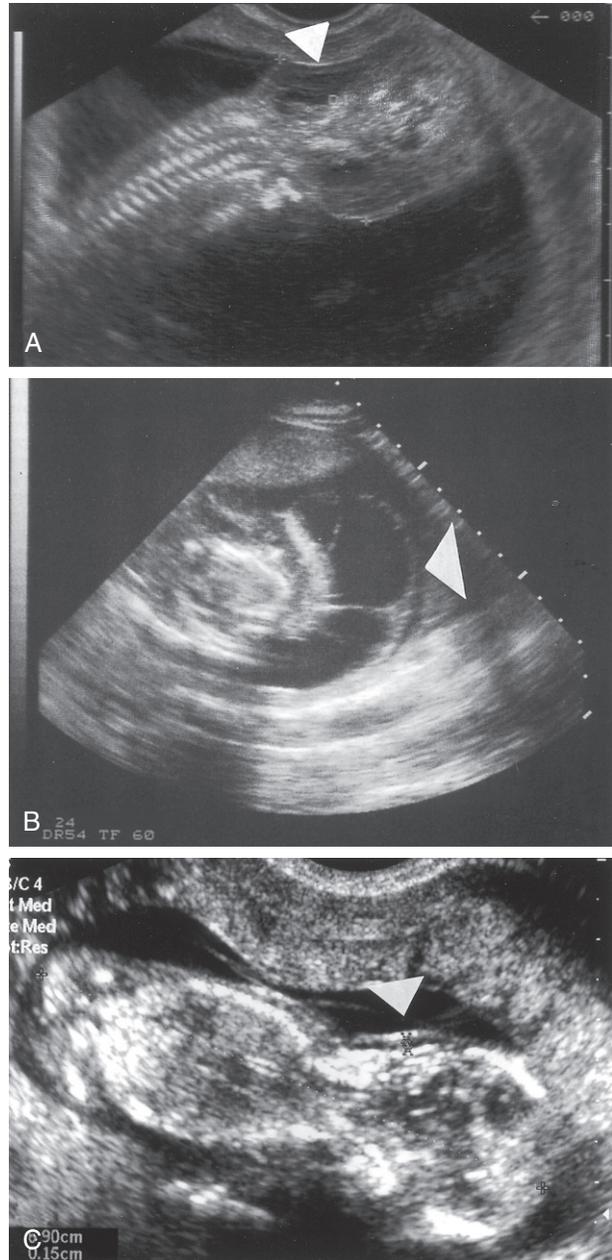


Fig. 17.5 A, A 14-week-old fetus with Turner syndrome and a cystic hygroma (arrow). B, A 13-week-old fetus with a normal karyotype and normal nuchal translucency of 1.5 mm (arrow). C, Same fetus as in view (A) in transverse plane. Large septated cystic hygroma (arrow) can be seen around the fetal neck. (Courtesy Pekka Taipale, MD, PhD, Kuppio University Hospital, Finland.)

the facial, cardiac, and development features are quite different between these two syndromes.⁶⁶

Prenatal Diagnosis

NIPT has changed the timing and accuracy of genetic prenatal screening, especially for aneuploidy syndromes, such as TS. NIPT is based on small fragments of circulating cell-free fetal deoxyribonucleic acid (ccfDNA) that originate from placental cells that are continuously released, and can be detected as early as 5 weeks of gestation.⁶⁷ Approximately 10% to 15% of cell free DNA in the maternal circulation is from the placenta, when measured between 10 and 20 weeks of gestation,⁶⁸ and the half-life of ccfDNA is less than a day.⁶⁹ There are a number of methods using NIPT to screen for TS prenatally, and most techniques count the number of copies of sequenced DNA fragments using next generation sequencing techniques.

In a Cochrane database systematic review,⁷⁰ a pooled analysis of over 7000 high-risk pregnancies involving 45,X, in 12 studies using massive parallel shotgun sequencing, showed a clinical sensitivity of 91.7% (95% confidence interval [CI], 78.3–97.1) and a clinical specificity of 99.6% (95% CI, 98.9–99.8). The Cochrane database study found similar results for roughly 1000 high-risk individuals in four studies, using targeted massive parallel sequencing, which only evaluates select loci of the genome.⁷⁰ Even with high sensitivity and specificity, the positive predictive values for NIPT for TS may be as low as 23% to 26%, which strongly reinforces the need for confirmatory testing.^{70–72} The American College of Obstetricians and Gynecologists recommends that all women with a positive cell free DNA result have a diagnostic procedure before clinical measures, such as pregnancy termination is taken.⁷³

The diagnosis of monosomy X is possible in embryos formed after in vitro fertilization (IVF) and is termed *preimplantation genetic diagnosis (PGD)* or *preimplantation genetic screening*. A biopsy of a blastomere at the cleavage stage or a trophoblast biopsy at the blastocyst stage allows for an aneuploidy assessment, using techniques including FISH, chromosomal microarray, and next generation sequencing. PGD analysis of IVF embryos is motivated by the belief that selection of euploid embryos will improve IVF outcome. However, to date, there is no evidence that PGD improves the live birth rate after IVF.^{74–76} More research is needed in PGD applications to aneuploidy, especially with respect to diagnosing TS.

CLINICAL CHARACTERISTICS AND COMORBIDITIES

Individuals with TS may present with a variety of phenotypic features: some patients have few and others are characterized by a multiplicity of findings. The typical physical characteristics can vary significantly between patients with similar karyotype (Fig. 17.6), and some of the clinical signs may be subtle. Table 17.1 summarizes the clinical features most commonly encountered in girls and women with TS.

Lymphedema

Lymphedema is relatively common in TS and begins in utero. Fig. 17.7 demonstrates the generalized edema that can be observed in many 45,X fetuses. It is the consequence of malformations of the lymphatic vessels, resulting in lymphatic hypoplasia and obstruction.⁷⁷ A cystic hygroma is a fluid-filled sac that often is located in the head/neck area, but may occur in other parts of the body, and results from abnormal communication between the jugular and central lymphatics draining into

the heart. Peripheral lymphatic hypoplasia or aplasia has also been demonstrated. Using lymphangiography hypoplasia, and even aplasia, can also be observed in the peripheral lymphatic vessels of the extremities.^{78,79}

Clinical features resulting from nuchal lymphatic obstruction include webbing of neck, also called *pterygium colli*, and is observed in up to 25% of TS patients. Additional features that appear to result from this tenting and stretching of facial/scalp and neck skin is thought to result in low-set, rotated external ears, ptosis and downslowing of the eyelids, and the development of a low posterior hairline, with upward growing hairs (see Fig. 17.6). The occurrence of thick hair growth, including thick eyelashes and eyebrows, may also be, at least in part, a consequence of the distended cutaneous structures during fetal

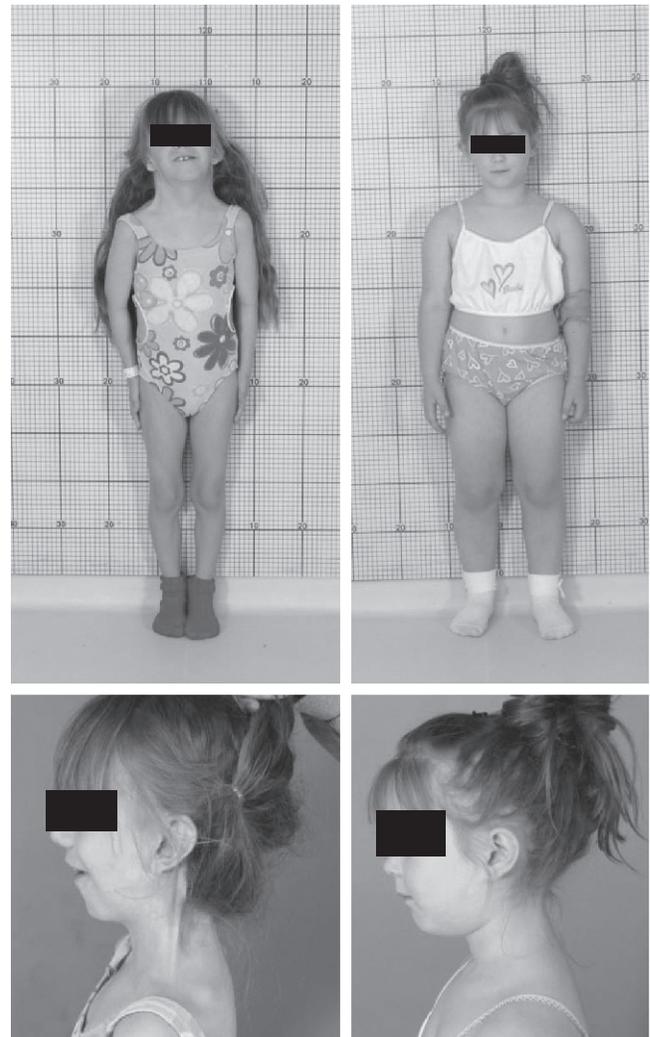


Fig. 17.6 Phenotypic variability in Turner syndrome. Both of these 7-year-old girls with short stature have Turner syndrome with a 45, X karyotype confirmed in analysis of 50 lymphocytes. The girl on the left was diagnosed at birth because of prominent neck webbing and low-set and posteriorly rotated ears. She also has micrognathia and a low posterior hairline. In contrast, the girl on the right was diagnosed at age 7 years because of short stature without “classical” stigmata of Turner syndrome, and she is more typical of the clinical presentation of the majority of girls with Turner syndrome diagnosed in the 21st century. (This image can be viewed in full color online at ExpertConsult.)

TABLE 17.1 Clinical Features in Girls With Turner Syndrome

Physical Diagnosis		Percentage Affected	
Skeletal	Short stature	100	
	Short neck	40	
	↑ Upper:lower body ratio	97	
	Cubitus valgus	47	
	Short metacarpal	37	
	Scoliosis	12.5	
	Madelung deformity	7.5	
	Micrognathia/high palate	60	
	Lymphatic obstruction	Neck webbing	25
		Low posterior hairline	42
Edema of hands/feet		22	
Nail dysplasia		13	
Other	Strabismus	18	
	Ptosis	11	
	Multiple nevi	26	
SCREENING EVALUATION			
Cardiovascular anomaly	All	44	
	Bicuspid aortic valve	30	
	Aortic coarctation	12	
	Dilated aorta	11	
	Other ^a	12	
Renal anomaly	All	18	
	Horseshoe kidney	11	
	Duplicated collecting ducts	4	
Liver disorder	Unilateral agenesis	3	
	All	36	
	Abnormal liver function tests (LFTs)	27	
Hypertension	Fatty infiltration	19	
	All	34	
	Prehypertension	14	
Autoimmunity	Overt hypertension	20	
	All	51	
	Hashimoto thyroiditis	51	
	Graves disease	1	
	Type 1 diabetes	0	
	Celiac	5	
	Inflammatory bowel	3	

"Physical Diagnosis" describes findings from physical examinations of more than 200 girls seen by Drs. Barbara Lippe and Paul Saenger between 1985 and 2000. Information on pubertal development is not included because many girls were not old enough to assess. The "screening evaluation" data are from 100 girls aged 7 to 17 years, who underwent standardized imaging and laboratory testing as part of the National Institutes of Health natural history study, performed between 2001 and 2008. The cardiovascular evaluation included magnetic resonance imaging and cardiac echo.

^aUnder the cardiovascular listing, the "Other" category included partial anomalous pulmonary veins, aberrant right subclavian artery, and atrial septal defects. Blood pressure was measured on 24-hour ambulatory monitors. All patients also had renal and hepatic ultrasound studies. Abnormal liver function was defined as greater than 10% elevation of aminotransferase(s). Hashimoto was defined by history of clinical hypothyroidism or elevation of circulating thyroid antibodies.

life. Swelling of the dorsum of the hands and feet at birth indicates the presence of a nonpitting type of peripheral edema. The lymphedema usually improves over the first 1 to 2 years of postnatal life. It may even resolve, but in many patients it persists to some degree.⁷⁹ Some TS individuals demonstrate intermittent worsening of their peripheral edema, and this appears to be associated with puberty or the introduction of growth

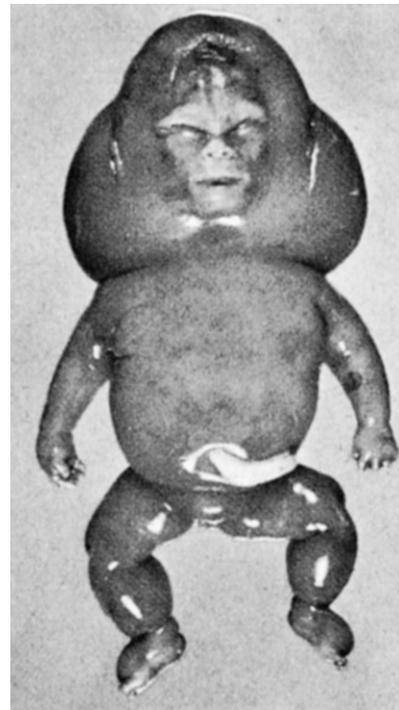


Fig. 17.7 A 45,X abortus demonstrating generalized lymphedema. Note the distended cervical region. With resolution of the edema, the redundant skin may cicatrize, resulting in a webbed neck. The edema of the hands and feet may persist and be present at birth. (From Gellis, S. S., & Feingold, M. (1978). Picture of the month. *Am J Dis Child*, 132, 417. Copyright © 1978, American Medical Association.)

hormone (GH) or sex steroid therapy (both somewhat salt-retaining).

Growth failure, Short Stature, and Skeletal Anomalies

Short stature is the most common physical abnormality seen in TS. Poor statural growth affects essentially every patient to some degree. Their growth failure is disproportionate and they have a relatively large trunk with broad shoulders and pelvis; together with the reduced height this renders them with a "stocky" appearance.⁸⁰ They often have a short neck because of hypoplasia of one or several cervical vertebrae.⁸¹ Long bone growth is preferentially impaired: patients do have relatively large hands and feet relative to their height, but the relatively short lower extremities lead to an increased upper-to-lower segment ratio.⁸² Selected bones can be more affected than others: an increased carrying angle at the elbow joint, also called *cubitus valgus*, is observed in nearly half of TS individuals. This carrying angle is the angle of intersection of the long axis of the upper arm with the long axis of the fully extended and supinated forearm. In adult normal females, this elbow angle is approximately 12 degrees (twice the size as seen in males).⁸³ This angle is between 15 and 30 degrees (Fig. 17.8) in many girls and women with TS because of abnormal development of the head of the trochlear bone.

The finding of a shortened fourth metacarpal, as shown on a bone age radiograph (Fig. 17.9A) is also quite common. On physical examination, it manifests as absence or less prominence of the fourth knuckle when making a fist. Sometimes the

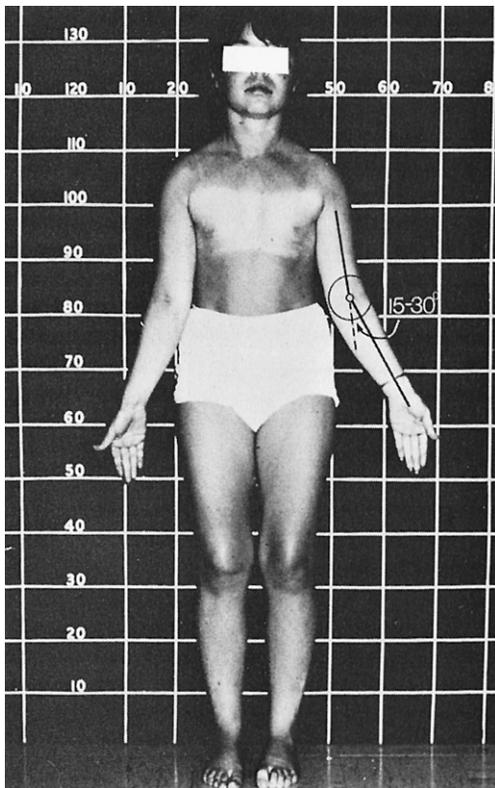


Fig. 17.8 A 16-year-old girl with Turner syndrome and absence of puberty. Note absence of most characteristic stigmata save short stature and an increased carrying angle (cubitus valgus).

fifth knuckle is also affected. Short fourth metatarsals can be found in some patients. The Madelung deformity is less common and is caused by a curvature of the distal radius in addition to a dorsal subluxation of the distal ulna (Fig. 17.10).⁸⁴ This anomaly also occurs as part of Leri-Weill dyschondrosteosis, and in both conditions is an indicator of a deficiency of SHOX. The previously described *cubitus valgus* and short metacarpals are also the result of SHOX deficiency.

At least 10%, and possibly as many as 20% of TS girls between 5 and 20 years of age develop scoliosis, defined as a lateral curve greater than 10 degrees. Kyphosis may develop in up to 40% of patients.⁸⁵ Both findings likely are the result of vertebral development anomalies. Abnormalities of the development of facial bones and skull base result in a micrognathic and retrognathic mandible and/or high arched palate in 60% of patients.

Osteoporosis and fractures are more common in women with TS. Hand and wrist radiographs may reveal mild osteopenia (Fig. 17.9B),⁸⁶ a coarse trabecular bone pattern, and ballooning of the tips of the terminal phalanges.⁸⁷ This osteoporotic appearance is observed during childhood and adolescence, suggesting it may be related to a developmental role of SHOX inherent to TS rather than to primary estrogen deficiency, which may still be a contributory factor. The fact that cortical bone mineralization is selectively reduced in TS, independent of estrogen exposure, is consistent with this.⁸⁸ Fractures of the long bones not associated with significant trauma are more frequently observed among girls with TS.^{89,90} It should be noted, however, that interpretation of studies of bone mineralization in young individuals with TS is difficult because areal instead of volumetric bone mineral density is measured. Nevertheless, prepubertal TS girls have decreased markers of bone formation because of a state of low-bone turnover and decreased bone deposition.

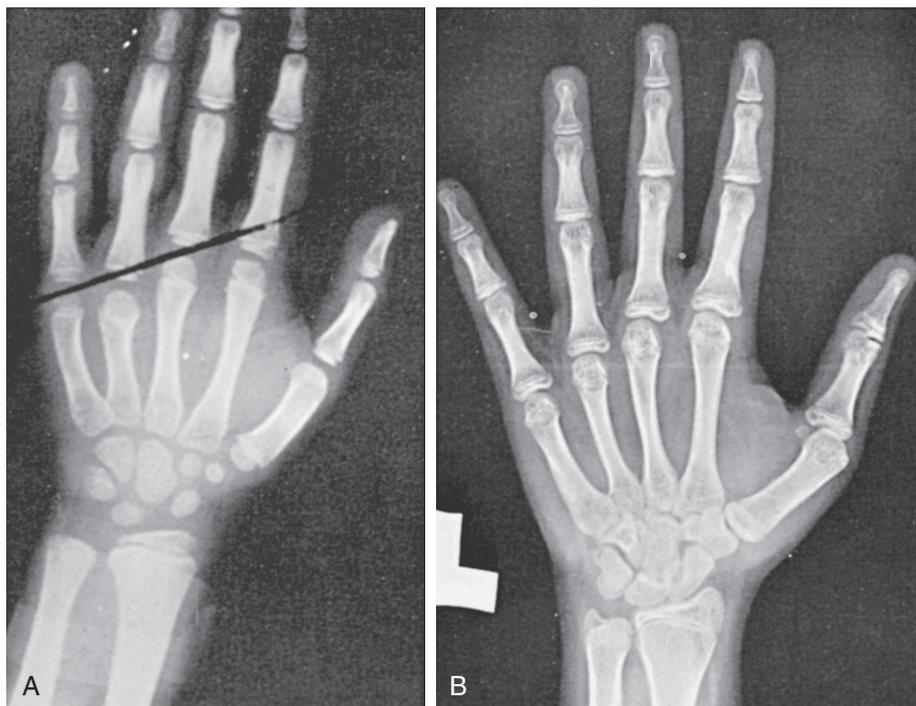


Fig. 17.9 Two characteristic hand radiographs. A, Short fourth metacarpal, the tip falling below a straight line drawn between the third and fifth metacarpals. B, Generalized lacy ("fish net") appearance of the carpals and tufting of the distal phalanges, characteristic of the osteoporotic appearance of the bones of patients with Turner syndrome.



Fig. 17.10 A 19-year-old-patient with Turner syndrome and bilateral bayonet-like Madelon deformities of the wrists.

Poor linear growth in childhood and adolescence leads to adult TS women ending up about 20 cm shorter than their non-Turner female peers. The short stature in TS can be attributed to the deleterious effect of SHOX haploinsufficiency.

One of the first large-scale assessments of the growth failure present in TS was reported by Ranke et al. in 1983.⁹¹ Fig. 17.11 shows cross-sectional height and height velocity data from 150 TS children who did not receive growth-promoting therapy. Davenport and coworkers⁹² further elaborated on the different stages of impaired growth throughout a TS patient's life. Prenatally, there is a mild degree of growth restriction resulting in a length at birth that is about 1 standard deviation (SD) below the mean. During infancy and up to 3 years of age, a further mild growth deceleration can be seen.⁹³ Continued subnormal growth is observed throughout childhood, so that between ages 3 and 13 years, TS girls develop more deflection from both their target percentile and the normal height curves. There is failure to experience a normal pubertal growth spurt, and slow growth may be prolonged for several more years, potentially into the early 20s.

Height at diagnosis,⁹⁴ ultimate height achieved, and mid-parental height are positively correlated.⁹⁵ The adult height deficit is approximately 20 cm and this is irrespective of patients' ethnic background.⁹⁶ TS-specific growth curves have been developed by Lyon and associates,⁹⁷ based on the data from Ranke and colleagues, as well as other European centers. These curves provide mean height and SD values for age and indicate a mean adult height of 143.1 cm (about 56.5 inches). A strong correlation between the initial height on these Turner curves and the adult height was also observed by Lyon and colleagues, independently of bone age at the time of the first height.⁹⁷ This essentially allows for the adult height prediction of any girl with TS based on her height at an earlier age.

Ovarian Insufficiency

Initial reports on the presence of gonadal pathology described TS individuals as having streak ovaries. These small fibrous gonadal structures have few if any oocytes present. It appeared as if the ovaries had failed to develop normally. Subsequent studies revealed that early ovarian development actually occurs in a normal manner, with adequate numbers of oocytes and primordial follicles present in 14- to 16-week-old Turner fetuses.⁹⁸ However, later in development, Turner ovaries become relatively depleted of oocytes and have fewer developing follicles. This suggests a process of accelerated oocyte demise and follicular atresia.^{99,100} Despite this, some TS girls have follicles in various stages of development, even during the second decade of life.¹⁰¹ Fig. 17.12 shows pelvic ultrasound studies comparing the pelvic structures of TS individuals with those of a normal pubertal females. The cause for the high rate of oocyte attrition in most girls with TS is unknown. One possible explanation is that premature oocyte depletion is caused by abnormal meiosis because of the aneuploidal status.¹⁰² Another possible explanation is that normal oocyte generation and survival require diploid expression of certain genes on the X chromosome. The chromosomal location of putative genes important for fertility has yet to be defined. This is because POI/gonadal failure is frequently observed in Xp deletions with an intact Xq complement, but also in some cases of Xq deletion with normal Xp complement.¹⁰³ In addition, it has been reported that terminal Xq deletions can be associated with POI in women who have few, if any features of TS (see earlier section on Genetics, for more detailed description).¹⁰⁴

Because there is a broad range of gonadal dysfunction in TS individuals, it is hard to predict which patient will spontaneously enter puberty, and who will not, or to determine which women could become pregnant. Based on information from ovarian biopsies the strongest predictor for the presence of ovarian follicles is a karyotype with mosaicism for 45,X and 46,XX cell lines. TS arising from 45,X monosomy or structural defects of one X chromosome are much less likely to be associated with the presence of follicles in the ovaries.¹⁰⁵ Additional positive predictors of follicle presence include both normal follicle-stimulating (FSH) and anti-Müllerian hormone (AMH) concentrations—see also Figs. 17.13 and 17.14.^{106,107} Although less robust than the blood karyotype, the occurrence of spontaneous puberty is also a positive predictor.¹⁰⁵ Spontaneous puberty was observed in approximately 15% of girls with 45,X monosomy and in 30% of girls with a second cell line with more than one X chromosome (i.e., 45,X/46,XX; 45,X/47,XXX)—based on the findings of a study that included more than 500 girls with TS.¹⁰⁸ Even when spontaneous puberty occurs, it may not progress normally, and menarche may be delayed or does not happen. Even if menarche occurs, oligomenorrhea and anovulation are common, so that less than 5% of 20-year-old TS women end up having normal menstrual cycles.

Spontaneous pregnancy may occur in 2% to 3% of women with TS.^{108–110} It is more common in women with 45,X/46,XX (or 45,X/47,XXX) cell line mosaicism. However, spontaneous pregnancies occurring in 45,X women, without evidence of mosaicism, are well-documented.^{110–112} In the initial literature on spontaneous pregnancy in TS individuals, increased fetal mortality or malformations were reported.¹¹³ However, this has not been observed in more recent, population-based studies^{110,114}—even for women with X monosomy.^{110,115} If the case where a TS mother has a sex chromosome structural abnormality, such abnormal chromosome may be passed on to her offspring. The risk of

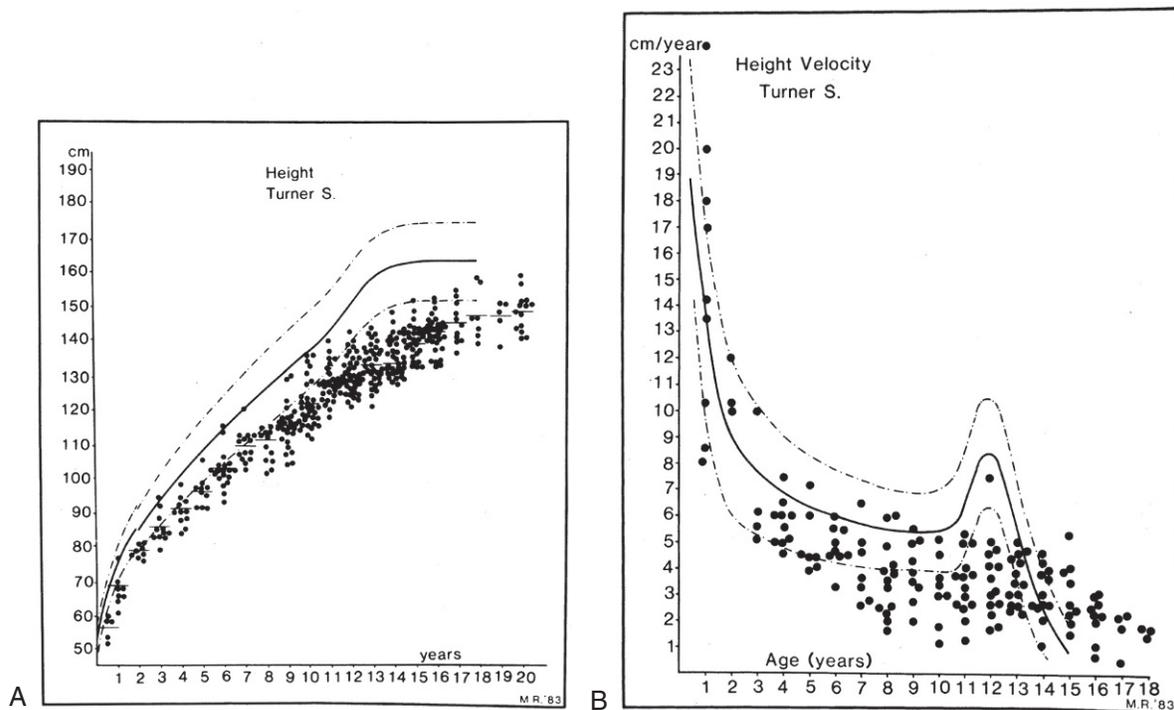


Fig. 17.11 Height and height velocity in Turner syndrome. A, Some 384 single measurements of height for 150 children with Turner syndrome. B, Height velocity from a total of 159 measurements. The normal ranges are shown by the *heavy* and *dashed* lines. (From Tanner, J. M., Whitehouse, R. H., & Takaishi, M. (1965). Standards from birth to maturity for height, weight, height velocity and weight velocity: British children. *Arch Dis Child*, 41, 454, 613; Ranke, M. D., Pfluger, H., Rosendahl, W., et al. (1983). Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Paediatr*, 141, 81.)

maternal complications with spontaneous or assisted pregnancies is quite high for women with TS.

Gonadoblastoma

A gonadoblastoma is a rare benign gonadal tumor, and is almost always found in a (dysgenetic) gonad with Y chromosome material. This tumor has a distinctive histological appearance, which distinguishes it from other gonadal neoplasms. The gonadoblastoma is composed of large germ cells surrounded by sex cord derivatives that resemble small cells with variable granulosa, lutein, or immature Sertoli-like morphology. Because it strongly resembles the histology of a developing gonad, it was assigned the name "gonadoblastoma." Although the tumor is considered a benign growth, it has both the potential for steroid production (can produce both testosterone and estrogen), as well as for malignant transformation (into a dysgerminoma).¹¹⁶ Gonadoblastomas are found in approximately 40% of females with 46,XY mixed gonadal dysgenesis.¹¹⁷ The frequency among girls with TS and Y chromosomal material is between 10% and 30%, but is likely closer to the lower end of this range.¹¹⁶ These data are based on histologic examination of ovaries that were "prophylactically" removed from girls who carried Y chromosome material. Analyses of Danish health registry data did not find increased morbidity or mortality related to any type of ovarian tumor among women with TS.^{118,119} A large British registry study ascertained gonadoblastoma diagnoses in 8% of women with Y chromosome in their peripheral blood karyotype.¹²⁰

Prophylactic gonadectomy has been standard practice since the time that an excess of gonadal tumors was first observed in girls and women who had both Y chromosome material and intraabdominal gonads.¹²¹ Such a surgical approach was taken because these gonads tended to be nonfunctional, and caused an increased risk for tumor formation. However, spontaneous puberty and even pregnancy may occur in individuals with TS and Y chromosome material.¹²²⁻¹²⁴ Portnoi et al. reported on an 8-year-old TS girl who was found to have a translocation of Y chromosome material onto the X chromosome (see Fig. 17.2). The patient did not undergo gonadectomy, developed spontaneous puberty, and eventually had a well-supervised pregnancy with good outcome.¹²² It should also be noted that our clinical experience in TS associated with gonadoblastoma and/or dysgerminoma is based on patients whose karyotype included visible Y chromosome material; in some cases, there even was clinical evidence of virilization. Because of this, the recommendation for ovariectomy was initially only applicable to individuals with visible Y chromosome material or virilization.^{125,126} With the introduction of newer molecular technologies, several reports indicate detection of Y chromosome sequences in 45,X girls without evidence of Y chromosome material on their karyotype. In some of these patients, gonadoblastoma has been detected after gonadectomy. It has been suggested by some that all patients with 45,X monosomy undergo molecular screening for cryptic Y chromosome material. Because it has been shown that PCR amplification may yield false positive results,¹²⁷ the risk of overtreatment is a real concern. In addition, immature ovaries may contain nests of germinal cells that are benign but resemble a gonadoblastoma.

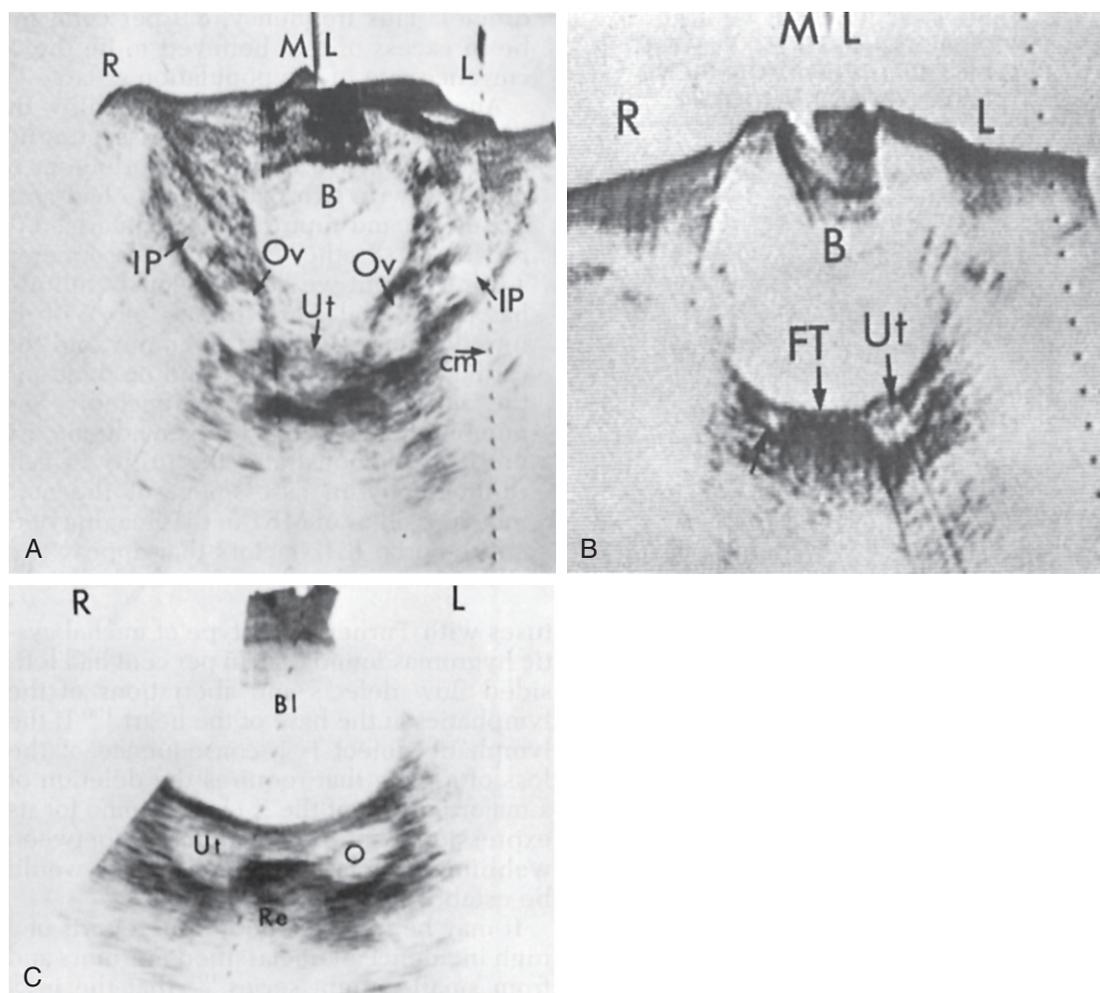


Fig. 17.12 Examples of pelvic ultrasound studies. A, Normal pubertal female demonstrating ovaries of adult size. B, Patient with Turner syndrome. The corpus of the uterus is seen slightly to the left of the midline. The fallopian tube can be followed into the right adnexa and observed to terminate in a small structure (*arrow*) believed to be the fimbriated end of the tube. No ovaries are identified. C, Patient with 45,X/46,XY Turner syndrome previously treated with estrogen. The corpus of the uterus is enlarged to adult size. In the left adnexa, a large gonadal mass (O) is seen. Histologically, this was identified as a gonadoblastoma. The images are transverse, oriented right (R) and left (L) of the midline (ML). The dotted scales are in centimeters. B, BL, bladder; FT, fallopian tube; IP, iliopsoas; O, gonadoblastoma; OV, ovary; Re, rectum; Ut, uterus.

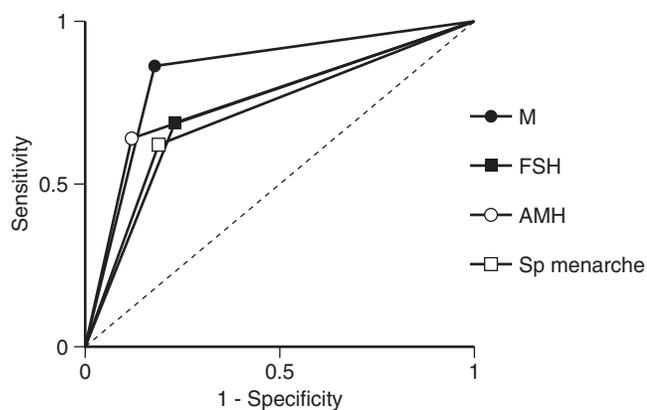


Fig. 17.13 Receiver operating characteristic curves showing the relationship between sensitivity and specificity regarding the four investigated variables with the highest sensitivity. (From Borgström, B., Hreinsson, J., Rasmussen, C., et al. (2009). Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab*, 94, 74–80. Erratum in *J Clin Endocrinol Metab*, 94, 1478, Figure 2.)

There is currently a paucity of information on the use of imaging or serum markers for surveillance of potential gonadal tumors in TS. In any case where Y chromosomes/sequences are detected and gonadoblastoma is suspected, there needs to be education and counseling of patients or family concerning gender identity, sexual functioning, and reproductive consequences relevant to the decision for gonadectomy. Preservation of follicles or oocytes may be an option for some patients undergoing gonadectomy. Further discussion of gonadoblastoma issues is found under the Patients with Y Chromosome section presented later in the chapter.

Cardiovascular System Anomalies

Congenital Cardiovascular Malformations in Turner Syndrome

Congenital cardiovascular malformations (CVMs) are the most serious, life-threatening consequences of X chromosome monosomy.^{118,120,128} Coarctation of the aorta is one of the first CVMs documented in patients with TS.^{5,129–131} The frequency and spectrum of TS-specific CVMs, based on data combining chromosomal genotyping and imaging, is summarized in

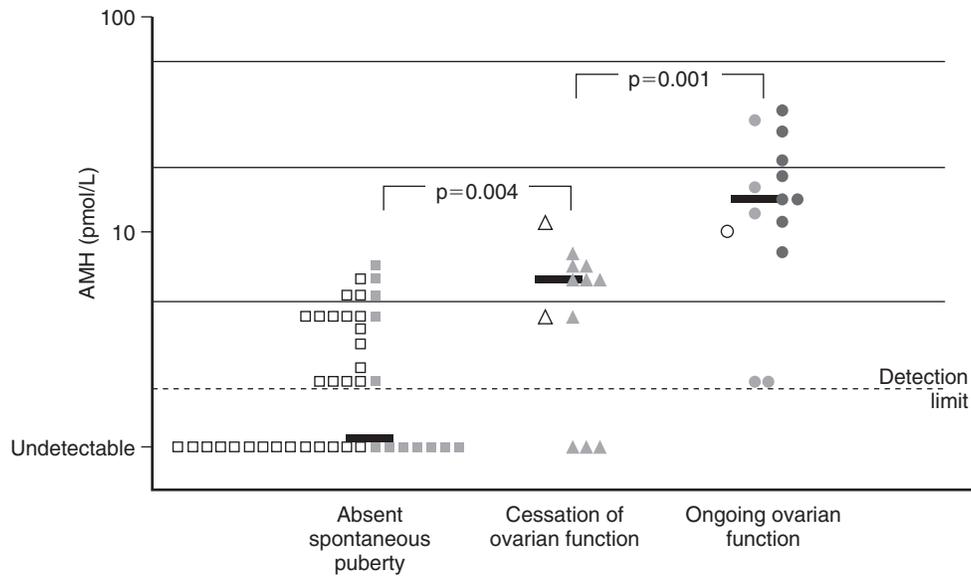


Fig. 17.14 Anti-Müllerian hormone (AMH) levels and ovarian function at time of AMH measurement in patients with Turner syndrome, aged 12 to 25 years. Dotted line represents the detection limit of the assay; squares represent patients with absent puberty; triangles represent patients with cessation of ovarian function; circles represent patients with ongoing ovarian function. Thick black bars, median of AMH; black, patients with 45,X. (From Hagen, C. P., Aksglaede, L., Sorensen, K., et al. (2010). Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab*, 95, 5003–5010. Figure 3. This image can be viewed in full color online at ExpertConsult.)

Table 17.2. Approximately 75% of spontaneously aborted TS fetuses and 30% of living patients have a clinically apparent CVM. Obstructive lesions of the left ventricular outflow tract (LVOT) predominate, ranging in severity from nonstenotic bicuspid aortic valve (BAV) to aortic stenosis, coarctation of the aorta, ascending aortic aneurysm, and mitral valve anomalies (see Table 17.2).¹³² The most severe form of left-sided CVM, with poor prognosis, is hypoplastic left heart syndrome.^{133,134} The association of TS and left-sided CVM is characteristic among malformation syndromes.

There exists a significant association between neck webbing and aortic coarctation in girls with TS.¹³⁵ This is supported by: (1) observations from aborted fetuses,¹³⁶ and (2) additional studies reporting a much higher prevalence of aortic coarctation and BAV in girls with neck webbing.^{137,138} The underlying pathophysiologic mechanism proposes that lymphectasia causes a mechanical defect: engorged fetal lymphatic structures compress the developing ascending aorta, which affects intracardiac blood flow, and ultimately leads to a spectrum LVOT anomalies.¹³⁵ Another potential explanation is derived from observations in 45,X fetuses with severe aortic arch defects and aortic valve abnormality. It invokes generalized hypoplasia of the fourth branchial arch tissues associated with defective

migration of neural crest cells.¹³⁹ LVOT anomaly defects also occur in TS patients without neck webbing, and neck webbing is observed in TS girls with normal cardiovascular structure and function.¹⁴⁰ Taken together, both explanations may play a role here, and haploinsufficiency for certain genes on the X chromosome could lead to both severe fetal lymphedema and aortic heart developmental defects, independent of each other. How manifest the CVM ultimately becomes may be further influenced by genetic variations on the autosomes with/without additional environmental effects.

Nonsyndromic LVOT defects (e.g., aortic coarctation and BAV), in the general population, are not associated with lymphedema or neck webbing. They are also more commonly observed in males than in females by a ratio close to 3:1. This CVM phenotype is observed in individuals with TS who present with either a deletion of just the short arm of the X or Y chromosome.^{129,141} This indicates that loci important for LVOT development are likely present on both Xp and Yp. The putative candidate gene for LVOT development would then have to escape X inactivation in females and be expressed from both the X and Y chromosomes in males. The Yp allele may be more prone to disruption in males, as a consequence of inherent Y chromosome instability.¹⁴² Localization of an LVOT-related

TABLE 17.2 Congenital Cardiovascular Malformations in Turner Syndrome

	Mode	BAV	COA	ASD/VSD	PAPVC	ARSC	ETA	Any
Gotzsche ¹⁵⁶	Echo	14%	10%	0	1%	NR	NR	26%
Mazzanti ¹³²	Echo	12%	7%	1%	5%	NR	NR	23%
Volkl ¹⁵⁷	Echo	18%	18%	8%	5%	NR	NR	30%
NIH ^{136,158}	MRA/Echo	30%	12%	1%	13%	13%	49%	50%
Kim ¹⁵⁹	MRA	39%	16%	NR	16%	6%	31%	NR

ARSC, Aberrant right subclavian artery; ASD/VSD, atrial or ventricular septal defects; BAV, bicuspid aortic valve; COA, aortic coarctation; ETA, elongated transverse arch of the aorta; MRA, magnetic resonance angiography; NR, not reported; PAPVC, partial anomalous venous connection.

gene on the PAR1 of the X chromosome may explain the greater prevalence of LVOT anomalies in males, because the meiotic recombination rate for PAR1 is sevenfold greater in males than females,¹⁴³ increasing the overall risk for *Yp* gene disruption.

Disease of the Aorta

Complications of CVMs are the leading cause of morbidity, as well as premature and increased mortality in TS.^{119,120,128} The major cardiovascular complications include aortic valve disease and aortic dilation, dissection, or even rupture. Approximately 30% of girls with TS are born with a congenitally abnormal aortic valve. Because careful appropriate screening for the presence of, for example a BAV, is sometimes lacking, congenitally abnormal valves are often not detected until the third decade of life or later.^{141,144} Carolyn Bondy's group studied 74 patients with BAV, at a median age of 30 years (range 7–67 years). Of these women, 55% had normal valve function, 30% had mild aortic regurgitation, and 15% had moderate to severe aortic regurgitation.¹⁴¹ In this BAV cohort, aortic stenosis was present in only 2/74 patients. The prevalence of BAV was equal in pediatric and adult subgroups, but adults were more likely to have functionally abnormal valves. A large National Institutes of Health screening study using advanced imaging technology (magnetic resonance angiography or cardiac MRA) confirmed a BAV prevalence of 34% of girls with TS.¹⁴⁵ In a more recent study using similar imaging techniques, Kim et al. report a prevalence of BAV as high as 39%.¹⁴⁶ It is imperative to screen all girls and women with TS for aortic valve pathology, as the aortic valve may deteriorate over time, leading to heart failure. In addition, aortic valve abnormalities are linked to further aorta pathology, with increased risk for the development of aortic dilation, dissection, and rupture.^{147–154}

In individuals who have an abnormal aortic valve, this is often associated with relative dilation of the ascending part of the aorta, using age and body surface area nomograms.^{141,152} The magnitude of ascending aortic dilation is greatest in patients whose valve is also functionally abnormal,^{141,152} although individuals with normal valve function may also develop dilation.¹⁵⁵ In about 10% of patients who have a normal tricuspid aortic valve (and normal blood pressure), mild-moderate ascending aortic dilation can also be found.^{141,152} There is an increasing body of evidence for the existence of a generalized vasculopathy in individuals with TS. Some of the observations supportive of this include an enlarged diameter of the carotid and brachial arteries,¹⁵⁶ as well as aneurysms of other arteries.^{157,158} The term *aortopathy* has come into wider use and describes the presence of aortic disease of diverse etiologies, including Marfan syndrome and vascular disorders not associated with valvular disease. *Aortopathy* exists in addition to aortic disease associated with BAV.¹⁵⁹

From the analysis of epidemiologic data obtained from the Danish registry we can safely state that the risk for serious aortic complications is 100-fold or greater increased in girls and women with TS, compared with the general female population.¹⁵¹ For example, the median age of aortic dissection was 35 years (in the general female population, this is an event usually observed in the seventh or eighth decade of life), with the few pediatric cases clearly associated with a history of more severe aortic valve disease or coarctation. Aortic valve abnormalities and aortic coarctation, with or without surgical repair, are important predisposing factors for aortic dissection. Hypertension is another risk factor for aortic disease, in the general population, as well as in TS individuals. The presence of aortic dilation is a strong risk factor for aortic dissection in TS, although dissection can occur in women with TS who do

not harbor any of the known aforementioned risk factors. Nevertheless, cardiology follow-up in these patients should always include direct and reliable measurements of ascending aortic diameters at the sinuses of Valsalva, sinotubular junction, and ascending aorta for all patients, together with body surface area and age.¹⁶⁰ Spontaneous and assisted pregnancies are associated with a further increased risk for life-threatening aortic complications in TS, and the French College of Obstetrics and Gynecology,¹⁶¹ as well as the American Society of Reproductive Medicine,¹⁶² have issued stringent guidelines on pre-conception screening and maternal care aimed at reducing these risks. In the past, too often TS women went through a pregnancy without such detailed evaluations.

Other Cardiovascular Health Issues

Aortic valve disease and risk for aortic complications are major concerns in TS, but other CVMs are also observed with a higher frequency. Partial anomalous pulmonary venous connection (PAPVC), or return (PAPVR), is increased in TS;^{163,164} it has a reported prevalence between 1% and 16%.^{137,165–168} The higher prevalence is found after using contrast cardiovascular MRA for screening purposes, and is likely a reflection of the true increased prevalence, because echocardiography is a suboptimal technology to study the anatomy of the pulmonary veins.^{167,168} This diagnosis should especially be pursued in girls with exercise intolerance, unexplained pulmonary issues (reactive airway disease), or evidence of pulmonary hypertension or right heart hypertrophy. The significance of incidentally discovered PAPVC in the screening of asymptomatic patients is unclear. Only 9% to 14% of PAPVC patients reported from two case series had a clinically significant left-to-right shunt necessitating surgical intervention.^{167,168} Aberrant right subclavian artery is another relatively common anomaly (5%–10%; Fig. 17.15)^{167,168} that may compress the esophagus, leading to dysphagia and chest pain.¹⁶⁹ One important clinical note relates to the origin of an aberrant right subclavian artery from the descending aorta: this could mask the presence of aortic coarctation if the right upper extremity alone is chosen to screen for upper versus lower extremity blood pressure difference. Atrial or ventricular septal defects and mitral valve disease are more common than in the general female population, but less frequent than aortic defects and PAPVC (see Table 17.2).

Minor electrocardiographic findings, including right axis deviation, T wave abnormalities, bundle branch block, QTc prolongation, and increased heart rate variability are more common among girls (30%) and adults (>50%) with TS, compared with age-matched female controls.^{170,171} Most of these findings are of uncertain significance. Specifically for QTc prolongation, there does not appear to be a worsening of this finding with increasing age. It is recommended to obtain a screening electrocardiogram (ECG) on all patients with TS, and recommend those with a prolonged age-specific QTc to avoid medications associated with QTc prolongation.¹²⁶ An ECG should also be done after initiation of QTc prolonging drugs, if these cannot be avoided. However, unlike in familial long QT syndrome, there is no evidence that prolonged QTc in TS is associated with sudden cardiac death. In case right axis deviation is detected on ECG, PAPVC, or PAPVR needs to be considered. Impaired autonomic responses are suggested by resting tachycardia, reduced heart rate variability, and the absence of the expected nocturnal dip in blood pressure.^{172,173} Systemic hypertension is more common in girls and women with TS, compared with age-matched female controls: at least 25% of patients.^{174–176} The higher prevalence of hypertension

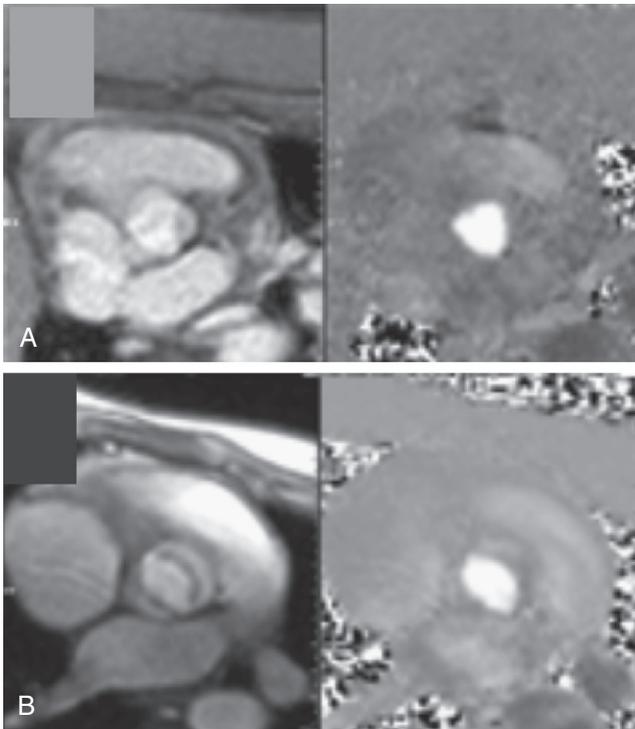


Fig. 17.15 Aortic valve structure shown by cardiac magnetic resonance imaging (MRI). Anatomy is shown on the left and blood flow on the right side of each panel. Cardiac MRI is able to visualize the aortic valve more consistently than transthoracic cardiac echo. This figure shows normal tricuspid (A) and bicuspid (B) aortic valves.

appears independent of renovascular or aortic disease¹⁷⁷ — although secondary hypertension may occur if vascular pathology or renovascular disease coexists (rare). The hypertension appears not adversely impacted by the usual recommended estrogen treatment regimens.^{176,178} In the majority of patients, the cause of the hypertension is unknown, and it probably has a multifactorial etiology. Sympathetic hyperactivity, increased activity of the renin-angiotensin-aldosterone system, and estrogen deficiency may all be contributory. Further studies are needed to determine ideal systemic blood pressures for girls and women with TS given their smaller stature and increased risk for aortic and also atherosclerotic disease. Moreover, further investigation is required to identify beneficial pharmacotherapy for patients with hypertension or BAV and aortic dilation.¹⁷⁹ We currently define hypertension in TS using the guidelines for the general population and use corrected norms for age, sex, and height when evaluating children.¹⁷

Cardiovascular Screening in Turner Syndrome

When a girl with TS is diagnosed with a CVM, either in infancy or in childhood, it is recommended she is followed by the pediatric cardiology team, ideally at a specialized tertiary care center, and preferably by a cardiologist with special expertise in following congenital heart disease. Most girls diagnosed with TS later in childhood or adolescence do not present with clinically obvious cardiovascular disease. Close cardiovascular examination and consultation with pediatric cardiology are required at the time of diagnosis however, with particular attention to blood pressure measurements in all extremities. The mode of imaging and the frequency of follow-up are determined by the clinical situation in each case. Transthoracic

echocardiography has been the standard approach to screen for congenital CVM for decades, but this modality has important limitations related to operator dependency and limited acoustic windows, especially in the context of abnormal chest anatomy and obesity, which are common physical features seen in TS.¹⁸⁰ Cardiovascular magnetic resonance (CMR) imaging provides excellent visualization of cardiovascular anatomy and function, with dimensions of ascending, transverse, and descending aorta, in any desired imaging plane (see Fig. 17.15 and Fig. 17.16). Importantly, CMR may detect significant cardiovascular anomalies, including chronic aortic dissection, aortic coarctation and dilation, aortic valve abnormality, and PAPVR (PAPVR) not always so easily demonstrated by routine echocardiography.^{153,167,168,180–182}

Asymptomatic BAV or aortic dilation and some cases of coarctation pose risks for aortic complications, but may escape detection with standard evaluation and echocardiography. Current recommendations include the more advanced imaging (i.e., CMR) when possible, without excessive risks of sedation or radiation exposure, usually by age 12 years.^{17,126} The detection of asymptomatic BAV and aortic dilation is crucial to assess the individual's risk for aortic complications, as well as the requirement for ongoing cardiac monitoring during adulthood. In addition, since 2017 new guidelines are available that help educate the young patient and her family about the risks of high-impact or high-resistance exercise and the knowledge of coexisting CVMs will enhance counseling in this area.¹⁷

Ongoing Cardiac Care

Pediatric TS patients with known congenital CVMs require continued care by the pediatric cardiology team and should be transferred to adult congenital heart disease specialists when appropriate for age.^{17,142} Patients who have had aortic coarctation repair in childhood need ongoing surveillance for detection of hypertension, development of aortic valve disease, as well as imaging of the aorta to monitor for restenosis, dilatation, or true aneurysm formation.^{183,184} Patients with BAV or aortic dilation need regular and continued monitoring for hypertension, valve function, and potential progression of aortic dilation. The optimal frequency of monitoring is not exactly known because it is uncertain if there is continuous progressive dilation of the aorta, as seen in Marfan syndrome, or whether changes occur by leaps rather than by gradual transition.¹⁸⁵ The latter process could be influenced, for example, by a stressor, such as a motor vehicle accident, participation in high-resistance exercise, or an associated pregnancy. Available evidence describes a small annual rate of dilation near the detection limit of current imaging methods.^{186,187} Further studies are required to identify parameters that may be more useful in predicting imminent aortic decompensation, such as aortic wall thickness, compliance, or circulating markers (e.g., natriuretic peptide).¹⁸⁸ The aforementioned 2017 guidelines provide a cardiac imaging and cardiology evaluation follow-up protocol, which was arrived at by reviewing the current available literature, combined by consensus agreement from experts in the field of TS cardiac disease.¹⁷

Beta-blockers and angiotensin receptor blockade have shown some efficacy in preventing aortic dilation in Marfan syndrome, but neither form of treatment has been carefully investigated in affected TS patients. Children at increased risk for aortic dissection because of BAV and aortic dilation, with or without hypertension, and their families should be counseled about possible presenting symptoms (e.g., chest or back pain). Wearing a medical identification bracelet indicating the presence of aortic disease could be of benefit in case the family

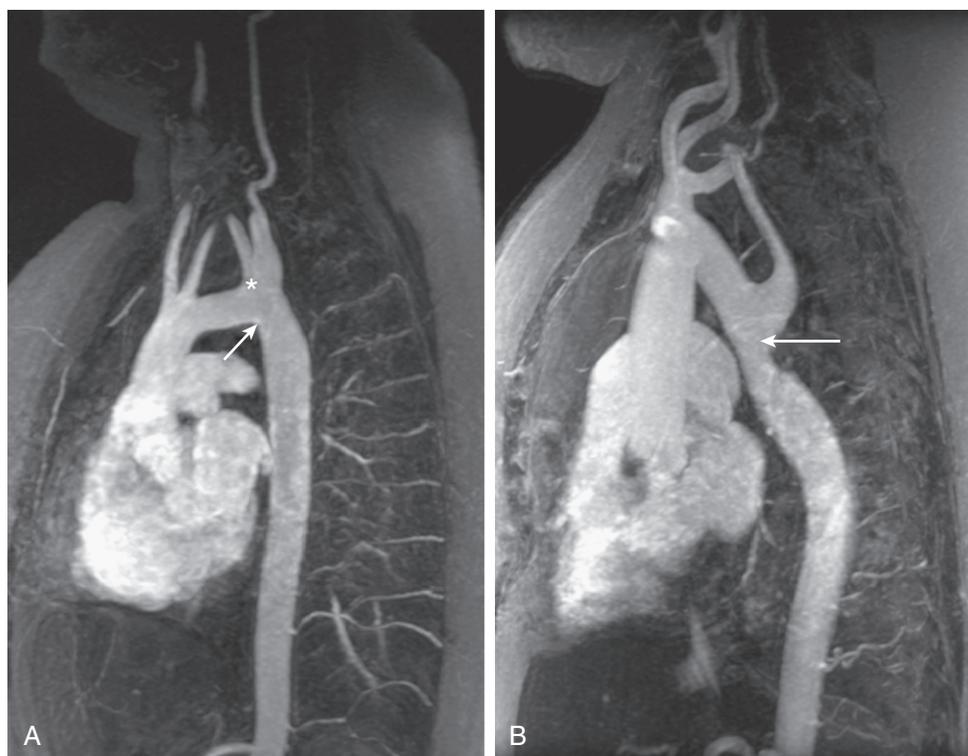


Fig. 17.16 Cardiac magnetic resonance imaging (MRI) aortograms in Turner syndrome. The image on the left (A) shows a squared off, elongated transverse aortic arch (ETA) with a kink in the lesser curvature (*arrow*). This kink has been called *pseudocoarctation* and is associated with risk for type B dissection. This patient also has a dilated origin of the left subclavian artery (*) and an aberrant origin of the right subclavian artery. The image on the right (B) shows a tortuous aortic arch with coarctation just below the take-off of the left subclavian. There is moderate dilation of the ascending aorta and poststenotic dilation of the descending aorta as well. This image was obtained from a 41-year-old woman with long-standing hypertension who had been followed with cardiac echo over the years.

is not able to advocate for their child in distress. Delayed or missed diagnosis is the most important cause of fatality in acute aortic dissection, a major concern if the emergency department staff fails to consider dissection in an atypical patient presenting with clinical features consistent with acute aortic dissection. The most common presenting symptom should be pain, often of abrupt onset. Typically, the pain is increasing in intensity and may be described as sharp. The pain is typically felt in the anterior chest but may be experienced in the back in case of descending aorta dissection.

For the patient with no identified cardiovascular defects after adequate cardiovascular evaluation and imaging, routine pediatric care with continued monitoring of blood pressure is advised. It seems also prudent to reevaluate aortic dimensions at 5- to 10-year intervals (see later). Heart-healthy moderate aerobic exercise is emphasized and should be encouraged. As noted before, eligibility for competitive sports for all with TS should be determined by a cardiologist, after a comprehensive evaluation that includes magnetic resonance imaging (i.e., CMR) of the aorta.

Risk for Premature Atherosclerotic Disease

Epidemiologic analysis of registry data from Denmark described a 3-fold increased risk of coronary and cerebrovascular disease among women with TS.¹¹⁸ It was not determined if the coronary disease and stroke were secondary to atherosclerosis or to underlying congenital CVMs. Adults with nonsyndromic aortic coarctation have an increased risk of hypertension, coronary disease, and stroke—despite apparently successful coarctation repair.¹⁴² Total cholesterol concentrations are often elevated in untreated girls with TS, compared with age-matched

females,¹⁸⁹ with improvements in the metabolic profile with GH treatment.¹⁹⁰ Atherogenic risk factors, including abdominal adiposity and lipid profile, are higher in women monosomic for the maternal X chromosome versus those with retained paternal X chromosome,¹⁹¹ but they are not usually increased into the atherogenic range.¹⁷⁵ Recommendations for preventing atherosclerosis apply equally to girls with TS and the general pediatric population and include promoting a healthy lifestyle, monitoring blood pressure, and preventing obesity. Although many pediatricians initiate lipid screening around 10 years of age, with repeat assessment in the transition years (between ages 17 and 21 years), the recent guidelines recommend a lipid profile to be performed in individuals who have at least one risk factor for cardiovascular disease and starting at 18 years.¹⁷

Renal Anomalies

Some 20% to 30% of girls with TS will have a renal abnormality.^{192,193} Duplication of the pelvocaliceal collecting system, a horseshoe kidney, renal ectopia, and unilateral renal agenesis are the more commonly occurring anomalies. The etiology of the renal anomalies is unknown. There is no apparent correlation between the renal anomalies and other features of TS, such as neck webbing or congenital CVMs.¹³⁸ However, a horseshoe kidney is thought to occur more commonly in patients with a 45,X karyotype.¹⁹⁴ Most TS children with congenital renal anomalies have normal renal function, but some are at increased risks for urinary tract infections. Pyelonephritis can occur if there is evidence of obstructive collecting system lesions, and this may require

surgical repair. It is recommended that all patients undergo ultrasound imaging of the renal system at diagnosis. Additional tests (e.g., voiding cystourethrogram or renal scintigraphy) may be required in patients with (recurrent) urinary tract infections. Renal function should be tested on a regular basis for those individuals with a horseshoe or single kidney. Additional studies are necessary to determine if renal anomalies diagnosed in TS patients are associated with gradual worsening of renal function or the development of proteinuria and hypertension during later life.

Otologic Disorders

Recurrent middle ear infections are one of the most common medical problems in young girls with TS. Anderson et al.¹⁹⁵ first described the presence of middle ear pathology in girls with TS: recurrent otitis media, eardrum perforations frequently requiring surgical intervention, and significant hearing loss occurring in up to one-quarter of affected patients.¹⁹⁶ Many other reports further address the conductive hearing loss that occurs secondary to refractory otitis media in TS, and having a karyotype with loss of the short arm of the X chromosome (Xp) seems to predispose to this.^{197–200} Many girls will require repeated tympanostomy tube placement. Despite this, some patients may develop complications, including the development of cholesteatoma.²⁰¹ The frequent otitis media may be the consequence of abnormalities in the shape and growth of the cranial base, leading to an abnormal relationship of the middle ear to the Eustachian tube. Together with anatomic abnormalities of the palate, a predisposition to fluid collection and secondary infection may be predisposing factors explaining the common occurrence of this problem, rather than a form of immunologic dysfunction, as initially entertained.

Although conductive hearing loss is most common and severe in children and is correlated with middle ear pathology,¹⁹⁷ sensorineural hearing loss is more common in adults with TS. This sensorineural hearing loss is usually bilateral and characterized by a dip in the mid-frequency range (between 500 and 2000 Hz) during audiology testing (Fig. 17.17). Sensorineural hearing loss seems progressive and is likely not a pure congenital abnormality. By middle age, more than 90% of women with TS have a hearing loss of more than 20 dB, and more than 25% will eventually require hearing aids. Significant conductive hearing loss in adults with TS may coexist, either as a consequence of scarring of the tympanic membrane, as a result of the frequent bouts of otitis during childhood, or it

may indicate ongoing middle ear pathology.¹⁹⁷ The aggressive treatment of ear, nose, and throat problems in childhood to avoid injury to the inner ear may reduce the risk of long-term hearing loss.

Autoimmunity

The most common autoimmune disorder encountered in TS is Hashimoto lymphocytic thyroiditis. Elevated antithyroid antibodies (antithyroid peroxidase, antithyroglobulin), with or without overt primary hypothyroidism, have been reported in as many as 50% of Turner patients, and this increases as patients get older.¹¹⁵ Although the prevalence of autoimmune thyroid disease is increased in all TS patients compared with the general female population, having an isochromosome X further increases the likelihood of developing autoimmune thyroiditis.^{200–205} The clinical picture of overt hypothyroidism in TS may be different from the general population because even severely affected individuals may not show any signs or symptoms of the disease.²⁰⁴ Coupled with the higher prevalence, annual screening of all TS patients for hypothyroidism is currently recommended—although it is unlikely that hypothyroidism will develop in early childhood. The prevalence of Graves disease is also slightly increased among TS girls.²⁰⁶ In one longitudinal study, 24% of 84 children with TS, who were followed for about 8 years, developed hypothyroidism, whereas 2.5% developed hyperthyroidism.²⁰⁷ Rarely, thyroid disease has been reported as early as 4 years.²⁰⁸ There is an overall consensus that women with TS are further at an increased risk of a variety of other autoimmune diseases. An association of juvenile rheumatoid arthritis (JRA) and TS was reported by Zulian et al.²⁰⁹ A 6-fold increase in JRA was detected based on a survey of 28 pediatric rheumatology centers (18 girls with TS had JRA among 15,000 JRA patients). Additional information on the occurrence of autoimmune disorders can be derived from a comprehensive evaluation of 204 TS adults in the United States: Hashimoto thyroiditis was noted in 37%, celiac disease in 2.7%, and inflammatory bowel disease in 4% of TS individuals compared with age-matched females with POI not caused by TS, and compared with the general female population.²⁰⁵

Disorders of the Gastrointestinal System

Liver Disease

Mild liver enzyme elevations are common in girls and women with TS. This is almost always not associated with manifest liver disease.²¹⁰ Approximately 20% to 25% of girls,²¹¹ and 40% of TS women²¹² may have increased aminotransferase concentrations, including aspartate-, alanine-, and gammaglutamyltransferases and, less commonly, alkaline phosphatase. The liver enzyme elevations may be associated with estrogen, progestin, or oxandrolone use,^{211,213} but over the long term, estrogen replacement therapy is thought to lead to normalization of the hepatic enzymes.²¹² In a cohort ($n = 100$) of 7- to 17-year-old TS girls, 27% had modest transaminase elevation (see Table 17.1) not correlated with GH or estrogen use. No evidence of fatty infiltration liver ultrasound was found.¹⁹² Aside from rare case reports, there appears no association between autoimmunity and hepatic abnormalities. In one study where liver biopsy was performed in TS adults who had the typical enzyme elevations described earlier, no diagnostic pathologic process has been identified to explain the transaminitis.²¹⁴ The authors suggested the hepatic disorder could be caused by intrinsic vascular changes/vasculopathy affecting normal liver physiology.

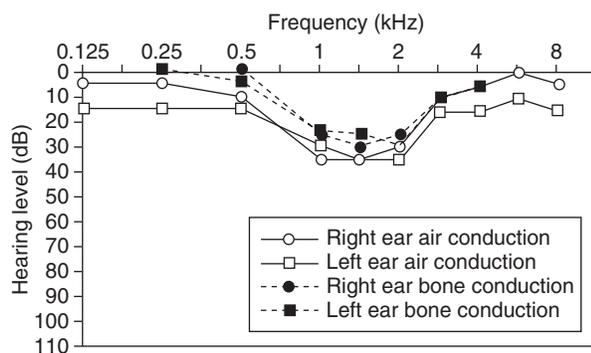


Fig. 17.17 Audiogram showing the typical dip with the peak of 35 dB in the 1.5-kHz frequency region in a 12-year-old girl with Turner syndrome (karyotype 45,X). This girl has no subjective hearing problems. (From Stenberg, A. E., Nylen, O., Windh, M., & Hultcrantz, M. (1998). Otological problems in children with Turner syndrome. *Hear Res*, 124, 85–90.)

Gastrointestinal Bleeding

Gastrointestinal bleeding occurring in patients with TS has been reported on several occasions.^{215,216} The bleeding episodes have been caused by intestinal telangiectasia, hemangiomatosis, or even angiodysplasia in the intestinal tract. Management of these cases may be problematic, but a conservative approach to avoid extensive bowel resection is preferred. Affected patients may require repeated transfusions to maintain a normal hemoglobin concentration. Estrogen replacement in these patients needs to be individualized.^{217,218} There is some anecdotal evidence that estrogen/progesterone supplementation is helpful in individuals affected by recurrent bleeding because of angiodysplasia.

Inflammatory Bowel Disease

Girls and women with TS have an increased risk for inflammatory bowel disease (IBD) and ulcerative colitis.^{219–223} Because growth failure and delayed pubertal development are manifestations of both IBD and TS, a detailed history and review of systems is needed to differentiate between the two, and to avoid attributing delayed sexual maturation to bowel disease without ruling out other causes, such as TS.

The prevalence of celiac disease is also increased in individuals with TS.^{224–226} Growth failure and pubertal delay can also be manifestations of celiac disease and TS as well, and attention needs to be directed to short girls with celiac disease, with or without pubertal delay, to ascertain they do not have TS. TS girls should be screened by measurement of tissue transglutaminase immunoglobulin (Ig)A antibodies beginning at age 2 years, and repeated every 2 years; adults with suggestive symptoms should also be screened.^{17,227,228}

Abnormalities of Carbohydrate Metabolism

Decreased carbohydrate tolerance in TS individuals was first described in 1963,²²⁹ and glucose intolerance has been further reported in many studies. Although insulin deficiency,²³⁰ as well as insulin resistance have both been proposed as causative, the true etiology of this glucose intolerance is still uncertain.²³¹ Data from the Danish health registry indicate an increased incidence of both type 1 and type 2 diabetes mellitus,¹¹⁹ although an increased rate of type 1 diabetes has not been observed in other countries or other reports. Clinical studies have consistently shown hyperglycemia in response to glucose challenge with variable insulin responses. Most patients are not insulin dependent or ketosis prone.²¹⁶ Determining the exact etiology of glucose intolerance in TS is made difficult, as most studies do not include control groups appropriately matching the girls and women with TS. The latter usually have increased adiposity and are exposed to less to normal concentrations of ovarian hormones. In addition, many TS patients are treated with GH or anabolic steroids that alter metabolic responsiveness to carbohydrate loading. TS individuals with a maternally derived X chromosome show excess visceral adiposity compared with individuals with a paternally derived X chromosome.¹⁹¹ This distinction between the different genotypes is usually not taken into account when submitting TS to studies on carbohydrate metabolism.

Some studies do shed some light on the potential pathogenic mechanism underlying the disordered carbohydrate metabolism.²³² One such study evaluated insulin sensitivity via a euglycemic insulin clamp approach in girls with TS (compared with age, but not body mass index [BMI]–matched control girls) and showed that Turner girls had decreased insulin sensitivity.²³³ More recently, the decreased glucose intolerance appears to be caused by insulin deficiency rather than insensitivity based on several reports.^{178,234,235} Still, another study documented insulin resistance that was associated with

obesity.²³⁶ Another study compared nonobese women with TS to age-matched women with 46,XX premature ovarian failure. Both groups discontinued sex hormone replacement therapy for 2 weeks, prior and during testing procedures.²³⁷ Fasting glucose was normal in both groups, but glucose concentrations after both oral and intravenous glucose loads were significantly higher and glucose-stimulated insulin was lower in the Turner subgroup. This points toward impaired insulin secretion rather than insulin resistance as a contributory factor to the diabetes risk in TS patients.

Four hundred Turner patients were further evaluated from 2001 to 2010, and only one presented with type 1 diabetes.²³⁵ Type 2 diabetes mellitus was not found in any children. However, type 2 diabetes was present in 25% of the adults during the same follow-up. Type 2 diabetes was also more than twice as common among women with an isochromosome Xq compared with women with 45,X or with Xp deletion TS karyotypes.²³⁵ A different (cross-sectional) study found that girls with TS have decreased beta-cell function, causing impaired glucose tolerance with normal insulin sensitivity, despite increased adiposity and higher blood pressure.²³⁸ In conclusion, many girls and women with TS do not appear to exhibit a picture of insulin resistance associated with excess adiposity, as is observed in non-TS females.²³⁹

Neurocognition and Psychological Features

Intelligence of TS individuals is in the normal range and girls with TS are similar to their siblings in overall intelligence quotient (IQ). Significant developmental delays have been reported in individuals ascertained because of a severe neurodevelopmental phenotype: these patients had a ring chromosome X, without the unique characteristic features of TS.^{240–244} This high risk of significant developmental delay is caused by failure of inactivation of the ring X as a consequence of loss of the X inactivation center (XIST), which leads to dysomy of certain normally X-inactivated genes.

Many individuals with TS have selective impairments in visual-spatial reasoning and information processing, arithmetic skills, and the coordination of motor and visual-perceptual skills.^{245–247} In some, this is associated with hyperactivity.²³² The discrepancy between verbal and performance IQ has been documented in many studies, and ranges between 10 and 15 points—with verbal IQ higher than performance IQ.^{245–251} In addition, brain size has been correlated with the maternal X chromosome.⁵⁴

The parental origin of the single normal X chromosome has been implicated in certain aspects of the TS neurocognitive presentation. Skuse et al. investigated 80 females with TS, and 25 (the expected proportion) of them had an X chromosome of paternal origin (Xpat). These individuals showed satisfactory social adjustment and had higher verbal IQ and executive functional skills than the larger group of girls with the retained X chromosome of maternal origin (Xmat).⁵² In an extension study, the investigators studied if verbal and nonverbal memory characteristics were at all related to the origin of their X chromosome.²⁵² In this study 45,Xpat Turner females matched controls in verbal memory, but 45,Xmat females did not. However, the results of 45,Xmat patients matched those of controls in visual-spatial memory tests, but the 45,Xpat patients did not do so. These data indicate an imprinted locus for social cognition on the X chromosome that appears silenced for the X of maternal origin. This may result in difficulties with social and nonverbal communication, and even possible increased risk for autism spectrum disorder. Because the parental origin of the X chromosome has not been correlated with cognitive or behavioral phenotype in more recent studies,^{253–256} further work is needed to confirm the aforementioned described findings.

Estrogen deficiency (and replacement therapy) are important factors to consider as playing a role for the cognitive, social, and functional disposition of girls and women with TS. Estrogen replacement therapy (compared with placebo) appears to enhance motor speed, nonverbal processing, and memory in estrogen-treated TS patients.^{257,258}

The risk for psychiatric illness in TS has been studied in 100 adults between 2001 and 2003.²⁵⁹ The incidence of major depression was 5% and that of anxiety disorder was 8%. Women with TS reported a higher rate of lifetime depression compared with rates observed in community-based studies—but similar to those obtained from gynecology clinic patients. Affective symptomatology was prospectively compared with age-matched women who have POI not caused by TS.²⁵⁵ The two groups have similar levels of shyness and social anxiety higher than seen in normal controls. They had similar levels of self-esteem lower than in controls. This indicates that ovarian failure contributes to the neuropsychological profile of girls and women with TS. The first systematic investigation of the personality of girls and women with TS dates from the 1970s.²⁶⁰ From then, and from additional work in this area, it shows that TS individuals have a high stress tolerance, tend to be overcompliant, and demonstrate a higher need of dependence, with often limitations in emotional competence.^{248,252,261} Most women with TS report unambiguous female gender identification.²⁵³ Although delayed sexual debut is common,^{254,255} sexual fantasies are similar to what other women report. Sexual activity among married Turner women also appears similar to that reported by the general female population.^{262–266}

MEDICAL MANAGEMENT

Initial Evaluation

Important elements of the initial evaluation include the following: disclosure of the condition and discussion of etiology, a detailed phenotyping using a variety of methods to identify which features of TS are present and which are not, identification of any coexisting comorbidities and, the development of a management plan to address current concerns, as well as the risk for future disease. Patients come to be diagnosed with TS at a variety of ages, depending largely on the presence of the specific clinical features that provoke evaluation. Because the issues differ somewhat, depending on the age of diagnosis, the initial evaluation will be discussed separately for those diagnosed in infancy, childhood, and adolescence (Boxes 17.1 and 17.2 and Table 17.3).

Patients diagnosed in infancy, typically display physical features that suggest the diagnosis, such as lymphedema of the dorsae of the hands and feet or characteristic cardiac anomalies, as previously described. At this age, the main tasks are to identify any concomitant renal or cardiovascular abnormalities by ultrasound measures. The disclosure and discussion can be somewhat challenging, as the specifics of the future health and development of the child are unclear. Perhaps most difficult are those TS individuals who have been diagnosed via prenatal genetic testing and are yet to be born. It is important to remember that individuals with sex chromosome anomalies diagnosed prenatally may have very modest phenotypes, especially those with mosaicism.

During childhood (ages 1–10 years), the most common feature leading to a diagnosis of TS is short stature. In addition to evaluation for cardiac and renal anomalies, the approach broadens to include investigation of autoimmune conditions, cognitive and behavioral status, and assessment of hearing. After assessment of comorbidities, the management plan generally focuses on short stature and the options for treatment as described later. The issues of fertility status and puberty may

BOX 17.1 Screening at Diagnosis of Turner Syndrome in Children and Adults

ALL PATIENTS

- Cardiovascular evaluation by specialist^a
- Renal ultrasound
- Hearing evaluation by an audiologist
- Evaluation for scoliosis/kyphosis
- Evaluation for knowledge of Turner syndrome; referral to support groups
- Evaluation for growth and pubertal development

AGES 0–4 YEARS

- Evaluation for hip dislocation
- Eye examination by pediatric ophthalmologist (if age \geq 1 year)

AGES 4–10 YEARS

- Thyroid function tests (T₄, TSH) and celiac screen (TTG Ab)
- Educational/psychosocial evaluation
- Orthodontic evaluation (if age \geq 7 years)

AGES \geq 10 YEARS

- Thyroid function tests (T₄, TSH) and celiac screen (TTG Ab)
- Educational and psychosocial evaluations
- Orthodontic evaluation
- Evaluation of ovarian function/estrogen replacement
- LFTs, FBG, lipids, CBC, Cr, BUN
- BMD (if age 18 years)

^aSee Box 17.2.

BMD, Bone mineral density; BUN, blood urea nitrogen; CBC, complete blood count; Cr, creatinine; FBG, fasting blood glucose; LFTs, liver function tests.

(From Gravholt, C. H., Andersen, N. H., Conway, G. S., Dekkers, O. M., Geffner, M. E., Klein, K. O., et al. (2017). International Turner Syndrome Consensus Group. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol*, 177(3), G1–G70.)

BOX 17.2 Cardiovascular Screening and Monitoring Algorithm for Girls and Women With Turner Syndrome

SCREENING (ALL PATIENTS AT TIME OF DIAGNOSIS)

- Evaluation by cardiologist with expertise in congenital heart disease
- Comprehensive examination, including blood pressure in all extremities
- All require clear imaging of heart, aortic valve, aortic arch, and pulmonary veins
- Echocardiography is usually adequate for infants and young girls
- MRI and echo for older girls and adults
- ECG

MONITORING (FOLLOW-UP DEPENDS ON CLINICAL SITUATION)

- Patients with apparently normal cardiovascular system and blood pressure need reevaluation with imaging at timely occasions (e.g., at transition to adult clinic), before attempting pregnancy, or with appearance of hypertension; girls who have only had echocardiography should undergo MRI when old enough to cooperate with the procedure
- For asymptomatic adults, imaging every 5 to 10 years
- For patients with cardiovascular pathology, treatment and monitoring determined by the cardiologist

ECG, Electrocardiogram; MRI, magnetic resonance imaging.

(From Gravholt, C. H., Andersen, N. H., Conway, G. S., Dekkers, O. M., Geffner, M. E., Klein, K. O., et al. (2017). International Turner Syndrome Consensus Group. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol*, 177(3), G1–G70.)

TABLE 17.3 Comorbidity Evaluation and Monitoring for Girls and Women With Turner Syndrome

Disorder	Test	Birth to 1 Year	1–10 Years	10–18 Years	Adulthood
Thyroiditis	TSH/T4	PRN clinical suspicion	Annually	Annually	Annually
Diabetes	HgbA1c/ blood glucose	PRN clinical suspicion	PRN clinical suspicion	Annually	Annually
Transaminitis	ALT/AST/GGT/AlkP	PRN clinical suspicion	PRN clinical suspicion	Annually	Annually
Celiac Disease	Antibody panel	PRN clinical suspicion	Every 2 years beginning age 2 years	Every 2 years	In symptomatic individuals
Vitamin D Deficiency	25OH D	PRN clinical suspicion	PRN clinical suspicion	Every 2–3 years	Every 3–5 years
Hearing Loss	Audiometry	PRN clinical suspicion	At diagnosis, then q 3 years	At diagnosis, then q 3 years	At diagnosis, then q 5 years
Abnormalities of Renal Structure	Renal ultrasound	At diagnosis	At diagnosis	At diagnosis	At diagnosis
Abnormalities of Cardiac Conduction	Electrocardiogram	PRN clinical suspicion	At diagnosis	At diagnosis	At diagnosis
Abnormalities of Cardiovascular Structure	Echocardiography, cardiac MRI	Echocardiogram at diagnosis- Frequency of repeat measures or CMR depends on age and findings (see text)	Echocardiogram at diagnosis- Frequency of repeat measures or CMR depends on age and findings (see text)	Echocardiogram at diagnosis- Frequency of repeat measures or CMR depends on age and findings (see text)	Echocardiogram at diagnosis- Frequency of repeat measures or CMR depends on age and findings (see text)
Refractive errors, Amblyopia	Ophthalmologic evaluation	PRN clinical suspicion	At diagnosis	At diagnosis	At diagnosis
Osteopenia, Osteoporosis	DXA for bone mineral density	PRN clinical suspicion	PRN clinical suspicion	When adult sex hormone replacement attained and q 5 years	q 5 years and following withdrawal of sex hormone replacement (menopause)
Neurocognitive Deficit	Neuropsychological assessment by professional	PRN clinical suspicion	At start of primary school, e.g., age 5 years	At entry to high school or equivalent, e.g., age 15 years	PRN clinical suspicion

25 OHD, 25-Hydroxyvitamin D; AlkP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; CMR, cardiovascular magnetic resonance; DXA, dual energy x-ray absorptiometry; GGT, gammaglutamyl transferase; HgbA1c, hemoglobin A1c; MRI, magnetic resonance imaging; PRN, as needed; T4, thyroxine; TSH, thyroid stimulating hormone.

(Modified from: Gravholt, C.H., Andersen, N.H., Conway, G.S., Dekkers, O.M., Geffner, M.E., Klein, K.O., et al., International Turner Syndrome Consensus Group. (2017). Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol*, 177, G1–70.)

seem less important at this age and are not as easy to assess, although measurement of circulating AMH can give prognostic information concerning future ovarian function.^{266,267} Recurrent otitis media is common in this age group and referral to an ear nose and throat specialist is frequently indicated, as are myringotomy tubes.

Childhood is also the time when neurocognitive or behavioral problems, which are more common in girls with TS, may emerge. It is recommended that neuropsychological assessment by a professional be performed in those showing symptoms and at key transitional times during development. These would be when the child first enters school and again when advancing to middle and high school or other higher educational institutions.

Patients diagnosed during adolescence or later frequently come to attention because of pubertal delay or infertility. The initial approach is similar to that for those diagnosed during childhood; however, the focus will be more on the lack of fertility and the need for sex hormone replacement. In some cases, significant short stature will be present and the pros and cons of initiating estrogen therapy at this age versus later (see later) will need to be discussed openly with the patient.

A management plan for patients following diagnosis has been detailed in recent guidelines¹⁷ and is summarized in Table 17.4. As patients progress from early childhood through adolescence, their clinical status and plan should be revisited on a regular basis. Experienced genetic counselors or psychologists, working as part of a multidisciplinary care center, can

be helpful in this regard, as can patient support groups, which exist in several countries.

Cardiovascular Assessment and Monitoring

Congenital heart disease and anomalies of the vascular system are frequent in TS and are major causes of morbidity and mortality.^{268,269} Details of the impact of the CVMs on the quality of life for TS individuals are included in prior paragraphs describing the clinical features of TS. Appropriate medical management first involves defining the structure of the heart and major blood vessels, and ongoing monitoring of the aortic root, as progressive dilation of the aortic root is a precursor to aortic dissection and rupture. The extent and frequency of testing depends on the anomalies present, as well as the age of the patient. A monitoring protocol is also detailed in the most recent guidelines¹⁷ and can be summarized as follows: all patients receive transthoracic echocardiographic examination at diagnosis, along with an electrocardiogram. Those classified as low risk (no structural cardiac anomaly or hypertension and a normal sized aortic root) are monitored every 5 to 10 years, typically by transthoracic echocardiogram. Those in moderate risk categories (those with the beginnings of aortic dilation or structural abnormalities of the heart or great blood vessels) may be evaluated every year or two, and should be monitored by a pediatric cardiologist. Although the evaluation by echocardiogram is easier and less costly, there is evidence that CMR imaging is more sensitive for evaluation of

TABLE 17.4 Guidelines for Adult Health Surveillance

	Action	Suggested Frequency	Comments
Obesity	Weight	Annually	Many comorbidities are weight related, e.g., diabetes, elevated cholesterol, and liver dysfunction. Weight management is the most important health intervention at annual visits. Obesity may be caused by low physical fitness, sedentary lifestyle, and poor food choices.
Cardiovascular	Echocardiogram	3–5 years—yearly if aortic root >3 cm	Management shared with GUCH (Grown-ups with congenital heart defects) clinic preferable. Congenital malformations include bicuspid aortic valve, coarctation of the aorta, and aortic dilation
	MRI aorta	As appropriate	Some units use echocardiography for routine monitoring and reserve MRI for ambiguous findings or as part of prepregnancy assessment. The place of MRI scanning depends on expertise of the echocardiography service
	Blood pressure	Annually	Hypertension affects up to 50% of young adults and contributes to the risk of aortic dissection. Refer to age-specific reference data. Hypertension can be treated to normal guidelines including use of beta-blockers or angiotensin receptor blockers. For aortic root >3.0 cm and BAV, aim for systolic blood pressure <140 mmHg if tricuspid aortic valve or <120 mmHg if bicuspid valve
Bone metabolism	DXA scan	Every 5 years	Estrogen replacement required until ~50 years (or older if there have been many years of estrogen deficiency) to prevent osteoporosis. Bone density of spine reads low in short stature with DXA. Osteoporosis can be treated as in other situations
	Vitamin D and calcium profile	3–5 years	Monitor bone profile in those with low calcium and low vitamin D levels; exclude celiac disease (see later)
Liver	Liver function tests	Annually	Liver enzymes, especially gammaglutamyl transaminase (GGT), are commonly elevated. Slowly progressive, but improves with estrogen and weight loss. Consider viral screen for acute changes (rarely positive)
	Liver ultrasound	As appropriate	Liver ultrasound required for markedly raised (GGT), alkaline phosphatase, or transaminases. Consider special scans measuring fibrosis and steatosis, and biopsy if structural defects identified on US
Diabetes	HbA1c+ fasting plasma glucose	Annually	Consider OGTT if HbA1c is elevated. High risk of developing impaired glucose tolerance (50%) due a combination of insulin deficiency and insulin resistance. Fasting plasma glucose underestimates defect of insulin secretion
Fertility	Adoption and oocyte donation education	As appropriate	Spontaneous pregnancy occurs in 2%–5%. Ovarian failure occurs in 90%. Medical review on advisability of pregnancy with regard to risk of aortic dissection is required
	Uterine ultrasound	As appropriate	Ultrasound of the uterus should take place on arrival in the adult clinic and again during the workup for pregnancy
Psychological	Review psychological issues	As appropriate	Increased risk of social isolation, anxiety, and obsessive behavior. Higher levels of shyness and social anxiety, and reduced self-esteem. Review problems in workplace or in relationships. Problems are responsive to clinical psychology support.
Audiology	Audiogram ENT History	3–5 years	Deafness is common and under reported. Self-reporting unreliable. Otitis media is common in childhood (60%–80%), which can lead to conductive hearing loss. Sensorineural hearing loss common and progressive in adults
Dermatology	Skin inspection	Annually	Assess for keloid and changes in pigmented nevi
Orthodontics	Teeth inspection	Annually	Referral recommended if required
Blood tests	Thyroid function	Annually	Increased risk of autoimmune thyroiditis. In hypothyroidism (24%) or hyperthyroidism (2.5%) include TPO antibodies if previously negative
	Celiac screen	With suggestive symptoms	Increased risk (4%–6%) of celiac disease. Check transglutaminase IgA antibodies (and total IgA) and vitamin B ₁₂

BAV, Bicuspid aortic valve; DXA, dual energy x-ray absorptiometry; ENT, ear, nose, and throat; HbA1c, hemoglobin A1c; Ig, immunoglobulin; MRI, magnetic resonance imaging; OGGT, oral glucose tolerance test; TPO, thyroid peroxidase.

(From Gravholt, C.H., Andersen, N.H., Conway, G.S., Dekkers, O.M., Geffner, M.E., Klein, K.O., et al; International Turner Syndrome Consensus Group. (2017). Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol*, 177(3), G1–G70.)

aortic root structure and can detect anomalies, such as PAPVR that might be missed otherwise.^{270,271} Thus current guidelines recommend that patients receive a CMR examination as soon as this can be accomplished without general anesthesia.

Hypertension is relatively common in girls and women with TS and should be aggressively treated in those with aortic root abnormalities. Four-extremity blood pressure measurement at initial diagnosis is recommended because of the increased prevalence of coarctation of the aorta.

Patients with Y Chromosome

As discussed previously, patients with TS who harbor overt or cryptic portions of the Y chromosome are at increased risk for

gonadoblastoma. Although gonadoblastoma is not a threat in itself, concern about malignant transformation to dysgerminoma has led to the recommendation that gonadectomy be performed in patients with Y chromosome mosaicism or marker chromosomes derived from the Y chromosome.²⁷² The gonadectomy was justified because of the increased risk for tumor and the lack of any detriment to removing gonads with no prospect for function. Such recommendations still stand,¹⁷ but the situation is becoming more complicated because newer, PCR-based molecular techniques detect cryptic Y chromosome sequences in 5% to 10% of patients with TS, who are Y chromosome negative by standard tests,²⁷³ but also because the risk for dysgerminoma appears to be relatively low in the population of TS.²⁷⁴ Current guidelines recommend

using standard karyotype and FISH probes for determination of marker chromosome origin to exclude the possibility of Y chromosome material, but do not endorse using the more advanced molecular techniques for all patients with TS. Recent studies now suggest that the rate of gonadoblastoma, in patients with Y chromosome detected by molecular probe testing, may be equivalent to those with cytogenetically detectable Y chromosome.²⁷⁵ Note that any patient with TS who shows signs of masculinization or virilization should have more extensive evaluation, which could include either molecular specific probes and/or testing of additional cell types (e.g., skin fibroblast, buccal epithelium).

The age of gonadectomy varies widely among patients with TS. The risk of malignant transformation within a gonadoblastoma before pubertal age is extremely low and thus there is no urgency to perform such procedures in the very young.²⁵⁹ Observation might be warranted for patients with functioning ovarian tissue, given the low risk for dysgerminoma overall. A better delineation of the risk loci for gonadoblastoma/dysgerminoma hopefully will allow a better assessment of risk in the future.

Growth-Promoting Therapies

Short stature is a nearly universal feature of TS and remains a prominent concern of affected children and adults. Even though patients with TS are not classically GH deficient, it was postulated that administration of human GH to yield modestly supraphysiologic concentrations could overcome the growth deficit associated with TS. Initial studies with relatively small numbers of patients clearly demonstrated the capacity of GH to stimulate skeletal growth over the short term. Subsequent, larger studies proved efficacy beyond doubt, leading to approval by regulatory bodies for the treatment of short stature caused by TS. The goal of treatment, at a minimum, is to prevent functional impairment because of short stature. Ideally though, treatment should be initiated at an age young enough to allow the patient to maintain her stature within the range typical for peers and avoid delay in pubertal induction.

The first studies evaluated the potential use of GH were done in the 1980s.^{276,277} Since that time, there have been dozens of reports describing short- and long-term effects of GH administration. Despite the numerous studies, an optimal therapy remains elusive because the studies varied widely in the doses used, the ages of the patients at initiation, and procedures and timing of feminization therapy, all of which affect growth and adult height. Nevertheless, several conclusions concerning GH therapy can be made.

GH therapy has the potential to normalize the stature of most patients with TS when treatment is initiated in mid to early childhood and at sufficient dosage. Van Pareren et al.²⁷⁸ showed that when patients were treated with escalating doses of GH from around 6 years of age, most reached an adult height within the normal range, even when feminization therapy began at age 12 years. In that trial, GH was administered at 6 to 8 IU/m²/day (up to approx. 67–90 mcg/kg/day) for almost 9 years, resulting in an adult height of 12 to 17 cm above that expected in the absence of treatment. Studies that use more typical doses (50 mcg/kg/day) usually report around 7 to 8 cm of height gain or about 1 cm gain for every year of treatment.²⁷⁹ Factors that result in a favorable response include higher GH dose, longer duration of treatment before feminization, younger age, and greater height at the initiation of therapy.^{280,281} However, it should be noted that the individual responses are quite variable and the reasons for this remain incompletely understood.

Treatment with GH is commonly recommended for those with heights below the normal range for their unaffected peers

and who have a reasonable potential for further growth, for example, bone age 12 years or less. Very young patients who stand within the low-normal range may also be candidates for treatment if they display a growth velocity which would eventually lead to significant short stature, that is, a growth velocity less than the 25th percentile for age. See also Fig. 17.18 for the effect of GH therapy in toddlers with TS. Current guidelines recommend beginning therapy around 4 to 6 years of age when possible.¹⁷ Although the majority of individuals with TS will be short as adults without growth-promoting therapy, occasional patients, especially mosaic variants or those with tall parents, will reach heights in the low-normal range in the absence of any treatment.

A typical starting dose for GH is approximately 50 mcg/kg/day, given as a single daily subcutaneous injection. Higher doses are not routinely recommended, although increased doses might be considered for patients with the most severe short stature, or late diagnosis. Measurements of circulating GH and insulin-like growth factor-I (IGF-1) are not required before treatment, although thyroid function should be normal before initiating therapy. Growth-promoting therapy is typically continued until the bone age is greater than 13.5 to 14 years and the growth velocity less than 2 cm/year, or the patient is satisfied with her height.¹⁷

Administration of exogenous GH reduces insulin sensitivity in girls with TS, as has been observed in all other conditions where GH is used.²⁸² A doubling of the fasting insulin concentration is typical. However, dysglycemia is rare, with a modest increase in blood glucose concentrations reported in one study,²⁷³ but not in others.²⁸² Similarly, there is no adverse effect on lipid metabolism in the majority.²⁸³ Once GH therapy ceases, these metabolic changes seem to resolve.^{282,284}

GH treatment may also yield favorable effects on body composition. Two months of treatment reduces fat mass and increases lean body mass.²⁸³ Although the BMI of adults with TS, treated with GH in their youth, is no different than those who never received GH,²⁸⁵ there is evidence that some of the favorable effects may persist with a reduction in abdominal adiposity and improved glucose tolerance.²⁸⁶

Patients with TS have been shown to have measurable deficits in bone mineral content and structure; however the clinical significance of these findings is controversial.^{287,288} GH typically increases bone mineral content along with increases in bone length. Recent studies have borne this out and show that patients treated with GH during childhood and who receive appropriate estrogen therapy tend to have favorable changes in bone microarchitecture and probably are not at increased risk for fractures.^{289,290}

True adverse effects of GH are uncommon. Scoliosis, which is more prevalent in girls with TS, may be exacerbated by the rapid growth that occurs with GH therapy. Insulin resistance and dysglycemia are also more prevalent and may worsen with therapy. One study reported an increase in type 2 diabetes in GH-treated children,²⁹¹ whereas others have not found this to be the case.²⁹² Benign intracranial hypertension, pancreatitis, and slipped capital femoral epiphysis also seem to appear with greater frequency in GH-treated TS patients.²⁹³ No specific recommendations are made for monitoring other than standard clinical assessments.

Women with TS are at increased risk for cardiovascular events, including dissection of the ascending aorta. Prior GH treatment does not appear to increase cardiac dimensions above that expected with increased growth.²⁹⁴ However, one study has reported an association with increased aortic dilation over time in adults previously treated with GH.²⁹⁵ At present, there is no contraindication for using GH in girls with TS harboring the cardiovascular anomalies typical of TS.

Circulating concentrations of IGF-1 are in part GH dose dependent and increase during therapy. Guidelines suggest that

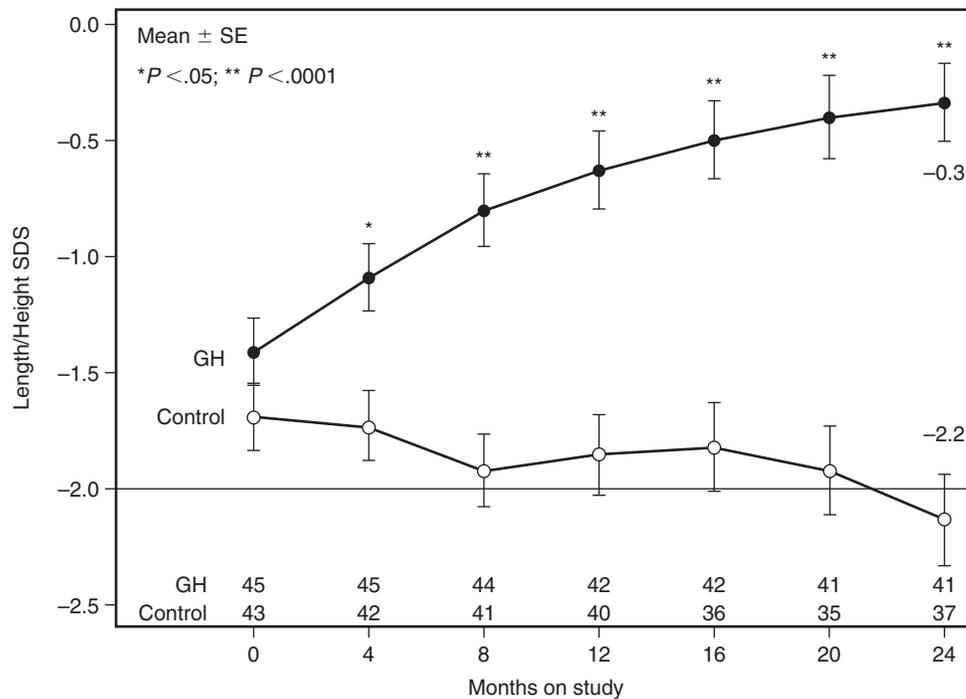


Fig. 17.18 Growth hormone treatment of toddlers with Turner syndrome. Length/height standard deviations (SDs) for the untreated control group (open symbols) and the growth hormone treatment group (filled symbols) during the 2-year study. Between-group difference at end point was 1.6 ± 0.6 SDs ($P < .0001$). Mean age at baseline was 24 ± 12.1 months. (From Davenport, M. L., Crowe, B. J., Travers, S. H., et al. (2000). Growth hormone treatment of early growth failure in toddlers with Turner syndrome: a randomized, controlled, multicenter trial. *JCEM*, 92, 3406–3416, Figure 1.)

circulating IGF-1 concentrations be measured during treatment and if they exceed $+3$ SD for age and sex, that a dose reduction be considered.¹⁷ Although perhaps prudent, there is little evidence to indicate that such a practice will reduce adverse events. In addition, it should be noted that most studies of GH safety in TS have focused on the during-treatment period. Longer follow-up studies are needed to assess the prevalence of late adverse effects.

Oxandrolone, a nonaromatizable, weak androgenic steroid was first used in TS as a sole growth-promoting agent with modest success.²⁹⁶ In fact, early GH studies demonstrated that combining GH with oxandrolone improved short-term height velocity and adult stature. The effect of GH combined with oxandrolone on adult height of girls with TS is shown in Fig. 17.19.²⁹⁷ Subsequent studies confirmed this, indicating that not only is interval growth more rapid, but that an additional inch (2.5 cm) or more can be added to adult height.²⁹⁸ Although mild virilization is a hazard (e.g., clitoromegaly, lowering pitch of the voice), these are uncommon when the drug is used as recommended. Current guidelines recommend using oxandrolone in those age 10 years and older, at a dose beginning at 0.03 mg/kg/day, and not to exceed 0.05 mg/kg/day.¹⁷ Patients for whom combination therapy could be considered would be those with more severe short stature or who were diagnosed relatively late. Oxandrolone can cause insulin resistance, although metabolic changes revert to baseline after withdrawal.^{299,300}

Estrogens also stimulate skeletal growth, but the problem is that the effect on skeletal maturation exceeds the growth stimulation, which shortens the period of growth and generally results in a reduction in stature. However, the maturing effects on the growth plate may be attenuated and a better growth response achieved when very low doses of estrogen are used. This was tested in a single randomized, placebo-controlled trial

where the effect of combining GH therapy with very low-dose estrogen in young patients was examined.³⁰¹ This study showed an enhanced growth response from the combined therapy. However, there have been no confirmatory studies and the GH dose used was lower than what is now standard practice. Thus using low-dose estrogen for growth promotion is not currently recommended for patients with TS.

The effects on growth and stature are clearer when estrogens are administered for the purposes of feminization. For those who begin treatment around age 12 years, one may expect a slight increase in growth velocity and 2 to 3 years of additional meaningful growth. As noted earlier, the duration of GH treatment before feminization is a significant predictor of adult stature in this population. One would expect that bone age at start, dosage, and perhaps form of estrogen would also influence the outcome, but this has not been sufficiently studied.

Short stature is commonly perceived by women with TS as leading to dissatisfaction in life.³⁰² It would seem obvious that an increase in stature would show measurable benefits in quality of life. However, studies thus far have not definitively shown this to be the case.³⁰³ Whether this reflects inadequacies in the quality of life assessment instruments used, the variability in the patients and the ages at which they are studied, or other factors, remains to be determined.

In summary, GH has been used to increase the stature of girls and women with TS for more than 3 decades. The effect on adult stature is clear and the safety profile is reassuring, but the degree to which the treatment confers long-term benefit requires further evaluation.

Puberty, Pubertal Induction, and Feminization

Ovarian failure in TS leads to estrogen and androgen insufficiency,³⁰⁴ whereas spontaneous pubertal development does

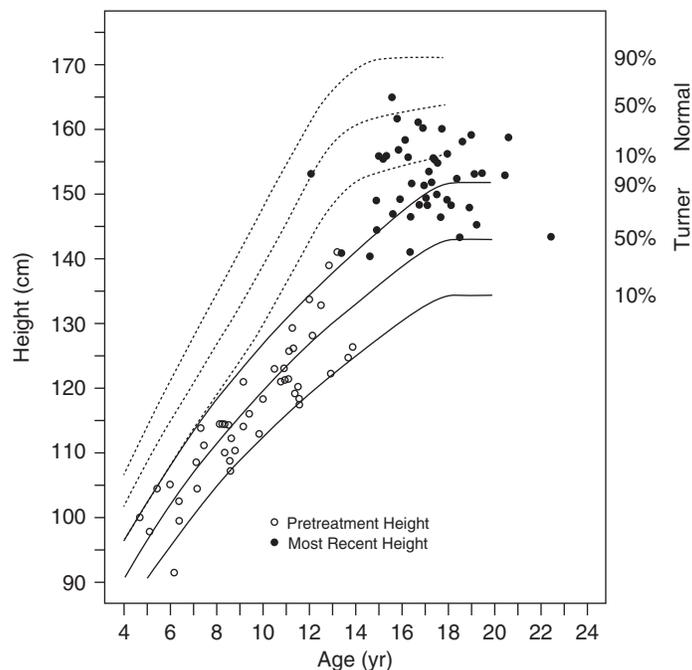


Fig. 17.19 Effect of human growth hormone combined with oxandrolone on adult height of girls with Turner syndrome. Growth centiles (10th–50th–90th, dotted lines) for normal females are from National Center for Health Statistics. Solid lines are Turner Syndrome specific. Open circles are for individuals before treatment, closed circles following treatment. Treatment duration was approximately 6 years on average. (From Rosenfeld, R. G., Attie, K. M., Frane, J. et al. (1998). Growth hormone therapy of Turner syndrome: beneficial effects on adult height. *J Pediatr*, 132(2), 319–324. With permission.)

occur and rarely spontaneous pregnancies.^{305,306} Spontaneous menarche presents in few (6%–9%) 45,X TS,^{307,308} and about one-third continue to have regular menstrual bleedings (2%–3%), but almost invariably with premature ovarian failure ensuing. Interestingly, mosaic TS (45,X/46,XX), with no structural abnormalities of the second X, presents with spontaneous menarche in 20% to 40% of the cases. Despite this, the great majority proceed to early POI. According to recent TS guidelines, female hormone replacement therapy (HRT) should be initiated³⁰⁹ in TS females with primary or secondary amenorrhea, preferably starting between 11 and 12 years of age, and continue until the normal age of natural menopause. Hormone replacement therapy is necessary to induce puberty, to maintain secondary sex characteristics, and to facilitate uterine growth, appropriate peak bone mass, and possibly neurocognitive function and improvement of the metabolic profile via a positive influence on body composition.³¹⁰ However, empirical data on the long-term effects of HRT in women with TS are limited, especially the impact on endocrine conditions, cardiovascular diseases, and mortality. Some have raised concern that HRT in TS may induce the development of deep venous thrombosis and lung emboli, although at present there is no data to support this notion.^{311,312} Additional studies will be necessary to clarify this point.

TS women are most often infertile, this being often the greatest factor influencing their quality of life.³¹³ Very few can become pregnant with their own oocytes. Most females with TS are likely to experience POI and are less likely to become pregnant. Spontaneous pregnancies occur in 4.8% to 7.6% of women with TS^{306,314}; however, one study showed that of the fertile women registered with 45,X/46,XX, several had 45,X in less than 10% of analyzed cells, indicating that some of these women might not have many, if any, signs of TS.³¹⁵ A recent study indicated that the frequency of 45,X/46,XX might be higher than previously anticipated. These individuals also show a phenotype that is distinct from the typical diagnosed

TS woman, and are often also often fertile.³¹⁶ With other words, patients with 45,X/46,XX diagnosed incidentally may be different from women with 45,X/46,XX diagnosed because of a clinical phenotype compatible with TS.

According to the new international TS guidelines,¹⁷ it is recommended to initiate estrogen replacement when the patient is between 11 and 12 years of age, increasing to adult dosage over 2 to 3 years. Low-dose estradiol is recommended, and transdermal administration is preferred because of a perceived more physiologic mode of delivery. There are no studies that firmly show that transdermal administration has fewer side effects compared with oral estradiol administration.³¹⁷ Once breakthrough bleeding occurs, progesterone should be added.

With adequate hormone replacement, it is possible to stimulate uterine growth, which can become susceptible to oocyte donation from a foreign donor. In mosaic TS women, pregnancy after FSH-stimulated oocyte retrieval, freezing of own oocytes until the female becomes older, followed by IVF treatment, is possible—if it is initiated at a young age before ovarian failure.^{318,319} Counseling not to postpone pregnancy is advisable. If pregnancy is accomplished, TS women experience a higher number of complications and miscarriages. A study reported a complication rate of up to 45%.^{306,314} It is also clear that pregnancy after oocyte donation carries a higher risk than the rare spontaneous pregnancy among TS women.³²⁰ Especially pregnancy-associated hypertensive disorder, gestational diabetes, and preeclampsia are frequent. We currently include prompt treatment of hypertension, diabetes, and prophylactic treatment with aspirin to reduce the risk of preeclampsia,³⁰⁹ and view pregnancy among women with TS as a high-risk situation.¹⁷

TRANSITION TO ADULT CARE

A myriad of diseases and conditions occur more frequently among females with TS, partly dependent on karyotype and

partly apparently independent of it. Knowledge of the breadth of the conditions is necessary when caring for adults with TS. The same is true when educating/counseling the young adult with TS to explain the need for continuing adherence to care in the outpatient clinic.

Both type 1 and type 2 diabetes occurs more frequently among women with TS and leads to increases in both morbidity and mortality.^{312,321–324} There is a significantly increased frequency of hypertension present in up to 50% of adults with TS at the age of 50 years.^{325,326} Hypertension often occurs already in childhood or adolescence.^{327,328} Other features of the metabolic syndrome, such as obesity^{310,325,329} and elevated cholesterol,³³⁰ can interact with CVMs and necessitate a multidisciplinary approach to the care of the adult woman with TS.

Liver enzymes are frequently elevated in TS adult life,³³¹ and this seems to be caused by dual influences from obesity and lack of estrogen. The transaminitis can be either completely or partly normalized by appropriate weight loss and substitution with estradiol therapy.^{332,333}

The current clinical care guidelines on TS recommend that diabetes, hypertension, and other features of the metabolic syndrome should be approached according to guidelines, as put forth by the specific relevant societies addressing these metabolic comorbidities.¹⁷

That all autoimmune conditions occur more frequently in TS³³⁴ is important to bear in mind. Especially, hypothyroidism is frequent and by the age of 50 years, it is estimated that about 50% will have acquired hypothyroidism.^{323,325,335} A recent meta-analysis of thyroid diseases among TS individuals showed a prevalence of 39% of all studied populations, including pediatric patients.³³⁶ Likewise, the occurrence of celiac disease is more frequent than among women in general.³³⁷

Osteoporosis and fractures were frequent in older populations of TS patients,^{323,338} but recent data indicate that with appropriate care, the prevalence is likely much lower.³²⁵ It is important to keep in mind that a considerable part of females with TS are diagnosed late,³²⁴ and thus women with TS diagnosed late will often present with osteopenia or frank osteoporosis.³³⁹ Appropriate hormone replacement therapy, even when instituted relatively late in life, can either partly or completely correct a bone mineral deficit³⁴⁰ (Fig. 17.20).

Hearing is a very frequent problem in any given TS cohort, often starting already in childhood. Approximately half of women with TS develop sensorineural hearing loss, which occurs when the inner ear or the neural pathways from the inner ear to the brain are damaged.³⁴¹ A sensorineural hearing deficit occurs often early in adult life and necessitates the use of hearing aids and even cochlear implants in some.³²⁵

Outpatient Care and Treatment

Guidelines for surveillance of adult patients with TS are noted in Table 17.4.

Given the increased risk of a number of diseases and conditions, it is recommended to follow-up all females with TS regularly in the outpatient clinic (see Table 17.4). The time interval (usually every 6–12 month) depends on the burden of comorbidities.

It is further recommended to establish a multidisciplinary setup, including an endocrinologist, a cardiologist with knowledge of congenital heart disease, a gynecologist, a psychologist, an otolaryngologist, a gastroenterologist, an MR radiologist, as

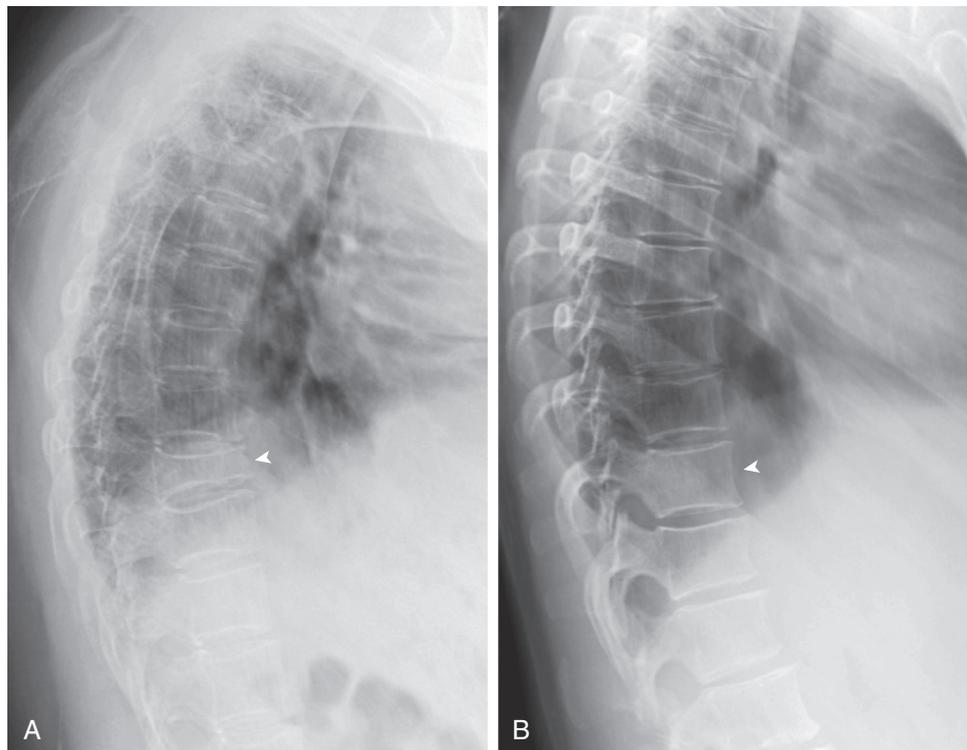


Fig. 17.20 Estrogen treatment prevents osteoporosis. These lateral chest x-rays are from two 30-year-old women with Turner syndrome. Both had a karyotype of 45,X, both received growth hormone treatment for several years during childhood, and both were started on estrogen treatment at age 12 years. One patient stopped taking estrogen at age 18 years (A), and the other adhered to treatment (B). The woman who discontinued estrogen treatment experienced height loss, dorsal kyphosis, and chronic pain caused by collapse and wedging of vertebral bodies. The arrowhead points to T12 in each woman.

TABLE 17.5 Some Common Low-Dose Estrogen Treatment Options for Pubertal Induction in TS and Considerations for Use

Preparation ^a	Doses Available, Frequency, Route	Starting Dose at Puberty	Dose Increase Approximately Every 6 Mo to Adult Dosing	Considerations for Use
Transdermal options (some brands)		3–7 mcg/day	25–100 mcg/day	See text on applying patches
Menostar (Bayer) (matrix)	14 mcg weekly TD	One-half patch weekly	Only used for low dosing, not full replacement	Easiest way to give low dose; once a week dosing
Vivelle-Dot (Novartis) (matrix)	25, 37.5, 50, 75, 100 mcg twice weekly	One-quarter patch weekly, or one patch per month (no patch other 3 weeks)	25–100 mcg twice weekly	Designed for twice-weekly dosing, but can give once per week to increase dose more slowly
Vivelle Mini (matrix)	25, 37.5, 50, 75, 100 mcg twice weekly	Too small to cut consistently	25–100 mcg twice weekly	Smaller size patch, but not smaller dosing
Generic (different brands in different countries)	25, 37.5, 50, 75, 100 mcg twice weekly	One-quarter patch weekly, or one patch per month (no patch other 3 wk)	25–100 mcg twice weekly	Once-weekly dosing can be used.
Estraderm (matrix)	50, 100 mcg twice weekly	Not small enough to initiate puberty	50–100 mcg twice weekly	Cannot use to initiate puberty
E ₂ gel		0.25 mg per pump	One pump daily	Only available in some countries at the low dose
Estragel (Ascend), 0.06%	0.75 mg E ₂ per pump			
Divigel (Vertical), 0.1%	0.25, 0.5, 0.1 mg E ₂ per pump			
Oral options				
17β-E ₂ (e.g., Estrace [Allergen], Cetura [ACE])	0.5, 1, 2, 4 mg/day	One-half pill daily	1–4 mg/day	Cheapest option, brands vary by country
EE		2 mcg/day	10–20 mcg/day	Not available in many countries
Premarin (Pfizer) (a CEE)	0.3, 0.625, 0.9, 1.25 mg/day	One-half pill daily	0.625–1.25 mg/day	Not available in many countries, not recommended based on safety
Depot options				
Depot E ₂ (E ₂ cypionate)	5 mg/mL	0.2 mg/mo	2 mg/mo	Not available in Europe

^aThe reader should be aware that availability and trade names differ among countries. The list is not all inclusive.

(Klein, K.O., Rosenfield, R.L., Santen, R.J., Gawlik, A.M., Bäckeljauw, P.F., et al. (2018) Estrogen replacement in turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab*, 103, 1790–1803.)

well as certain other medical specialties, dependent on the morbidity profile of the individual patient.

Appropriate female HRT is the mainstay of treatment to address the POI in adult life and has numerous beneficial effects on blood pressure,³²² lipid composition,³³⁰ bone,^{340,342} uterine size,³⁴³ possibly neurocognitive function,^{344–347} sexuality,³⁴⁶ and quality of life.³⁴⁷ One can get HRT in the form of oral tablets, transdermal patches, and as injectable depot formulations. Very few studies have made head-to-head comparisons of different routes of delivery and, presently, there is no firm evidence to suggest that any route is superior to others, although the transdermal route is seen as more physiologic because of its similarity with the physiologic delivery of estradiol from the ovaries.

The adult dose of estradiol replacement is quite individual and some women can be treated with estradiol 2 mg orally, whereas others may need a higher dose of up to estradiol 3 to 4 mg orally. Table 17.5 provides an overview of commonly used low-dose estrogen options for pubertal induction treatment in TS. If fertility is an issue, it is sometimes necessary to treat for periods of time with estradiol doses up to 8 mg to ensure adequate growth of the uterus and increase the yield for achieving a successful pregnancy.

REFERENCES

- Turner HH. A syndrome of infantilism, congenital webbed neck, and cubitus valgus. *Endocrinology*. 1938;23:566–574.
- Ullrich O. Über typische Kombinationsbilder multipler Abartungen. *Z Kinderheilk*. 1930;49:271–276.
- Ullrich O. Turner's syndrome and status bonnevie-ullrich; A synthesis of animal phenogenetics and clinical observations on a typical complex of developmental anomalies. *Am J Hum Genet*. 1949;1(2):179–202.
- Goldman ML, Schroeder HA, Futcher PH. Coarctation of the aorta associated with abnormal digits, ovarian insufficiency and shortness of stature. *J Clin Endocrinol Metab*. 1949;9(7):622–629.
- Polani PE, Hunter WF, Lennox B. Chromosomal sex in Turner's syndrome with coarctation of the aorta. *Lancet (London, England)*. 1954;267(6829):120–121.
- Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet (London, England)*. 1959;1(7075):711–713.
- Opitz JM, Pallister PD. Brief historical note: the concept of "gonadal dysgenesis" *Am J Med Genet*. 1979;4(4):333–343.
- Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*. 1959;1(7075):711–713.
- Ferguson-Smith MA. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. *J Med Genet*. 1965;2:142–155.
- Jacobs P, Dalton P, James R, Mosse K, Power M, Robinson D, Skuse D. Turner syndrome: a cytogenetic and molecular study. *Ann Hum Genet*. 1997;61(Pt 6):471–483.
- Templado C, Vidal F, Estop A. Aneuploidy in human spermatozoa. *Cytogenet Genome Res*. 2011;133(2–4):91–99.

12. Bean CJ, Hunt PA, Millie EA, Hassold TJ. Analysis of a malsegregating mouse Y chromosome: evidence that the earliest cleavage divisions of the mammalian embryo are non-disjunction-prone. *Hum Mol Genet.* 2001;10(9):963–972.
13. Wolff DJ, Miller AP, Van Dyke DL, Schwartz S, Willard HF. Molecular definition of breakpoints associated with human Xq isochromosomes: implications for mechanisms of formation. *Am J Hum Genet.* 1996;58(1):154–160.
14. Nielsen J, Wohler M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet.* 1991;87:81–83.
15. Gravholt CH, Juul S, Naeraa RW, Hansen J. Prenatal and postnatal prevalence of Turner's syndrome: a registry study. *BMJ.* 1996;312(7022):16–21.
16. Hook EB, Warburton D. The distribution of chromosomal genotypes associated with Turner's syndrome: livebirth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. *Hum Genet.* 1983;64(1):24–27.
17. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, et al. International Turner Syndrome Consensus Group. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol.* 2017;177(3):G1–G70.
18. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab.* 2006;91(10):3897–3902.
19. Sybert VP, McCauley E. Turner's syndrome. *N Engl J Med.* 2004;351(12):1227–1238.
20. Gunther DF, Eugster E, Zagar AJ, Bryant CG, Davenport ML, Quigley CA. Ascertainment bias in Turner syndrome: new insights from girls who were diagnosed incidentally in prenatal life. *Pediatrics.* 2004;114:640–644.
21. Chang HJ, Clark RD, Bachman H. The phenotype of 45,X/46,XY mosaicism: an analysis of 92 prenatally diagnosed cases. *Am J Hum Genet.* 1990;46(1):156–167.
22. Gravholt CH, Fedder J, Naeraa RW, Müller J. Occurrence of gonadoblastoma in females with Turner syndrome and Y chromosome material: a population study. *J Clin Endocrinol Metab.* 2000;85(9):3199–3202.
23. Mazzanti L, Cicognani A, Baldazzi L, Bergamaschi R, Scarano E, Stocchi S, et al. Gonadoblastoma in Turner syndrome and Y-chromosome-derived material. *Am J Med Genet A.* 2005;135(2):150–154.
24. Sallai A, Sólyom J, Dobos M, Szabó J, Halász Z, Ságodi L, et al. Y-chromosome markers in Turner syndrome: screening of 130 patients. *J Endocrinol Invest.* 2010;33(4):222–227.
25. Graves JA. Did sex chromosome turnover promote divergence of the major mammal groups?: de novo sex chromosomes and drastic rearrangements may have posed reproductive barriers between monotremes, marsupials and placental mammals. *Bioessays.* 2016;38(8):734–743.
26. Ng K, Pullirsch D, Leeb M, Wutz A. Xist and the order of silencing. *EMBO Rep.* 2007;8(1):34–39.
27. Balaton BP, Brown CJ. Escape artists of the X chromosome. *Trends Genet.* 2016;32(6):348–359. <https://doi.org/10.1016/j.tig.2016.03.007>. Epub 2016 Apr 18. Review. PMID: 27103486.
28. Helena Mangs A, Morris BJ. The human pseudoautosomal region (PAR): origin, function and future. *Curr Genomics.* 2007;8(2):129–136.
29. Tukiainen T, Villani A-C, Yen A, Rivas MA, Marshall JL, Satija R, et al. Landscape of X chromosome inactivation across human tissues. *Nature.* 2017;550:244–248.
30. Binder G. Short stature due to SHOX deficiency: genotype, phenotype, and therapy. *Horm Res Paediatr.* 2011;75:81–89.
31. Rappold G, Blum WF, Shavrikova EP, Crowe BJ, Roeth R, Quigley CA, et al. Genotypes and phenotypes in children with short stature: clinical indicators of SHOX haploinsufficiency. *J Med Genet.* 2007;44:306–313.
32. Zinn AR, Tonk VS, Chen Z, Flejter WL, Gardner HA, Guerra R, et al. Evidence for a Turner syndrome locus or loci at Xp11.2–p22.1. *Am J Hum Genet.* 1998;63(6):1757–1766.
33. Di Pasquale E, Beck-Peccoz P, Persani L. Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am J Hum Genet.* 2004;75(1):106–111.
34. Rossetti R, Di Pasquale E, Marozzi A, Bione S, Toniolo D, Grammatico P. BMP15 mutations associated with primary ovarian insufficiency cause a defective production of bioactive protein. *Hum Mutat.* 2009;30(5):804–810.
35. Castronovo C, Rossetti R, Rusconi D, Recalcati MP, Cacciatore C, Beccaria E, et al. Gene dosage as a relevant mechanism contributing to the determination of ovarian function in Turner syndrome. *Hum Reprod.* 2014;29(2):368–379.
36. Therman E, Laxova R, Susman B. The critical region on the human Xq. *Hum Genet.* 1990;85(5):455–461.
37. Toniolo D. X-linked premature ovarian failure: a complex disease. *Curr Opin Genet Dev.* 2006;3:293–300.
38. Mansouri MR, Schuster J, Badhai J, Stattin EL, Lösel R, Wehling M, et al. Alterations in the expression, structure and function of progesterone receptor membrane component-1 (PGRMC1) in premature ovarian failure. *Hum Mol Genet.* 2008;17(23):3776–3783.
39. ExAC Database accessed November 1, 2018
40. Lacombe A, Lee H, Zahed L, Choucair M, Muller JM, Nelson SF, et al. Disruption of POF1B binding to nonmuscle actin filaments is associated with premature ovarian failure. *Am J Hum Genet.* 2006;79(1):113–119.
41. Riva P, Magnani I, Fuhrmann Conti AM, Gelli D, Sala C, Toniolo D, Larizza L. FISH characterization of the Xq21 breakpoint in a translocation carrier with premature ovarian failure. *Clin Genet.* 1996;50(4):267–269.
42. Prueitt RL, Chen H, Barnes RI, Zinn AR. Most X;autosome translocations associated with premature ovarian failure do not interrupt X-linked genes. *Cytogenet Genome Res.* 2002;97(1–2):32–38.
43. Panda B, Rao L, Tosh D, Dixit H, Padmalatha V, Kanakavalli M, et al. Germline study of AR gene of Indian women with ovarian failure. *Gynecol Endocrinol.* 2010;8:572–578.
44. Shiina H, Matsumoto T, Sato T, Igarashi K, Miyamoto J, Takemasa S, et al. Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci USA.* 2006;1:224–229.
45. Van Esch H. The Fragile X premutation: new insights and clinical consequences. *Eur J Med Genet.* 2006;49(1):1–8.
46. Qin Y, Jiao X, Simpson JL, Chen ZJ. Genetics of primary ovarian insufficiency: new developments and opportunities. *Hum Reprod Update.* 2015;21(6):787–808.
47. Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet.* 2001;10:1449–1454.
48. Leppig KA, Sybert VP, Ross JL, Cunniff C, Trejo T, Raskind WH, Distechi CM. Phenotype and X inactivation in 45,X/46,X,r(X) cases. *Am J Med Genet.* 2004;128A:276–284.
49. Kubota T, Wakui K, Nakamura T, Ohashi H, Watanabe Y, Yoshino M, et al. The proportion of cells with functional X disomy is associated with the severity of mental retardation in mosaic ring X Turner syndrome females. *Cytogenet Genome Res.* 2002;99(1–4):276–284.
50. Trolle C, Nielsen MM, Skakkebaek A, Lamy P, Vang S, Hedegaard J, et al. Widespread DNA hypomethylation and differential gene expression in Turner syndrome. *Sci Rep.* 2016;6:34220.
51. Florijn BW, Bijkerk R, van der Veer EP, van Zonneveld, AJ. Gender and cardiovascular disease: are sex-biased microRNA networks a driving force behind heart failure with preserved ejection fraction in women? *Cardiovasc Res.* 2018;114(2):210–225. <https://doi.org/10.1093/cvr/cvx223>. Review.
52. Skuse DH, James RS, Bishop DVM, Coppin B, Dalton P, Aamodt-Leeper G, et al. Evidence from Turner syndrome of an imprinted X-linked locus affecting cognitive function. *Nature.* 1997;387(6634):705–708.
53. Bishop D, Canning E, Elgar K, Morris E, Jacobs P, Skuse D. Distinctive patterns of memory function in subgroups of females with Turnersyndrome: evidence for imprinted loci on the X-chromosome affecting neurodevelopment. *Neuropsychologia.* 2000;38:712–721.
54. Lepage JF, Hong DS, Mazaika PK, Raman M, Sheau K, Marzelli MJ, et al. Genomic imprinting effects of the X chromosome on brain morphology. *J Neurosci.* 2013;33(19):8567–8574.

55. Russell HF, Wallis D, Mazzocco MM, Moshang T, Zackai E, Zinn AR, et al. Increased prevalence of ADHD in Turner syndrome with no evidence of imprinting effects. *J Pediatr Psychol.* 2006;31(9):945–955.
56. Lee HJ, Jung HW, Lee GM, Kim HY, Kim JH, Lee SH, et al. No influence of parental origin of intact X chromosome and/or Y chromosome sequences on three-year height response to growth hormone therapy in Turner syndrome. *Ann Pediatr Endocrinol Metab.* 2014;19(3):127–134.
57. Wolff DJ, Van Dyke DL, Powell CM. Laboratory guideline for Turner syndrome. *Genet Med.* 2010;12:52–55.
58. Prakash S, Guo D, Maslen CL, Silberbach M, Milewicz D, Bondy CA. Single-nucleotide polymorphism array genotyping is equivalent to metaphase cytogenetics for diagnosis of Turner syndrome. *Genet Med.* 2014;14:53–59.
59. Murdock DR, Donovan FX, Chandrasekharappa SC, Banks N, Bondy C, Muenke M, Kruszka P. Whole-exome sequencing for diagnosis of Turner syndrome: toward next-generation sequencing and newborn screening. *J Clin Endocrinol Metab.* 2017;102(5):1529–1537.
60. Hook EB. Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *Am J Hum Genet.* 1977;29(1):94–97.
61. Papp C, Beke A, Mezei G, Szigeti Z, Bán Z, Papp Z. Prenatal diagnosis of Turner syndrome: report on 69 cases. *J Ultrasound Med.* 2006;25(6):711–717. quiz 718–720.
62. Schreurs L, Lannoo L, De Catte L, Van Schoubroeck D, Devriendt K, Richter J. First trimester cystic hygroma colli: Retrospective analysis in a tertiary center. *Eur J Obstet Gynecol Reprod Biol.* 2018;231:60–64.
63. Sparks TN, Thao K, Lianoglou BR, Boe NM, Bruce KG, Datkhaeva I, et al. University of California Fetal–Maternal Consortium (UCFC). Nonimmune hydrops fetalis: identifying the underlying genetic etiology. *Genet Med.* 2019;21(6):1339–1344.
64. Noonan JA. Hypertelorism with Turner phenotype. *Am J Dis Child.* 1968;116:373–380.
65. Celermajer JM, Bowdler JD, Cohen DH. Pulmonary stenosis in patients with the Turner phenotype in the male. *Am J Dis Child.* 1968;116:351–358.
66. Allanson JE, Roberts AE. Noonan syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, eds. *GeneReviews*[®] [Internet]. Seattle (WA): University of Washington, Seattle, p; 2011:1993–2018.
67. Birch L, English CA, O'Donoghue K, Barigye O, Fisk NM, Keer JT. Accurate and robust quantification of circulating fetal and total DNA in maternal plasma from 5 to 41 weeks of gestation. *Clin Chem.* 2005;51(2):312–320.
68. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. *Prenat Diagn.* 2013;33(7):667–674.
69. Yu SC, Lee SW, Jiang P, Leung TY, Chan KC, Chiu RW, et al. High-resolution profiling of fetal DNA clearance from maternal plasma by massively parallel sequencing. *Clin Chem.* 2013;59(8):1228–1237.
70. Badeau M, Lindsay C, Blais J, Nshimyumukiza L, Takwoingi Y, Langlois S, et al. Genomics-based non-invasive prenatal testing for detection of fetal chromosomal aneuploidy in pregnant women. *Cochrane Database Syst Rev.* 2017;11. CD011767.
71. Meck JM, Kramer Dugan E, Matyakhina L, Aviram A, Trunca C, Pineda-Alvarez D, et al. Noninvasive prenatal screening for aneuploidy: positive predictive values based on cytogenetic findings. *Am J Obstet Gynecol.* 2015;213(2):214.e1–5.
72. Petersen AK, Cheung SW, Smith JL, Bi W, Ward PA, Peacock S, et al. Positive predictive value estimates for cell-free noninvasive prenatal screening from data of a large referral genetic diagnostic laboratory. *Am J Obstet Gynecol.* 2017;217(6):691.e1–691.e6.
73. 2016. Practice Bulletin No. 163 Summary: screening for fetal aneuploidy. *Obstet Gynecol.* 127(5), 979–981.
74. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. *Hum Reprod Update.* 2011;17(4):454–466. Erratum in: *Hum Reprod Update.* 19(2), 206.
75. Gleicher N, Orvieto R. Is the hypothesis of preimplantation genetic screening (PGS) still supportable? A review. *J Ovarian Res.* 2017;10(1):21.
76. Griffin DK, Ogur C. Chromosomal analysis in IVF: just how useful is it? *Reproduction.* 2018;156(1):F29–F50.
77. van der Putte SC. Lymphatic malformation in human fetuses. A study of fetuses with Turner's syndrome or status Bonneville-Ullrich. *Virchows Arch, Pathol, Anat, Histol.* 1977;376(3):233–246.
78. Vittay P, Bosze P, Gaal M, Laszlo J. Lymph vessel defects in patients with ovarian dysgenesis. *Clin Genet.* 1980;18(5):387–391.
79. Atton G, Gordon K, Brice G, Keeley V, Riches K, Ostergaard P, et al. The lymphatic phenotype in Turner syndrome: an evaluation of nineteen patients and literature review. *Eur J Hum Genet.* 2015;23(12):1634–1639.
80. Collins E. The illusion of widely spaced nipples in the Noonan and the Turner syndromes. *J Pediatr.* 1973;83(4):557–561.
81. Felix A, Capek V, Pashayan HM. The neck in the XO and XX-XO mosaic Turner's syndrome. *Clin Genet.* 1974;5(1):77–80.
82. Neufeld ND, Lippe BM, Kaplan SA. Disproportionate growth of the lower extremities. A major determinant of short stature in Turner's syndrome. *Am J Dis Child.* 1978;132(3):296–298.
83. Beals RK. Orthopedic aspects of the XO (Turner's) syndrome. *Clin Orthopaed Relat Res.* 1973;1973(97):19–30.
84. Kaitila I, Leisti JT, Rimoin DL. Mesomelic skeletal dysplasias. *Clin Orthopaed Relat Res.* 1976;114:94–106.
85. Elder DA, Roper MG, Henderson RC, Davenport ML. Kyphosis in a Turner syndrome population. *Pediatrics.* 2002;109(6). e93.
86. Bercu BB, Kramer SS, Bode HH. A useful radiologic sign for the diagnosis of Turner's syndrome. *Pediatrics.* 1976;58(5):737–739.
87. Necic S, Grant DB. Diagnostic value of hand X-rays in Turner's syndrome. *Acta Paediatr Scand.* 1978;67(3):309–312.
88. Ari M, Bakalov VK, Hill S, Bondy CA. The effects of growth hormone treatment on bone mineral density and body composition in girls with Turner syndrome. *J Clin Endocrinol Metab.* 2006;91(11):4302–4305.
89. Ross JL, Long LM, Feuillan P, Cassorla F, Cutler Jr GB. Normal bone density of the wrist and spine and increased wrist fractures in girls with Turner's syndrome. *J Clin Endocrinol Metab.* 1991;73(2):355–359.
90. Wasserman H, Backeljauw PF, Khoury JC, Kalkwarf HJ, Gordon CM. Bone fragility in Turner syndrome: Fracture prevalence and risk factors determined by a national patient survey. *Clin Endocrinol (Oxf).* 2018;89(1):46–55.
91. Ranke MB, Pfluger H, Rosendahl W, Stubbe P, Enders H, Bierich JR, et al. (1983). Turner syndrome: spontaneous growth in 150 cases and review of the literature. *Eur J Pediatr.* 1983;141(2):81–88.
92. Davenport ML, Punyasavatsut N, Gunther D, Savendahl L, Stewart PW. Turner syndrome: a pattern of early growth failure. *Acta Paediatr (Oslo, Norway : 1992) Supplement.* 1999;88(433):118–121.
93. Even L, Cohen A, Marbach N, Brand M, Kauli R, Sippell W, et al. Longitudinal analysis of growth over the first 3 years of life in Turner's syndrome. *J Pediatr.* 2000;137(4):460–464.
94. Massa GG, Vanderschueren-Lodeweyckx M. Age and height at diagnosis in Turner syndrome: influence of parental height. *Pediatrics.* 1991;88(6):1148–1152.
95. Brook CG, Murset G, Zachmann M, Prader A. Growth in children with 45,XO Turner's syndrome. *Arch Dis Child.* 1974;49(10):789–795.
96. Ranke MB. Turner syndrome. *Eur J Endocrinol.* 1999;141(3):216–217.
97. Lyon AJ, Preece MA, Grant DB. Growth curve for girls with Turner syndrome. *Arch Dis Child.* 1985;60(10):932–935.
98. Singh RP, Carr DH. The anatomy and histology of XO human embryos and fetuses. *Anatomical Rec.* 1966;155(3):369–383.
99. Weiss L. Additional evidence of gradual loss of germ cells in the pathogenesis of streak ovaries in Turner's syndrome. *J Med Genet.* 1971;8(4):540–544.
100. Reynaud K, Cortvrint R, Verlinde F, De Schepper J, Bourgain C, Smitz J. Number of ovarian follicles in human fetuses with the 45, X karyotype. *Fertil Steril.* 2004;81(4):1112–1119.
101. Hreinsson JG, Otala M, Fridstrom M, Borgstrom B, Rasmussen C, Lundqvist M, et al. Follicles are found in the ovaries of adolescent girls with Turner's syndrome. *J Clin Endocrinol Metab.* 2002;87(8):3618–3623.
102. Speed RM. The possible role of meiotic pairing anomalies in the atresia of human fetal oocytes. *Hum Genet.* 1988;78(3):260–266.

103. Therman E, Susman B. The similarity of phenotypic effects caused by Xp and Xq deletions in the human female: a hypothesis. *Hum Genet.* 1990;85(2):175–183.
104. Mercer CL, Lachlan K, Karcianias A, Affara N, Huang S, Jacobs PA, et al. Detailed clinical and molecular study of 20 females with Xq deletions with special reference to menstruation and fertility. *European J Med Genet.* 2013;56(1):1–6.
105. Borgstrom B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, et al. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab.* 2009;94(1):74–80.
106. Hankus M, Soltysik K, Szeliga K, Antosz A, Drosdzol-Cop A, Wilk K, et al. (2018). Prediction of spontaneous puberty in turner syndrome based on mid-childhood gonadotropin concentrations, karyotype, and ovary visualization: a longitudinal study. *Horm Res Paediatr.* 2018;89(2):90–97.
107. Hamza RT, Mira MF, Hamed AI, Ezzat T, Sallam MT. Anti-Müllerian hormone levels in patients with turner syndrome: relation to karyotype, spontaneous puberty, and replacement therapy. *Am J Med Genet A.* 2018;176(9):1929–1934.
108. Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. *J Clin Endocrinol Metab.* 1997;82(6):1810–1813.
109. Hovatta O. Pregnancies in women with Turner's syndrome. *Ann Med.* 1999;31(2):106–110.
110. Hadnott TN, Gould HN, Gharib AM, Bondy CA. Outcomes of spontaneous and assisted pregnancies in Turner syndrome: the U.S. National Institutes of Health experience. *Fertil Steril.* 2011;95(7):2251–2256.
111. Cools M, Rooman RP, Wauters J, Jacqemyn Y, Du Caju MV. A nonmosaic 45,X karyotype in a mother with Turner's syndrome and in her daughter. *Fertil Steril.* 2004;82(4):923–925.
112. Mortensen KH, Rohde MD, Ulbjerg N, Gravholt CH. Repeated spontaneous pregnancies in 45,X Turner syndrome. *Obstet Gynecol.* 2010;115(2 Pt 2):446–449.
113. Tarani L, Lampariello S, Raguso G, Colloridi F, Pucarelli I, Pasquino AM, et al. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. *Gynecol Endocrinol.* 1998;12(2):83–87.
114. Hagman A, Kallen K, Barrenas ML, Landin-Wilhelmsen K, Hanson C, Bryman I, et al. Obstetric outcomes in women with Turner karyotype. *J Clin Endocrinol Metab.* 2011;96(11):3475–3482.
115. Mortensen KH, Cleemann L, Hjerrild BE, Nexø E, Locht H, Jeppesen EM, et al. Increased prevalence of autoimmunity in Turner syndrome—influence of age. *Clin Exp Immunol.* 2009;156(2):205–210.
116. Cools M, Drop SL, Wolffenbuttel KP, Oosterhuis JW, Looijenga LH. Germ cell tumors in the intersex gonad: old paths, new directions, moving frontiers. *Endocr Rev.* 2006;27(5):468–484.
117. Zelaya G, López Martí JM, Marino R, García de Dávila MT, Gallego MS. Gonadoblastoma in patients with Ullrich-Turner syndrome. *Pediatr Dev Pathol.* 2015;18(2):117–121.
118. Stochholm K, Juul S, Juul K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab.* 2006;91(10):3897–3902.
119. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. *J Clin Epidemiol.* 1998;51(2):147–158.
120. Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA. Mortality in women with turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab.* 2008;93(12):4735–4742.
121. Kwon A, Hyun SE, Jung MK, Chae HW, Lee WJ, Kim TH, et al. Risk of gonadoblastoma development in patients with Turner syndrome with cryptic Y chromosome material. *Horm Cancer.* 2017;8(3):166–173.
122. Portnoi MF, Chantot-Bastarud S, Christin-Maitre S, Carbonne B, Beaujard MP, Keren B, et al. Familial Turner syndrome with an X;Y translocation mosaicism: implications for genetic counseling. *European J Med Genet.* 2012;55(11):635–640.
123. Landin-Wilhelmsen K, Bryman I, Hanson C, Hanson L. Spontaneous pregnancies in a Turner syndrome woman with Y-chromosome mosaicism. *J Assist Reprod Genet.* 2004;21(6):229–230.
124. Wei F, Cheng S, Badie N, Elder F, Scott Jr C, Nicholson L, et al. A man who inherited his SRY gene and Leri-Weill dyschondrosteosis from his mother and neurofibromatosis type 1 from his father. *Am J Med Genet.* 2001;102(4):353–358.
125. Saenger P, Wikland KA, Conway GS, Davenport M, Gravholt CH, Hintz R, et al. Recommendations for the diagnosis and management of Turner syndrome. *J Clin Endocrinol Metab.* 2001;86(7):3061–3069.
126. Bondy CA. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab.* 2007;92(1):10–25.
127. Nishi MY, Domenice S, Medeiros MA, Mendonca BB, Billerbeck AE. Detection of Y-specific sequences in 122 patients with Turner syndrome: nested PCR is not a reliable method. *Am J Med Genet.* 2002;107(4):299–305.
128. Price WH, Clayton JF, Collyer S, De Mey R, Wilson J. Mortality ratios, life expectancy, and causes of death in patients with Turner's syndrome. *J Epidemiol Comm Health.* 1986;40(2):97–102.
129. Ferguson-Smith MA. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. *J Med Genet.* 1965;2(2):142–155.
130. Haddad HM, Wilkins L. Congenital anomalies associated with gonadal aplasia; review of 55 cases. *Pediatrics.* 1959;23(5):885–902.
131. Nora JJ, Torres FG, Sinha AK, McNamara DG. Characteristic cardiovascular anomalies of XO Turner syndrome, XX and XY phenotype and XO-XX Turner mosaic. *Am J Cardiol.* 1970;25(6):639–641.
132. Sigakis CJG, Browne LP, Bang T, Khanna A, Prunte R, Vargas D. Computed tomography and magnetic resonance imaging of cardiovascular anomalies associated with Turner syndrome. *J Thorac Imaging.* 2018;34(3):W23–W35.
133. Sybert VP. Cardiovascular malformations and complications in Turner syndrome. *Pediatrics.* 1998;101(1):E11.
134. Patel A, Hickey E, Mavroudis C, Jacobs JP, Jacobs ML, Backer CL, et al. Impact of noncardiac congenital and genetic abnormalities on outcomes in hypoplastic left heart syndrome. *Ann Thorac Surg.* 2010;89(6):1805–1813. discussion 13–14.
135. Clark EB. Neck web and congenital heart defects: a pathogenic association in 45 X-O Turner syndrome? *Teratology.* 1984;29(3):355–361.
136. Lacro RV, Jones KL, Benirschke K. Coarctation of the aorta in Turner syndrome: a pathologic study of fetuses with nuchal cystic hygromas, hydrops fetalis, and female genitalia. *Pediatrics.* 1988;81(3):445–451.
137. Mazzanti L, Cacciari E. Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). *J Pediatr.* 1998;133(5):688–962.
138. Localzo ML, Van PL, Ho VB, Bakalov VK, Rosing DR, Malone CA, et al. Association between fetal lymphedema and congenital cardiovascular defects in Turner syndrome. *Pediatrics.* 2005;115(3):732–735.
139. Miyabara S, Nakayama M, Suzumori K, Yonemitsu N, Sugihara H. Developmental analysis of cardiovascular system of 45,X fetuses with cystic hygroma. *Am J Med Genet.* 1997;68(2):135–141.
140. Bondy CA. Congenital cardiovascular disease in Turner syndrome. *Congenit Heart Dis.* 2008;3(1):2–15.
141. Sachdev V, Matura LA, Sidenko S, Ho VB, Arai AE, Rosing DR, et al. Aortic valve disease in Turner syndrome. *J Am Coll Cardiol.* 2008;51(19):1904–1909.
142. Bondy CA. Aortic coarctation and coronary artery disease: the XY factor. *Circulation.* 2012;126(1):5–7.
143. Flaquer A, Fischer C, Wienker TF. A new sex-specific genetic map of the human pseudoautosomal regions (PAR1 and PAR2). *Hum Hered.* 2009;68(3):192–200.
144. Donadille B, Rousseau A, Zenaty D, Cabrol S, Courtillot C, Samara-Boustani D, et al. Cardiovascular findings and management in Turner syndrome: insights from a French cohort. *Eur J Endocrinol.* 2012;167(4):517–522.
145. Miller MJ, Geffner ME, Lippe BM, Itami RM, Kaplan SA, DiSessa TG, et al. Echocardiography reveals a high incidence of bicuspid aortic valve in Turner syndrome. *J Pediatr.* 1983;102(1):47–50.

146. Kim HK, Gottliebson W, Hor K, Backeljauw P, Gutmark-Little I, Salisbury SR, et al. Cardiovascular anomalies in Turner syndrome: spectrum, prevalence, and cardiac MRI findings in a pediatric and young adult population. *AJR Am J Roentgenol.* 2011;196(2):454–460.
147. Lin AE, Lippe BM, Geffner ME, Gomes A, Lois JF, Barton CW, et al. Aortic dilation, dissection, and rupture in patients with Turner syndrome. *J Pediatr.* 1986;109(5):820–826.
148. Lin AE, Lippe B, Rosenfeld RG. Further delineation of aortic dilation, dissection, and rupture in patients with Turner syndrome. *Pediatrics.* 1998;102(1). e12.
149. Matura LA, Ho VB, Rosing DR, Bondy CA. Aortic dilatation and dissection in Turner syndrome. *Circulation.* 2007;116(15):1663–1670.
150. Carlson M, Silberbach M. Dissection of the aorta in Turner syndrome: two cases and review of 85 cases in the literature. *J Med Genet.* 2007;44(12):745–749.
151. Gravholt CH, Landin-Wilhelmsen K, Stochholm K, Hjerrild BE, Ledet T, Djurhuus CB, et al. Clinical and epidemiological description of aortic dissection in Turner's syndrome. *Cardiol Young.* 2006;16(5):430–436.
152. Lopez L, Arheart KL, Colan SD, Stein NS, Lopez-Mitnik G, Lin AE, et al. Turner syndrome is an independent risk factor for aortic dilation in the young. *Pediatrics.* 2008;121(6):e1622–e1627.
153. Ostberg JE, Brookes JA, McCarthy C, Halcox J, Conway GS. A comparison of echocardiography and magnetic resonance imaging in cardiovascular screening of adults with Turner syndrome. *J Clin Endocrinol Metab.* 2004;89(12):5966–5971.
154. Hjerrild BE, Mortensen KH, Sorensen KE, Pedersen EM, Andersen NH, Lundorf E, et al. Thoracic aortopathy in Turner syndrome and the influence of bicuspid aortic valves and blood pressure: a CMR study. *J Cardiovasc Magn Reson.* 2010;20(10):12.
155. Duijnhouwer AL, Bons LR, Timmers HJLM, van Kimmenade RRL, Snoeren M, Timmermans J, et al. Aortic dilatation and outcome in women with Turner syndrome. *Heart.* 2018. pii: heartjnl-2018-313716.
156. Ostberg JE, Donald AE, Halcox JP, Storry C, McCarthy C, Conway GS. Vasculopathy in Turner syndrome: arterial dilatation and intimal thickening without endothelial dysfunction. *J Clin Endocrinol Metab.* 2005;90(9):5161–5166.
157. Polkampally PR, Matta JR, McAreavey D, Bakalov V, Bondy CA, Charib AM. Aneurysmal dilatation of medium caliber arteries in Turner syndrome. *Congenit Heart Dis.* 2011;6(4):382–383.
158. De Groote K, Devos D, Van Herck K, De Wolf D, Van der Straaten S, Rietzschel E, et al. Increased aortic stiffness in prepubertal girls with Turner syndrome. *J Cardiol.* 2017;69(1):201–207.
159. Devos DG, De Groote K, Babin D, Demulier L, Taeymans Y, Westenberg JJ, et al. Proximal aortic stiffening in Turner patients may be present before dilation can be detected: a segmental functional MRI study. *J Cardiovasc Magn Reson.* 2017;19(1):27.
160. Bondy CA. Aortic dissection in Turner syndrome. *Curr Opin Cardiol.* 2008;23(6):519–526.
161. Cabanes L, Chalas C, Christine-Maitre S, Donadille B, Felten ML, Gaxotte V, et al. Turner syndrome and pregnancy: clinical practice. Recommendations for the management of patients with Turner syndrome before and during pregnancy. *Eur J Obstet Gynecol Reprod Biol.* 2010;152(1):18–24.
162. Practice Committee of American Society For Reproductive Medicine. Increased maternal cardiovascular mortality associated with pregnancy in women with Turner syndrome. *Fertil Steril.* 2012;97(2):282–284.
163. van Wassenaer AG, Lubbers LJ, Losekoot G. Partial abnormal pulmonary venous return in Turner syndrome. *Eur J Pediatr.* 1988;148(2):101–103.
164. Bechtold SM, Dalla Pozza R, Becker A, Meidert A, Dohlemann C, Schwarz HP. Partial anomalous pulmonary vein connection: an underestimated cardiovascular defect in Ullrich-Turner syndrome. *Eur J Pediatr.* 2004;163(3):158–162.
165. Gotzsche CO, Krag-Olsen B, Nielsen J, Sorensen KE, Kristensen BO. Prevalence of cardiovascular malformations and association with karyotypes in Turner's syndrome. *Arch Dis Child.* 1994;71(5):433–436.
166. Volkl TM, Degenhardt K, Koch A, Simm D, Dorr HG, Singer H. Cardiovascular anomalies in children and young adults with Ullrich-Turner syndrome the Erlangen experience. *Clin Cardiol.* 2005;28(2):88–92.
167. Ho VB, Bakalov VK, Cooley M, Van PL, Hood MN, Burklow TR, et al. Major vascular anomalies in Turner syndrome: prevalence and magnetic resonance angiographic features. *Circulation.* 2004;110(12):1694–1700.
168. Kim HK, Gottliebson W, Hor K, Backeljauw P, Gutmark-Little I, Salisbury SR, et al. Cardiovascular anomalies in Turner syndrome: spectrum, prevalence, and cardiac MRI findings in a pediatric and young adult population. *AJR Am J Roentgenol.* 2011;196(2):454–460.
169. Banerji D, Martinez F, Abbara S, Truong QA. Turner syndrome with aberrant right subclavian artery and partial anomalous pulmonary venous return. *J Cardiovasc Comput Tomogr.* 2011;5(3):189–191.
170. Bondy CA, Ceniceris I, Van PL, Bakalov VK, Rosing DR. Prolonged rate-corrected QT interval and other electrocardiogram abnormalities in girls with Turner syndrome. *Pediatrics.* 2006;118(4):e1220–e1225.
171. Dalla Pozza R, Bechtold S, Kaab S, Buckl M, Urschel S, Netz H, et al. QTc interval prolongation in children with Ullrich-Turner syndrome. *Eur J Pediatr.* 2006;165(12):831–837.
172. Zuckerman-Levin N, Zinder O, Greenberg A, Levin M, Jacob G, Hochberg Z. (2006). Physiological and catecholamine response to sympathetic stimulation in turner syndrome. *Clin Endocrinol.* 2006;64(4):410–415.
173. Gravholt CH, Hansen KW, Erlandsen M, Ebbelohj E, Christiansen JS. Nocturnal hypertension and impaired sympathovagal tone in Turner syndrome. *J Hypertens.* 2006;24(2):353–360.
174. Nathwani NC, Unwin R, Brook CG, Hindmarsh PC. Blood pressure and Turner syndrome. *Clin Endocrinol.* 2000;52(3):363–370.
175. Landin-Wilhelmsen K, Bryman I, Wilhelmsen L. Cardiac malformations and hypertension, but not metabolic risk factors, are common in Turner syndrome. *J Clin Endocrinol Metab.* 2001;86(9):4166–41670.
176. Elsheikh M, Dunger DB, Conway GS, Wass JA. Turner's syndrome in adulthood. *Endocr Rev.* 2002;23(1):120–140.
177. Nathwani NC, Unwin R, Brook CG, Hindmarsh PC. The influence of renal and cardiovascular abnormalities on blood pressure in Turner syndrome. *Clin Endocrinol.* 2000;52(3):371–377.
178. Gravholt CH, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, et al. Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care.* 1998;21(7):1062–1070.
179. De Groote K, Demulier L, De Backer J, De Wolf D, De Schepper J, T'sjoen G, De Backer T. Arterial hypertension in Turner syndrome: a review of the literature and a practical approach for diagnosis and treatment. *J Hypertens.* 2015;33(7):1342–1351.
180. Roest AA, de Roos A. Imaging of patients with congenital heart disease. *Nat Rev Cardiol.* 2011;9(2):101–115.
181. Dawson-Falk KL, Wright AM, Bakker B, Pitlick PT, Wilson DM, Rosenfeld RG. Cardiovascular evaluation in Turner syndrome: utility of MR imaging. *Australas Radiol.* 1992;36(3):204–209.
182. Castro AV, Okoshi K, Ribeiro SM, Barbosa MF, Mattos PF, Pagliare L, et al. Cardiovascular assessment of patients with Ullrich-Turner's Syndrome on Doppler echocardiography and magnetic resonance imaging. *Arq Bras Cardiol.* 2002;78(1):51–58.
183. Mebus S, Meierhofer C, Pringsheim M, Schoen P, Kaemmerer H, Hess J, et al. How late is too late? Giant balloon-like aneurysm of the ascending aorta. *Am J Med.* 2011;124(12):e3–e4.
184. Oza NM, Siegenthaler M, Horvath K, Rosing DR, Chen MY, Arai AE, et al. Serious aortic complications in a patient with Turner syndrome. *Eur J Pediatr.* 2013;172(5):703–705.
185. Pleskacova J, Ruckdova K, Popelova J, Cerny S, Syrucek M, Snajderova M, et al. Aortic dissection and rupture in a 16-year-old girl with Turner syndrome following previous progression of aortic dilation. *Eur J Pediatr.* 2010;169(10):1283–1286.
186. Lanzarini L, Larizza D, Prete G, Calcaterra V, Klersy C. Prospective evaluation of aortic dimensions in Turner syndrome: a 2-dimensional echocardiographic study. *J Am Soc Echocardiogr.* 2007;20(3):307–313.

187. Mortensen KH, Hjerrild BE, Stochholm K, Andersen NH, Sorensen KE, Lundorf E, et al. Dilation of the ascending aorta in Turner syndrome - a prospective cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson*. 2011;13:24.
188. Gutin LS, Bakalov VK, Rosing DR, Arai AE, Gharib AM, Bondy CA. N-terminal pro-brain natriuretic peptide levels and aortic diameters. *Am Heart J*. 2012;164(3):419–424.
189. Ross JL, Feuillan P, Long LM, Kowal K, Kushner H, Cutler Jr GB. Lipid abnormalities in Turner syndrome. *J Pediatr*. 1995;126(2):242–245.
190. Wooten N, Bakalov VK, Hill S, Bondy CA. Reduced abdominal adiposity and improved glucose tolerance in growth hormone-treated girls with Turner syndrome. *J Clin Endocrinol Metab*. 2008;93(6):2109–2114.
191. Van PL, Bakalov VK, Zinn AR, Bondy CA. Maternal X chromosome, visceral adiposity, and lipid profile. *JAMA*. 2006;295(12):1373–1374.
192. McCarthy K, Bondy CA. Turner syndrome in childhood and adolescence. *Exp Rev Endocrinol Metab*. 2008;3(6):771–775.
193. Lippe B, Geffner ME, Dietrich RB, Boechat MI, Kangaroo H. Renal malformations in patients with Turner syndrome: imaging in 141 patients. *Pediatrics*. 1988;82(6):852–856.
194. Bilge I, Kayserili H, Emre S, Nayir A, Sirin A, Tukul T, et al. Frequency of renal malformations in Turner syndrome: analysis of 82 Turkish children. *Pediatr Nephrol*. 2000;14(12):1111–1114.
195. Anderson H, Filipsson R, Fluor E, Koch B, Lindsten J, Wedenberg E. Hearing impairment in Turner's syndrome. *Acta Oto-laryngol, Suppl*. 1969;247:1–26.
196. Bonnard Å, Hederstierna C, Bark R, Hultcrantz M. Audiometric features in young adults with Turner syndrome. *Int J Audiol*. 2017;56(9):650–656.
197. King KA, Makishima T, Zalewski CK, Bakalov VK, Griffith AJ, Bondy CA, et al. Analysis of auditory phenotype and karyotype in 200 females with Turner syndrome. *Ear Hearing*. 2007;28(6):831–841.
198. Barenas ML, Nylen O, Hanson C. The influence of karyotype on the auricle, otitis media and hearing in Turner syndrome. *Hearing Res*. 1999;138(1–2):163–170.
199. Stenberg AE, Nylen O, Windh M, Hultcrantz M. Otological problems in children with Turner's syndrome. *Hearing Res*. 1998;124(1–2):85–90.
200. Dhooge IJ, De Vel E, Verhoye C, Lemmerling M, Vinck B. Otolologic disease in turner syndrome. *Otol Neurotol*. 2005;26(2):145–150.
201. Bois E, Nassar M, Zenaty D, Léger J, Van Den Abbeele T, Teissier N. Otolologic disorders in Turner syndrome. *Eur Ann Otorhinolaryngol Head Neck Dis*. 2018;135(1):21–24.
202. Sparkes RS, Motulsky AG. Hashimoto's disease in Turner's syndrome with isochromosome X. *Lancet (London, England)*. 1963;1(7287):947.
203. Elsheikh M, Wass JA, Conway GS. Autoimmune thyroid syndrome in women with Turner's syndrome—the association with karyotype. *Clin Endocrinol*. 2001;55(2):223–226.
204. Radetti G, Mazzanti L, Paganini C, Bernasconi S, Russo G, Rigon F, et al. Frequency, clinical and laboratory features of thyroiditis in girls with Turner's syndrome. The Italian Study Group for Turner's Syndrome. *Acta Paediatr (Oslo, Norway : 1992)*. 1995;84(8):909–912.
205. Bakalov VK, Gutin L, Cheng CM, Zhou J, Sheth P, Shah K, et al. Autoimmune disorders in women with turner syndrome and women with karyotypically normal primary ovarian insufficiency. *J Autoimmun*. 2012;38(4):315–321.
206. Brooks WH, Meek JC, Schimke RN. Gonadal dysgenesis with Graves's disease. *J Med Genet*. 1977;14(2):128–129.
207. El-Mansoury M, Bryman I, Berntorp K, Hanson C, Wilhelmsen L, Landin-Wilhelmsen K. Hypothyroidism is common in turner syndrome: results of a five-year follow-up. *J Clin Endocrinol Metab*. 2005;90(4):2131–2135.
208. Livadas S, Xekouki P, Fouka F, Kanaka-Gantenbein C, Kaloumenou I, Mavrou A, et al. Prevalence of thyroid dysfunction in Turner's syndrome: a long-term follow-up study and brief literature review. *Thyroid*. 2005;15(9):1061–1066.
209. Zulian F, Schumacher HR, Calore A, Goldsmith DP, Athreya BH. Juvenile arthritis in Turner's syndrome: a multicenter study. *Clin Exp Rheumatol*. 1998;16(4):489–494.
210. Calanchini M, Moolla A, Tomlinson JW, Cobbold JF, Grossman A, Fabbri A, Turner HE. Liver biochemical abnormalities in Turner syndrome: a comprehensive characterization of an adult population. *Clin Endocrinol (Oxf)*. 2018;89(5):667–676.
211. Salerno M, Di Maio S, Gasparini N, Rizzo M, Ferri P, Vajro P. Liver abnormalities in Turner syndrome. *Eur J Pediatr*. 1999;158(8):618–623.
212. Koulouri O, Ostberg J, Conway GS. Liver dysfunction in Turner's syndrome: prevalence, natural history and effect of exogenous oestrogen. *Clin Endocrinol*. 2008;69(2):306–310.
213. Wemme H, Pohlenz J, Schonberger W. Effect of oestrogen/gestagen replacement therapy on liver enzymes in patients with Ullrich-Turner syndrome. *Eur J Pediatr*. 1995;154(10):807–810.
214. Roulot D. Liver involvement in Turner syndrome. *Liver Int*. 2013;33(1):24–30.
215. Rosen KM, Sirota DK, Marinoff SC. Gastrointestinal bleeding in Turner's syndrome. *Ann Intern Med*. 1967;67(1):145–150.
216. Salomonowitz E, Staffen A, Potzi R, Czembirek H, Meryn S. Angiographic demonstration of phlebectasia in a case of Turner's syndrome. *Gastrointest Radiol*. 1983;8(3):279–281.
217. van Cutsem E, Rutgeerts P, Vantrappen G. Treatment of bleeding gastroduodenal vascular malformations with oestrogen-progesterone. *Lancet (London, England)*. 1990;335(8695):953–955.
218. O'Hare JP, Hamilton M, Davies JD, Corral RJ, Mountford R. Oestrogen deficiency and bleeding from large bowel telangiectasia in Turner's syndrome. *J Royal Soc Med*. 1986;79(12):746–747.
219. Arulanantham K, Kramer MS, Gryboski JD. The association of inflammatory bowel disease and X chromosomal abnormality. *Pediatrics*. 1980;66(1):63–67.
220. Price WH. A high incidence of chronic inflammatory bowel disease in patients with Turner's syndrome. *J Med Genet*. 1979;16(4):263–266.
221. Knudtzon J, Svane S. Turner's syndrome associated with chronic inflammatory bowel disease. A case report and review of the literature. *Acta Med Scand*. 1988;223(4):375–378.
222. Manzione NC, Kram M, Kram E, Das KM. Turner's syndrome and inflammatory bowel disease: a case report with immunologic studies. *Am J Gastroenterol*. 1988;83(11):1294–1297.
223. Hayward PA, Satsangi J, Jewell DP. Inflammatory bowel disease and the X chromosome. *QJM*. 1996;89(9):713–718.
224. Bonamico M, Bottaro G, Pasquino AM, Caruso-Nicoletti M, Mariani P, Gemme G, et al. Celiac disease and Turner syndrome. *J Pediatr Gastroenterol Nutr*. 1998;26(5):496–499.
225. Ivarsson SA, Carlsson A, Bredberg A, Alm J, Aronsson S, Gustafsson J, et al. Prevalence of coeliac disease in Turner syndrome. *Acta paediatrica (Oslo, Norway : 1992)*. 1999;88(9):933–936.
226. Schewior S, Brand M, Santer R. Celiac disease and selective IgA deficiency in a girl with atypical Turner syndrome. *J Pediatr Gastroenterol Nutr*. 1999;28(3):353–354.
227. Bonamico M, Pasquino AM, Mariani P, Danesi HM, Culasso F, Mazzanti L, et al. Prevalence and clinical picture of celiac disease in Turner syndrome. *J Clin Endocrinol Metab*. 2002;87(12):5495–5498.
228. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*. 2005;40(1):1–19.
229. Forbes AP, Engel E. The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. *Metab Clin Exp*. 1963;12:428–439.
230. AvRuskin TW, Crigler Jr JF, Soeldner JS. Turner's syndrome and carbohydrate metabolism. I. Impaired insulin secretion after tolbutamide and glucagon stimulant tests: evidence of insulin deficiency. *Am J Med Sci*. 1979;277(2):145–152.
231. Neufeld ND, Lippe B, Sperling MA. Carbohydrate (CHO) intolerance in gonadal dysgenesis: a new model of insulin resistance. *Diabetes Care*. 1980;25:379.
232. Ibarra-Gasparini D, Altieri P, Scarano E, Perri A, Morselli-Labate AM, Pagotto U, et al. New insights on diabetes in Turner syndrome: results from an observational study in adulthood. *Endocrine*. 2018;59(3):651–660.
233. Caprio S, Boulware S, Diamond M, Sherwin RS, Carpenter TO, Rubin K, et al. Insulin resistance: an early metabolic defect of

- Turner's syndrome. *J Clin Endocrinol Metab.* 1991;72(4):832–836.
234. Bakalov VK, Cooley MM, Quon MJ, Luo ML, Yanovski JA, Nelson LM, et al. Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab.* 2004;89(7):3516–3520.
 235. Bakalov VK, Cheng C, Zhou J, Bondy CA. X-chromosome gene dosage and the risk of diabetes in Turner syndrome. *J Clin Endocrinol Metab.* 2009;94(9):3289–3296.
 236. Salgin B, Amin R, Yuen K, Williams RM, Murgatroyd P, Dunger DB. Insulin resistance is an intrinsic defect independent of fat mass in women with Turner's syndrome. *Horm Res.* 2006;65(2):69–75.
 237. Bakalov VK, Cooley MM, Troendle J, Bondy CA. The prevalence of diabetes mellitus in the parents of women with Turner's syndrome. *Clin Endocrinol.* 2004;60(2):272. 2004.
 238. O'Gorman CS, Syme C, Lang J, Bradley TJ, Wells GD, Hamilton JK. An evaluation of early cardiometabolic risk factors in children and adolescents with Turner syndrome. *Clin Endocrinol.* 2013;78(6):907–913.
 239. Ostberg JE, Attar MJ, Mohamed-Ali V, Conway GS. Adipokine dysregulation in turner syndrome: comparison of circulating interleukin-6 and leptin concentrations with measures of adiposity and C-reactive protein. *J Clin Endocrinol Metab.* 2005;90(5):2948–2953.
 240. Van Dyke DL, Wiktor A, Palmer CG, Miller DA, Witt M, Babu VR, et al. Ullrich-Turner syndrome with a small ring X chromosome and presence of mental retardation. *Am J Med Genet.* 1992;43(6):996–1005.
 241. Van Dyke DL, Wiktor A, Roberson JR, Weiss L. Mental retardation in Turner syndrome. *J Pediatr.* 1991;118(3):415–417.
 242. Grompe M, Rao N, Elder FF, Caskey CT, Greenberg F. 45,X/46,X,+r(X) can have a distinct phenotype different from Ullrich-Turner syndrome. *Am J Med Genet.* 1992;42(1):39–43.
 243. Migeon BR, Luo S, Jani M, Jeppesen P. The severe phenotype of females with tiny ring X chromosomes is associated with inability of these chromosomes to undergo X inactivation. *Am J Hum Genet.* 1994;55(3):497–504.
 244. Jani MM, Torchia BS, Pai GS, Migeon BR. Molecular characterization of tiny ring X chromosomes from females with functional X chromosome disomy and lack of cis X inactivation. *Genomics.* 1995;27(1):182–188.
 245. Silbert A, Wolff PH, Lilienthal J. Spatial and temporal processing in patients with Turner's syndrome. *Behav Genet.* 1977;7(1):11–21.
 246. Nyborg H, Nielsen J. Sex chromosome abnormalities and cognitive performance: III. Field dependence, frame dependence, and failing development of perceptual stability in girls with Turner's syndrome. *J Psychol.* 1977;205–211.
 247. Waber DP. Neuropsychological aspects of Turner's syndrome. *Dev Med Child Neurol.* 1979;21(1):58–70.
 248. McCauley E, Kay T, Ito J, Treder R. The Turner syndrome: cognitive deficits, affective discrimination, and behavior problems. *Child Dev.* 1987;58(2):464–473.
 249. Rovet J, Netley C. Processing deficits in Turner's syndrome. *Dev Psychol.* 1982;18:77.
 250. Temple CM, Carney RA. Intellectual functioning of children with Turner syndrome: a comparison of behavioural phenotypes. *Dev Med Child Neurol.* 1993;35(8):691–698.
 251. Ross JL, Stefanatos G, Roeltgen D, Kushner H, Cutler Jr GB. Ullrich-Turner syndrome: neurodevelopmental changes from childhood through adolescence. *Am J Med Genet.* 1995;58(1):74–82.
 252. Bishop DV, Canning E, Elgar K, Morris E, Jacobs PA, Skuse DH. Distinctive patterns of memory function in subgroups of females with Turner syndrome: evidence for imprinted loci on the X-chromosome affecting neurodevelopment. *Neuropsychologia.* 2000;38(5):712–721.
 253. Ross JL, Roeltgen D, Kushner H, Wei F, Zinn AR. The Turner syndrome-associated neurocognitive phenotype maps to distal Xp. *Am J Hum Genet.* 2000;67(3):672–681.
 254. Russell HF, Wallis D, Mazzocco MM, Moshang T, Zackai E, Zinn AR, et al. Increased prevalence of ADHD in Turner syndrome with no evidence of imprinting effects. *J Pediatr Psychol.* 2006;31(9):945–955.
 255. Schmidt PJ, Cardoso GM, Ross JL, Haq N, Rubinow DR, Bondy CA. Shyness, social anxiety, and impaired self-esteem in Turner syndrome and premature ovarian failure. *JAMA.* 2006;295(12):1374–1376.
 256. Hong DS, Reiss AL. Cognition and behavior in Turner syndrome: a brief review. *Pediatr Endocrinol Rev.* 2012;9(Suppl 2):710–712.
 257. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler Jr GB. Effects of estrogen on nonverbal processing speed and motor function in girls with Turner's syndrome. *J Clin Endocrinol Metab.* 1998;83(9):3198–3204.
 258. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler Jr GB. Use of estrogen in young girls with Turner syndrome: effects on memory. *Neurology.* 2000;54(1):164–170.
 259. Cardoso G., Daly R., Haq, N.A., Hanton, L., Rubinow, D.R., Bondy, C.A., et al. Current and lifetime psychiatric illness in women with Turner syndrome. *Gynecol Endocrinol.* 2004;19(6):313–319.
 260. Money J, Mittenenthal S. Lack of personality pathology in Turner's syndrome: relation to cytogenetics, hormones and physique. *Behav Genet.* 1970;1(1):43–56.
 261. Downey J, Ehrhardt AA, Gruen R, Bell JJ, Morishima A. Psychopathology and social functioning in women with Turner syndrome. *J Nerv Ment Dis.* 1989;177(4):191–201.
 262. McCauley E, Sybert VP, Ehrhardt AA. Psychosocial adjustment of adult women with Turner syndrome. *Clin Genet.* 1986;29(4):284–290.
 263. Garron DC, Vander Stoep LR. Personality and intelligence in Turner's syndrome. A critical review. *Arch Gen Psychiatry.* 1969;21(3):339–346.
 264. Pavlidis K, McCauley E, Sybert VP. Psychosocial and sexual functioning in women with Turner syndrome. *Clin Genet.* 1995;47(2):85–89.
 265. Carel JC, Elie C, Ecosse E, Tauber M, Leger J, Cabrol S, et al. Self-esteem and social adjustment in young women with Turner syndrome—influence of pubertal management and sexuality: population-based cohort study. *J Clin Endocrinol Metab.* 2006;91(8):2972–2979.
 266. Sheaffer AT, Lange E, Bondy CA. Sexual function in women with Turner syndrome. *J Woman Health (2002).* 2008;17(1):27–33.
 267. Lunding SA, Akglaede L, Anderson RA, Main KM, Juul A, Hagen CP, et al. AMH as predictor of premature ovarian insufficiency: a longitudinal study of 120 Turner syndrome patients. *J Clin Endocrinol Metab.* 2015;100(7):E1030–E1038.
 268. Schoemaker M, Swerdlow A, Higgins C, Wright A, Jacobs P. Mortality in women with Turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab.* 2008;93:4735–4742.
 269. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab.* 2006;91(10):3897–3902.
 270. Gutmark-Little I, Backeljauw P. Cardiac magnetic resonance imaging in Turner syndrome. *Clin Endocrinol.* 2013;78:646–658.
 271. Nejatian A, Yu J, Geva T, White M, Prakash A. Aortic measurements in patients with aortopathy are larger and more reproducible by cardiac magnetic resonance compared with echocardiography. *Pediatr Cardiol.* 2015;36:1761–1773.
 272. Bondy CA. Care of girls and women with Turner syndrome: A guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab.* 2007;92(1):10–25.
 273. Freriks K, Timmers H, Netea-Maier R, Beerendonk C, Otten B, Alfens-van der Velden J, et al. Buccal cell FISH and blood PCR-Y detect high rates of X chromosomal mosaicism and Y chromosomal derivatives in patients with Turner syndrome. *European J Med Genet.* 2013;56:497–501.
 274. Gravholt CH, Fedder J, Naeraa RW, Muller J. Occurrence of gonadoblastoma in females with Turner syndrome and Y chromosome material: a population study. *J Clin Endocrinol Metab.* 2000;85(9):3199–3202.
 275. Kwon A, Hyun SE, Jung MK, Chae HW, Lee WJ, Kim TH, et al. Risk of gonadoblastoma development in patients with Turner syndrome with cryptic Y chromosome material. *Horm Cancer.* 2017;8(3):166–173.
 276. Rosenfeld RG, Hintz RL, Johanson AJ, Brasel JA, Burstein S, Chermak SD, et al. Methionyl human growth hormone and oxandrolone in Turner syndrome: preliminary results of a prospective randomized trial. *J Pediatr.* 1986;109(6):936–943.
 277. Vanderschueren-Lodeweyckx M, Massa G, Maes M, Craen M, van Vliet G, Heinrichs C, et al. Growth-promoting effect of growth

- hormone and low dose ethinyl estradiol in girls with turner's syndrome. *J Clin Endocrinol Metab.* 1990;70(1):122–126.
278. van Pareden YK, de Muinck Keizer-Schrama SM, Stijnen T, Sas TC, Jansen M, Otten BJ, et al. Final height in girls with turner syndrome after long-term growth hormone treatment in three dosages and low dose estrogens. *J Clin Endocrinol Metab.* 2003;88(3):1119–1125.
 279. Li P, Cheng F, Xiu L. Height outcome of the recombinant human growth hormone treatment in Turner syndrome: a meta-analysis. *Endocr Connect.* 2018;7(4):573–583.
 280. Chernausek SD, Attie KM, Cara JF, Rosenfeld RG, Frane J. Growth hormone therapy of Turner syndrome: the impact of age of estrogen replacement on final height. Genentech, Inc., Collaborative Study Group. *J Clin Endocrinol Metab.* 2000;85(7):2439–2445.
 281. Ranke M, Lindberg A, Brosz M, Kaspers S, Loftus J, Wollmann H, et al. Accurate long-term prediction of height during the first four years of growth hormone treatment in prepubertal children with growth hormone deficiency or Turner Syndrome. *Horm Res Paediatr.* 2012;78:8–17.
 282. Sas TC, de Muinck Keizer-Schrama SM, Stijnen T, Aanstoot HJ, Drop SL. Carbohydrate metabolism during long-term growth hormone (GH) treatment and after discontinuation of GH treatment in girls with Turner syndrome participating in a randomized dose-response study. Dutch Advisory Group on Growth Hormone. *J Clin Endocrinol Metab.* 2000;85(2):769–775.
 283. Gravholt CH, Naeraa RW, Brixen K, Kastrup KW, Mosekilde L, Jorgensen JO, et al. Short-term growth hormone treatment in girls with Turner syndrome decreases fat mass and insulin sensitivity: a randomized, double-blind, placebo-controlled, crossover study. *Pediatrics.* 2002;110(5):889–896.
 284. Van Pareden Y, de Muinck Keizer-Schrama S, Stijnen T, Sas T, Drop S. Effect of discontinuation of long-term growth hormone treatment on carbohydrate metabolism and risk factors for cardiovascular disease in girls with Turner syndrome. *J Clin Endocrinol Metab.* 2002;87:5442–5448.
 285. Baldin AD, Fabbri T, Siviero-Miachon AA, Spinola-Castro AM, de Lemos-Marini SH, Baptista MT, et al. Growth hormone effect on body composition in Turner syndrome. *Endocrine.* 2011;40(3):486–491.
 286. Wooten N, Bakalov VK, Hill S, Bondy CA. Reduced abdominal adiposity and improved glucose tolerance in growth hormone-treated girls with Turner syndrome. *J Clin Endocrinol Metab.* 2008;93(6):2109–2114.
 287. Faienza MF, Ventura A, Colucci S, Cavallo L, Grano M, Brunetti G. Bone fragility in turner syndrome: mechanisms and prevention strategies. *Front Endocrinol (Lausanne).* 2016;7:34.
 288. Wasserman H, Bäckeljauw PF, Khoury JC, Kalkwarf HJ, Gordon CM. Bone fragility in Turner syndrome: Fracture prevalence and risk factors determined by a national patient survey. *Clin Endocrinol (Oxf).* 2018;89(1):46–55.
 289. Nour MA, Burt LA, Perry RJ, Stephure DK, Hanley DA, Boyd SK. Impact of growth hormone on adult bone quality in Turner syndrome: a HR-pQCT Study. *Calcif Tissue Int.* 2016;98(1):49–59.
 290. Soucek O, Schonau E, Lebl J, Willnecker J, Hlavka Z, Sumnik Z. A 6-year follow-up of fracture incidence and volumetric bone mineral density development in girls with Turner syndrome. *J Clin Endocrinol Metab.* 2018;103(3):1188–1197.
 291. Cutfield W, Wilton P, Bennmarker H, Albertsson-Wikland K, Chatelain P, Ranke M, et al. Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet.* 2000;355:610–613.
 292. Stochholm K, Kiess W. Long-term safety of growth hormone-A combined registry analysis. *Clin Endocrinol (Oxf).* 2018;88(4):515–528.
 293. Bolar K, Hoffman A, Maneatis T, Lippe B. Long-term safety of recombinant human growth hormone in Turner syndrome. *J Clin Endocrinol Metab.* 2008;93:344–351.
 294. Matura LA, Sachdev V, Bakalov VK, Rosing DR, Bondy CA. Growth hormone treatment and left ventricular dimensions in Turner syndrome. *J Pediatr.* 2007;150(6):587–591.
 295. Duijnhouwer AL, Bons LR, Timmers H, van Kimmenade RRL, Snoeren M, Timmermans J, et al. Aortic dilatation and outcome in women with Turner syndrome. *Heart.* 2018;105(9):693–700.
 296. Urban MD, Lee PA, Dorst JP, Plotnick LP, Migeon CJ. Oxandrolone therapy in patients with Turner syndrome. *J Pediatr.* 1979;94(5):823–827.
 297. Rosenfeld RG, Attie KM, Frane J, Brasel JA, Burstein S, Cara JF, et al. Growth hormone therapy of Turner syndrome: beneficial effect on adult height. *J Pediatr.* 1998;132:319–324.
 298. Sheanon NM, Bäckeljauw PF. Effect of oxandrolone therapy on adult height in Turner syndrome patients treated with growth hormone: a meta-analysis. *Int J Pediatr Endocrinol.* 2015;1:18.
 299. Freriks K, Sas TC, Traas MA, Netea-Maier RT, den Heijer M, Hermus AR, et al. Long-term effects of previous oxandrolone treatment in adult women with Turner syndrome. *Eur J Endocrinol.* 2013;168(1):91–99.
 300. Wilson DM, Frane JW, Sherman B, Johanson AJ, Hintz RL, Rosenfeld RG. Carbohydrate and lipid metabolism in Turner syndrome: effect of therapy with growth hormone, oxandrolone, and a combination of both. *J Pediatr.* 1988;112(2):210–217.
 301. Ross JL, Quigley CA, Cao D, Feuillan P, Kowal K, Chipman JJ, et al. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. *N Engl J Med.* 2011;364(13):1230–1242.
 302. Jez W, Tobiasz-Adamczyk B, Brzyski P, Majkiewicz M, Pankiewicz P, Irzyniec TJ. Social and medical determinants of quality of life and life satisfaction in women with Turner syndrome. *Adv Clin Exp Med.* 2018;27(2):229–236.
 303. Reis CT, de Assumpcao MS, Guerra-Junior G, de Lemos-Marini SHV. Systematic review of quality of life in Turner syndrome. *Qual Life Res.* 2018;27(8):1985–2006.
 304. Gravholt CH, Svenstrup B, Bennett P, Christiansen JS. Reduced androgen levels in adult Turner syndrome: influence of female sex hormones and growth hormone status. *Clin Endocrinol Oxf.* 1999;50:791–800.
 305. Hadnott TN, Gould HN, Gharib AM, Bondy CA. Outcomes of spontaneous and assisted pregnancies in Turner syndrome: the U.S. National Institutes of Health experience. *Fertil Steril.* 2011;95:2251–2256.
 306. Bernard V, Donadille B, Zenaty D, Courtillot C, Salenave S, Brac dIP, et al. Spontaneous fertility and pregnancy outcomes amongst 480 women with Turner syndrome. *Hum Reprod.* 2016;31:782–788.
 307. Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. *J Clin Endocrinol Metab.* 1997;82:1810–1813.
 308. Tanaka T, Igarashi Y, Ozono K, Ohya K, Ogawa M, Osada H, et al. Frequencies of spontaneous breast development and spontaneous menarche in Turner syndrome in Japan. *Clin Pediatr Endocrinol.* 2015;24:167–173.
 309. National Collaborating Centre for Women's and Children's Health (UK). Hypertension in pregnancy: the management of hypertensive disorders during pregnancy. *NICE Clinical Guideline, No. 107. London.*
 310. Gravholt CH, Hjerrild BE, Mosekilde L, Hansen TK, Rasmussen LM, Frystyk J, et al. Body composition is distinctly altered in Turner syndrome: relations to glucose metabolism, circulating adipokines, and endothelial adhesion molecules. *Eur J Endocrinol.* 2006;155:583–592.
 311. Stochholm K, Hjerrild B, Mortensen KH, Juul S, Frydenberg M, Gravholt CH. Socio-economic parameters and mortality in Turner syndrome. *Eur J Endocrinol.* 2012;166:1013–1019.
 312. Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA. Mortality in women with Turner syndrome in Great Britain: a national cohort study. *J Clin Endocrinol Metab.* 2008;93:4735–4742.
 313. Sutton EJ, McInerney-Leo A, Bondy CA, Gollust SE, King D, Biesecker B. Turner syndrome: four challenges across the lifespan. *Am J Med Genet A.* 2005;139A:57–66.
 314. Bryman I, Sylven L, Berntorp K, Innala E, Bergstrom I, Hanson C, et al. Pregnancy rate and outcome in Swedish women with Turner syndrome. *Fertil Steril.* 2011;95:2507–2510.
 315. Birkebaek N, Cruger D, Hansen J, Nielsen J, Bruun-Petersen G. Fertility and pregnancy outcome in Danish women with Turner syndrome. *Clin Genet.* 2002;61:35–39.
 316. Tuke MA, Ruth KS, Wood AR, Beaumont RN, Tyrrell J, Jones SE, et al. Mosaic Turner syndrome shows reduced penetrance in an adult population study. *Genet Med.* 2018;10–0271.

317. Klein KO, Rosenfield RL, Santen RJ, Gawlik AM, Backeljauw PF, et al. Estrogen replacement in turner syndrome: literature review and practical considerations. *J Clin Endocrinol Metab.* 2018;103:1790–1803.
318. Borgstrom B, Hreinsson JG, Rasmussen C, Sheikhi M, Fried G, Keros V, et al. Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab.* 2009;94:74–80.
319. Hovatta O. Ovarian function and in vitro fertilization (IVF) in Turner syndrome. *Pediatr Endocrinol Rev.* 2012;(9 Suppl 2):713–717 713–7.
320. Chevalier N, Letur H, Lelannou D, Ohl J, Cornet D, Chalas-Boissonnas C, et al. Materno-fetal cardiovascular complications in turner syndrome after oocyte donation: insufficient prepregnancy screening and pregnancy follow-up are associated with poor outcome. *J Clin Endocrinol Metab.* 2011;96:E260–E267.
321. Bakalov VK, Cooley MM, Troendle J, Bondy CA. The prevalence of diabetes mellitus in the parents of women with Turner's syndrome. *Clin Endocrinol (Oxf).* 2004;60:272.
322. Gravholt CH, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, Christiansen JS. Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. The impact of sex hormone replacement. *Diabetes Care.* 1998;21:1062–1070.
323. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. *J Clin Epidemiol.* 1998;51:147–158.
324. Stochholm K, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab.* 2006;91:3897–3902.
325. Cameron-Pimblett A, La RC, King TFJ, Davies MC, Conway GS. The Turner Syndrome Life Course Project: Karyotype-phenotype analyses across the lifespan. *Clin Endocrinol (Oxf).* 2017;87:532–538.
326. Landin-Wilhelmsen K, Bryman I, Wilhelmsen L. Cardiac malformations and hypertension, but not metabolic risk factors, are common in Turner syndrome. *J Clin Endocrinol Metab.* 2001;86:4166–4170.
327. Nathwani NC, Unwin R, Brook CG, Hindmarsh PC. Blood pressure and Turner syndrome. *Clin Endocrinol (Oxf).* 2000;52:363–370.
328. Brun S, Cleemann L, Holm K, Salskov G, Erlandsen M, Berglund A, et al. Five-year randomized study demonstrates blood pressure increases in young women with Turner syndrome regardless of estradiol dose. *Hypertension.* 2019;73:1–7.
329. Corrigan EC, Nelson LM, Bakalov VK, et al. Effects of ovarian failure and X-chromosome deletion on body composition and insulin sensitivity in young women. *Menopause.* 2006;13:911–916.
330. Gravholt CH, Klausen IC, Weeke J, Christiansen JS. Lp(a) and lipids in adult Turner's syndrome: impact of treatment with 17beta-estradiol and norethisterone. *Atherosclerosis.* 2000;150:201–208.
331. Roulot D. Liver involvement in Turner syndrome. *Liver Int.* 2013;33:24–30.
332. Elsheikh M, Hodgson HJ, Wass JA, Conway GS. Hormone replacement therapy may improve hepatic function in women with Turner's syndrome. *Clin Endocrinol (Oxf).* 2001;55:227–231.
333. Gravholt CH, Poulsen HE, Ott P, Christiansen JS, Vilstrup H. Quantitative liver functions in Turner syndrome with and without hormone replacement therapy. *Eur J Endocrinol.* 2007;156:679–686.
334. Jorgensen KT, Rostgaard K, Bache I, Biggar RJ, Nielsen NM, Tommerup N, Frisch M. Autoimmune diseases in women with Turner's Syndrome. *Arthritis Rheum.* 2010;62:658–666.
335. El Mansoury M, Bryman I, Berntorp K, Hanson C, Wilhelmsen L, Landin-Wilhelmsen K. Hypothyroidism is common in turner syndrome: results of a five-year follow-up. *J Clin Endocrinol Metab.* 2005;2131–2135.
336. Mohamed SOO, Elkhidir IHE, Abuzied AIH, Nouredin AAMH, Ibrahim GAA, Mahmoud AAA. Prevalence of autoimmune thyroid diseases among the Turner Syndrome patients: meta-analysis of cross sectional studies. *BMC Res Notes.* 2018;11:842–3950.
337. Mortensen KH, Cleemann L, Hjerrild BE, et al. Increased prevalence of autoimmunity in Turner syndrome—influence of age. *Clin Exp Immunol.* 2009;156:205–210.
338. Gravholt CH, Vestergaard P, Hermann AP, Mosekilde L, Brixen K, Christiansen JS. Increased fracture rates in Turner's syndrome: a nationwide questionnaire survey. *Clin Endocrinol (Oxf).* 2003;59:89–96.
339. Hansen S, Brixen K, Gravholt CH. Compromised trabecular microarchitecture and lower finite element estimates of radius and tibia bone strength in adults with turner syndrome: a cross-sectional study using high-resolution-pQCT. *J Bone Miner Res.* 2012;27:1794–1803.
340. Cleemann L, Hjerrild BE, Lauridsen AL, et al. Long-term hormone replacement therapy preserves bone mineral density in Turner syndrome. *Eur J Endocrinol.* 2009;161:251–257.
341. Bois E, Nassar M, Zenaty D, Leger J, Van Den Abbeele T, Teissier N. Otorologic disorders in Turner syndrome. *Eur Ann Otorhinolaryngol Head Neck Dis.* 2018;135:21–24.
342. Cleemann L, Holm K, Kobbarnagel H, et al. Dosage of estradiol, bone and body composition in Turner syndrome: a 5-year randomized controlled clinical trial. *Eur J Endocrinol.* 2017;176:233–242.
343. Cleemann L, Holm K, Fallentin E, Skouby SO, Smedegaard H, Moller N, et al. Uterus and ovaries in girls and young women with Turner syndrome evaluated by ultrasound and magnetic resonance imaging. *Clin Endocrinol (Oxf).* 2011;756–761.
344. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler Jr GB. Effects of estrogen on nonverbal processing speed and motor function in girls with Turner's syndrome. *J Clin Endocrinol Metab.* 1998;83:3198–3204.
345. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler Jr GB. Use of estrogen in young girls with Turner syndrome: effects on memory. *Neurology.* 2000;54:164–170.
346. Carel JC, Elie C, Ecosse E, Tauber M, Leger J, Cabrol S, et al. Self-esteem and social adjustment in young women with Turner syndrome—influence of pubertal management and sexuality: population-based cohort study. *J Clin Endocrinol Metab.* 2006;91:2972–2979.
347. Carel JC, Ecosse E, Bastie-Sigeac I, Cabrol S, Tauber M, Leger J, et al. Quality of life determinants in young women with turner's syndrome after growth hormone treatment: results of the StaTur population-based cohort study. *J Clin Endocrinol Metab.* 2005;90:1992–1997.

18

Puberty and Its Disorders in the Male

Mark R. Palmert, Yee-Ming Chan, Leo Dunkel

CHAPTER OUTLINE

DEVELOPMENT OF THE REPRODUCTIVE

ENDOCRINE SYSTEM 661

Development of the Gonadotropin-Releasing
Hormone Neuronal Network 661

Pituitary Development 662
Testicular Development 663

PHYSIOLOGY OF PUBERTY 663

Hypothalamic KNDy and Gonadotropin-Releasing
Hormone Neurons 663

Pituitary Gonadotropins 665
Gonadal Hormone Production 665

Putting It All Together: the Pubertal Reactivation
of the Gonadotropic Axis 666

Somatic Changes 668

REGULATION OF THE TIMING OF PUBERTY 669

Secular Trends in the Timing of Puberty 669
Genetic Contributions to Variation in Normal
Puberty 669

Other Factors That Modulate the Timing of
Puberty 670

PRECOCIOUS PUBERTY 671

Gonadotropin-Releasing Hormone-Dependent
Forms of Precocious Puberty 672

Gonadotropin-Releasing Hormone-Independent
Forms of Precocity 672
Premature Adrenarche 673

Consequences of Precocious Puberty 673

Evaluation of the Boy With Precocious Development
of Secondary Sexual Characteristics 674

Treatment of the Child With Precocious Puberty 674

DELAYED PUBERTY 675

Etiologies of Delayed Puberty 676

Persistent Hypogonadotropic Hypogonadism 676

Self-Limited Delayed Puberty or Constitutional
Delay of Growth and Puberty 678

Functional Hypogonadotropic
Hypogonadism 679

Hypergonadotropic Hypogonadism 679
Consequences of Delayed Puberty 680

EVALUATION OF THE YOUNG MAN WITH

DELAYED PUBERTY 681

Initial Evaluation 681

History 681

Physical Examination 682

Testing 683

Additional Evaluation 684

TREATMENT OF DELAYED PUBERTY 684

GYNECOMASTIA 686

CONCLUSION 686

References 686

Puberty is the process through which children attain adult secondary sexual characteristics and reproductive capability.¹ In humans, two distinct processes of sexual maturation are recognized: gonadarche and adrenarche. Gonadarche is defined as the growth and maturation of the gonads, resulting in increased sex steroid secretion (most notably testosterone in boys) and spermatogenesis. Gonadarche requires an intact hypothalamic-pituitary-gonadal (HPG) axis, and any disruption of this axis can result in temporary or permanent disorders of reproductive endocrine function. Adrenarche is defined as maturation of the adrenal cortex, leading to increased secretion of adrenal androgens and development of pubic and axillary hair, apocrine body odor, and acne. The physiologic foundations for puberty begin in utero with the development of the neurobiologic structures that comprise the hypothalamic-pituitary component of the HPG axis and with the differentiation and development of the gonads. The entire process, extending from fetal life through achievement of reproductive competence, results from the dynamic and coordinated interactions of an expanding list of genes, proteins, signaling molecules, endocrine and paracrine factors, and epigenetic events.

DEVELOPMENT OF THE REPRODUCTIVE ENDOCRINE SYSTEM

Development of the Gonadotropin-Releasing Hormone Neuronal Network

In postnatal life, the gonadotropin-releasing hormone (GnRH) neurons are located in the hypothalamus. These neurons produce intermittent discharges of GnRH into the hypophysial-portal circulation to stimulate the pituitary gonadotropes to synthesize and secrete the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

The development of the HPG axis is exceptional in that GnRH neurons develop in metazoan embryos outside the central nervous system (CNS). Immature GnRH neuronal precursors become detectable in the olfactory placode and in the anlage of the nose (vomeronasal organ) from an early embryologic stage (around the sixth week of gestation in human and embryonic days E10.5–E11 in mice) and then begin their complex journey toward the hypothalamus. The embryonic migration of GnRH neurons to the hypothalamus is key for the

development of the neuroendocrine network that allows normal pubertal development.²

The pathway along which the GnRH neurons travel includes scaffolds formed by olfactory, vomeronasal, and terminal nerves 3 and 4. Migratory GnRH neurons are known to have receptors for at least 20 signaling molecules. They receive a plethora of guidance and movement-inducing messages during their journey, which appear to be distinct depending on the stage of their migration.² These signals may act directly on GnRH neurons or indirectly on olfactory axons, as disruption of the olfactory tract “scaffolds” themselves can disrupt GnRH migration. The molecular signals involved include adhesion molecules (e.g., neuronal cell adhesion molecule [N-CAM]), extracellular matrix molecules (e.g., heparin sulphotransferases), cytokines (e.g., leukemia inhibitory factor, hepatocyte growth factor), those controlling cell-to-cell interactions (membrane receptors [e.g., neuropilin-2]), and transcription factors (e.g., Ebf2,) as well as both chemoattractants and chemorepellents (e.g., Reelin). Gradients of chemokines (e.g., CXCL12) may be particularly important for promoting and guiding the movement of GnRH neurons. This combination of factors has a high degree of redundancy, such that loss of any one factor may not fully disrupt GnRH neuronal migration.

The neurons begin their migration in the nasal compartment in or around the vomeronasal organ, pass the cribriform plate and penetrate the CNS in close proximity to the olfactory bulbs, and finally migrate apposed to a subset of vomeronasal nerves that diverge caudally into the basal areas of the forebrain. At the end of their journey, GnRH neurons diverge from guiding axons to disperse into their final positions in the septohypothalamic region, including the diagonal of Broca, the medial septum, and the preoptic areas of the hypothalamus. Eventually, these neurons reach the hypothalamus where they extend projections to the median eminence to form a network, which completes this aspect of GnRH neuronal development.

The whole process of migration involves no more than a few hundred neurons per hemisphere in mice and several thousand in primates or humans. The absolute number of GnRH neurons required for pubertal development is not known, but there appears to be a degree of redundancy in the system. Rodent studies suggest that around 12% of the GnRH neuron population is sufficient for pulsatile gonadotropin secretion and puberty onset, whereas between 12% and 34% are required for estrous cycling and ovulation in adult female mice. In addition, adult *Reeler* mice (which were initially identified based on an ataxia phenotype) have significantly fewer GnRH neurons in the hypothalamus and display a phenotype of delayed pubertal maturation and low fertility.

GnRH neurons extend their neurites to the median eminence under the control of factors that remain mostly unknown. Fibroblast growth factor receptor 1 (FGFR1) signaling is known to be important for this process of axon extension, as evidenced by reduced projections to the median eminence in transgenic mice expressing a dominant negative FGFR1 (dnFGFR1) in GnRH neurons.³

The final stage of GnRH neuronal development comprises functional activity. By the 15th week of human gestation, the GnRH pulse generator is modulating the function of the fetal gonadotropes. The entire HPG axis is functionally active for the first time during fetal development, and after a brief perinatal pause, it continues to function in infancy (during the so-called *minipuberty*), until it enters a relative quiescent state, often referred to as the *juvenile pause* or *prepuberty*.

As discussed subsequently, pathological abnormalities of pubertal development have been identified with each aspect of GnRH neuronal development: (1) defects in the synthesis of GnRH, which result mainly from an abnormal migration

of GnRH neurons from the olfactory placode toward the hypothalamus during the first trimester of fetal life; (2) defect in the maturation and function of the GnRH neuronal network; (3) loss of function of GnRH itself or its receptor also known as a *defect of the bioactivity of GnRH*⁴ (Fig. 18.1).

As a prismatic example, *ANOS1*, previously known as *KAL1*, encodes anosmin-1, an extracellular matrix protein that regulates axonal pathfinding and cellular adhesion. Anosmin-1 promotes branching of olfactory bulb neurons. Subjects with *ANOS1* loss-of-function mutations have arrest of both olfactory bulb neurons and GnRH neurons at the level of the cribriform plate, resulting in abnormal sense of smell and hypogonadotropic hypogonadism (HH). Additional genetic factors involved in GnRH neuronal development and function are described in detail in the “Delayed Puberty, Hypogonadotropic Hypogonadism” section later in this chapter.⁵

Pituitary Development

The pituitary consists of the anterior lobe (or adenohypophysis) and the posterior lobe (or neurohypophysis), as well as the pars intermedia and the infundibulum (or pituitary stalk). The anterior lobe derives from epithelial precursors/oral ectoderm and the posterior lobe derives from neural ectoderm, both under the control of a cascade of transcription factors. The spatial and temporal expression of these factors is critical, and mutations in the genes encoding these factors can lead to HH, as well as combined/multiple pituitary hormone deficiencies (see “Delayed Puberty” section later; reviewed in^{6,7}).

Organogenesis of the pituitary gland begins during the fourth week of gestation with an upward extension of the oral ectoderm (to form Rathke’s pouch) toward the neural ectoderm. Simultaneously, the neural ectoderm of the ventral diencephalon extends downward, allowing for the connection of these two elements and leading to the eventual formation of the composite, two-lobe structure of the adult pituitary.⁷

Initiation of organogenesis and formation of Rathke’s pouch involves signaling molecules, such as *Hex1*, *Otx2*, *Pitx1/2/3*, *Sox2*, *FGF8*, and *Lhx3*. Subsequent cell lineage determination depends on molecules, such as *Prop1*, *POU1F1* (*Pit1*), and *Nr5a1* (*SF1*) among others.⁷ Because of the complex pattern transcription factor control, different molecules are key to the development of different cell lineages at different times. For example, *Lhx3* and *Hex1* affect the development of all lineages as does *Pitx1/2/3*; *Prop1* affects differentiation of gonadotrophs, thyrotrophs, somatotrophs, lactotrophs but perhaps plays a less independent role in development of corticotrophs; *POU1F1* regulates differentiation of thyrotrophs, somatotrophs, and lactotrophs; *Nr5a1* largely affects gonadotrophs. As noted, in the absence of functional redundancy, mutations in the genes for these regulatory factors can lead to pituitary hormone deficiencies, with the hormones affected largely predicted by the cell types whose development is dependent on the particular transcription factor. However, some mutations, such as in *HESX1* or *OTX2*, can lead to combined pituitary hormone deficiencies, as expected, but also to isolated growth hormone (GH) deficiency and to septooptic dysplasia.⁶

The pituitary develops early in gestation and, similar to the GnRH neuronal network, becomes functional in utero. The gonadotrophs are the last anterior pituitary cell types to develop,⁶ but gonadotropin release is detectable by week 14 in response to GnRH secretion. Gonadotropin secretion peaks around 20 to 22 weeks in utero and subsequently increases and decreases in accordance with the dynamic hypothalamic GnRH secretion, described earlier, and with positive and negative feedback loops, described later.

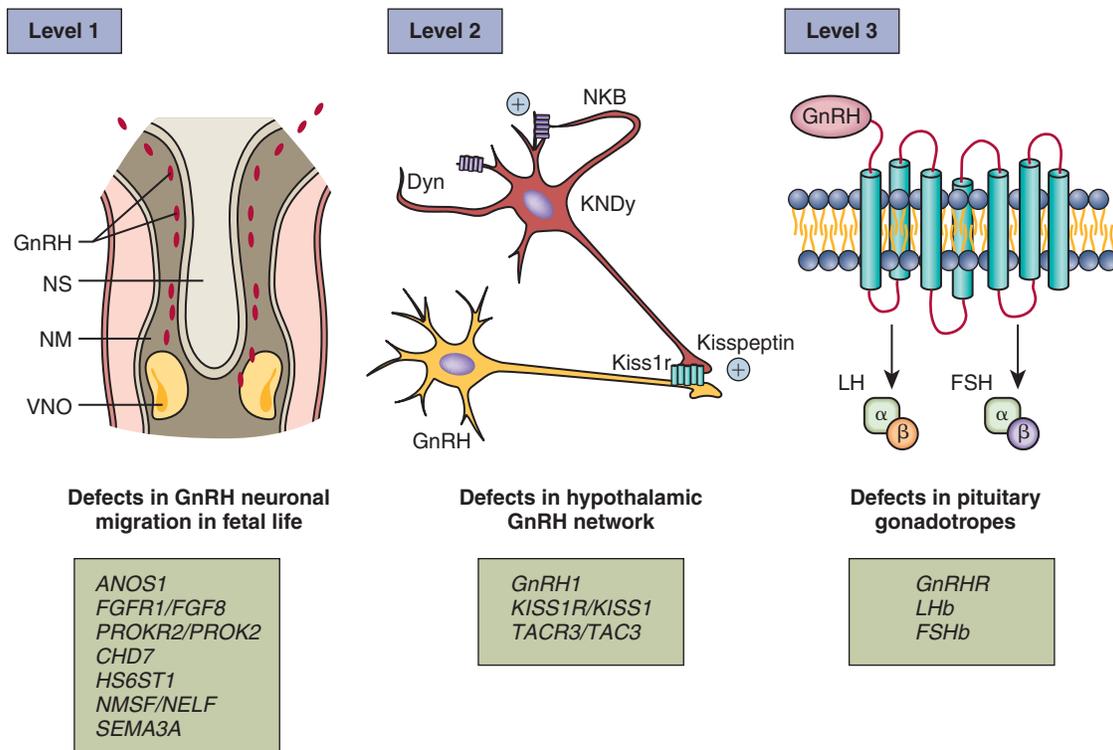


Fig. 18.1 Mutations in single genes at three different levels of the hypothalamic-pituitary-gonadal (HPG) axis can cause hypogonadotropic hypogonadism. Schematic of the three levels along with genes that affect HPG function at each level are shown. See text for details regarding each gene. *ARC*, Arcuate nucleus; *Dyn*, Dynorphin; *ME*, median eminence; *NKB*, neurokinin B; *NM*, nasal mesenchyme; *NS*, nasal septum; *POA*, preoptic area; *VNO*, vomeronasal organ.

Testicular Development

A brief summary of prenatal testicular differentiation and development follows; a more detailed description of testicular differentiation and sex development can be found in [Chapter 6](#). Beginning at approximately 4 to 6 weeks of gestation, the primordial bipotential gonad arises from a condensation of the mesoderm of the urogenital ridge. During this time, the primordial germ cells proliferate and migrate from the hindgut to colonize the developing gonad. In the human male fetus, the testicular compartments, tubular and interstitial components, and specific cell types, Leydig, Sertoli, and germ cells can be visualized by 11 weeks of gestation. During early gestation, placental human chorionic gonadotropin (hCG) governs Leydig cell proliferation and secretion of testosterone and insulin-like factor 3 (INSL3); endogenous LH begins to regulate these activities in midgestation. Because of the role of placental hCG during early gestation, gonadotropin deficiency does not influence male sexual differentiation. However, LH secretion does influence the number of fetal Leydig cells, as demonstrated by a reduced number in anencephalic fetuses and an increased number in 46,XY fetuses who have elevated gonadotropin concentrations secondary to complete androgen insensitivity.⁸ Testosterone production secondary to fetal LH secretion is also critical for phallic growth during the second and third trimesters; males with LH deficiency typically have normally formed but small penises at birth. FSH secretion influences Sertoli cell differentiation and anti-Müllerian hormone (AMH) and inhibin B secretion.⁹

Testicular descent occurs in two phases. The transabdominal phase begins at approximately 12 weeks of gestation and is influenced by the Leydig cell product, INSL3, and its cognate receptor, leucine-rich repeat-containing G-protein-coupled receptor 8 (LGR8). The second androgen-dependent phase,

descent of the testes through the inguinal canal, is usually accomplished by the end of week 35.¹⁰

Following a brief pause in activity after birth, the hypothalamic-pituitary-testicular axis is active during the first few months of life with testosterone concentrations peaking at 1 to 2 months of age.¹¹ By approximately 6 months of age, testosterone concentrations decline to prepubertal levels. During the brief period of increased neonatal HPG activity, sexual hair does not develop and gametogenesis does not occur because of limited androgen receptor (AR) signaling in certain tissues (e.g., Sertoli cells). During infancy and childhood, seminiferous cords are solid and generally filled with immature Sertoli cells. The germ cells are limited to spermatogonia and Leydig cells are rarely visualized.¹² Inhibin B and AMH continue to be secreted during childhood and serve as valuable markers of Sertoli cell function.

PHYSIOLOGY OF PUBERTY

Hypothalamic KNDy and Gonadotropin-Releasing Hormone Neurons

GnRH release is coordinated by inhibitory and excitatory neuronal and glial inputs¹³ (Fig. 18.2). Retrograde tracing studies in mice have demonstrated that GnRH neurons are controlled by a complex neuronal network of inputs from many regions of the brain, including the brain stem, limbic system, hypothalamic nuclei, motor and sensory circuits, and basal ganglia.

Among the various regulators of GnRH neurons, kisspeptins and neurokinin B are the most essential. The distribution of kisspeptin neurons in the hypothalamus varies with species. Kisspeptin neurons have been anatomically mapped in mice to reside within the hypothalamus caudally, in the mediobasal hypothalamus, which includes the arcuate nucleus (ARC)/

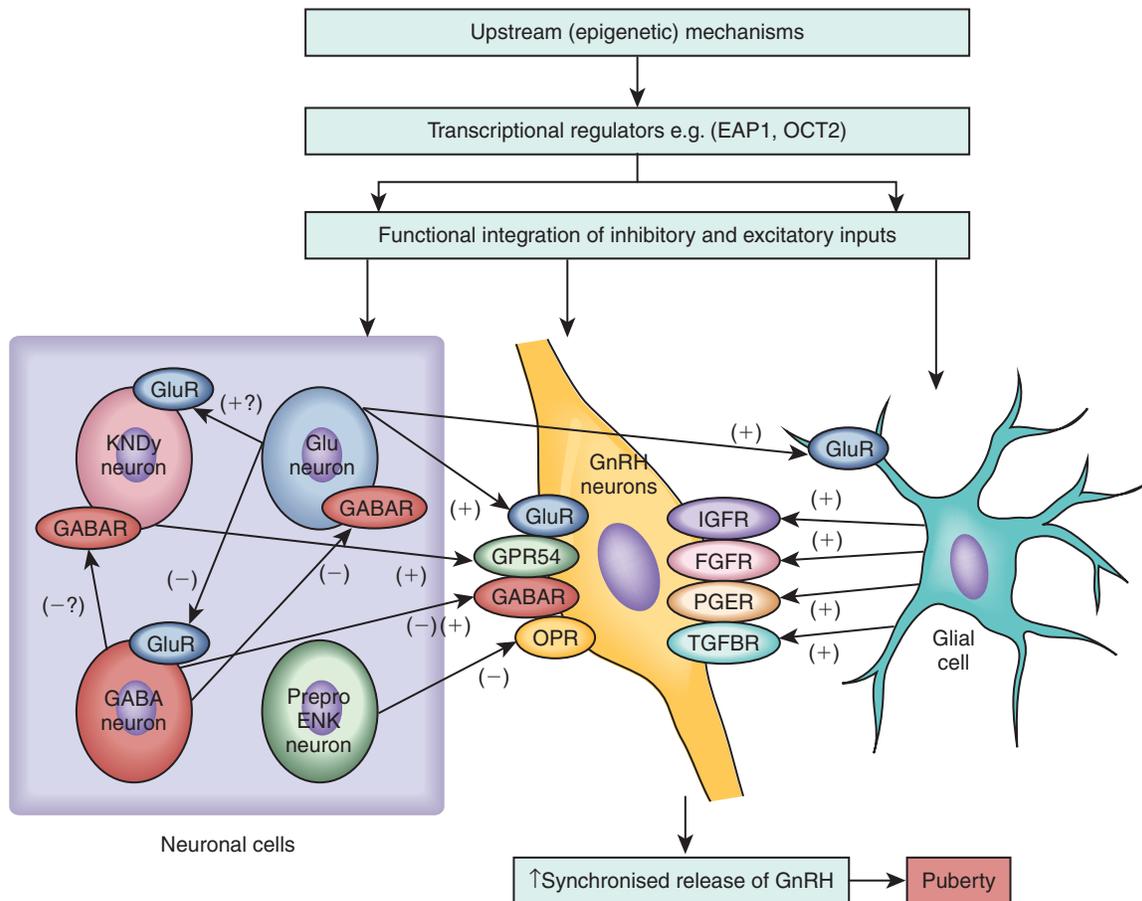


Fig. 18.2 Regulators of gonadotropin-releasing hormone (GnRH) neuronal function. GnRH neurons are regulated by a network including epigenetic and transcriptional factors, as well as transsynaptic and glial modifiers during puberty. (Modified from Ojeda, S.R., Lomniczi, A., Mastronardi, C., Heger, S., Roth, C., Parent, A.S., et al. (2006). Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology*, 147:1166–1174.¹³)

infundibular region. In mice, a second population is located in the rostral part of the hypothalamus called the *anteroventral periventricular* (AVPV) region. In rats, monkeys, and humans, this regional distribution of kisspeptin neurons is less clear. The two populations of kisspeptin neurons in mice exhibit marked functional differences, with, for example, the AVPV kisspeptin neurons having a role in mediating the positive-feedback effects of estrogen, leading to the GnRH/LH surge that triggers ovulation, and the ARC kisspeptin neurons having a role in mediating negative-feedback effects of sex steroids on tonic activity of the HPG axis. AVPV kisspeptin neurons exhibit clear sexual dimorphism, with female rodents at puberty having a much greater number of kisspeptin neurons in this area.

Kisspeptin, an excitatory neuropeptide, was identified as a vital permissive factor in puberty onset by the discovery of patients with GnRH deficiency with loss-of-function mutations in the kisspeptin receptor, *KISS1R*, previously known as *GPR54*.^{14,15} Subsequently, an activating mutation in *KISS1R* was identified in a girl with central precocious puberty,¹⁶ and an activating mutation in *KISS1*, which encodes kisspeptin itself, has been reported in a child with central precocious puberty.¹⁷ Moreover, mice with knockout of *Kiss1r* are infertile, despite normal distribution of GnRH neurons and normal hypothalamic GnRH levels,¹⁵ and mice with *Kiss1* knockout also have a phenotype consistent with normosmic GnRH deficiency.

Kisspeptins are synthesized by hypothalamic neurons that contact GnRH neurons and their projections. Kisspeptin

neurons are located outside the blood-brain barrier and therefore come directly in contact with peripheral sex hormones. Most GnRH neurons express the kisspeptin receptor and kisspeptin neurons express steroid receptors (estrogen receptor alpha, androgen receptor, and progesterone receptor) and are the main relay for the negative and positive feedback of steroid hormones on the gonadotropic axis.

Kisspeptin signals directly to GnRH neurons to control pulsatile GnRH release. Kisspeptin is upregulated in both primates and mice in the peripubertal period, and its administration to pubertal rodents advances the onset of sexual maturation. Interestingly, kisspeptin also appears to be downregulated in functional amenorrhea, suggesting its role as a mediator of environmental factors, such as emotional well-being and nutritional status. In addition, as noted, kisspeptin has been shown to be an important neuroendocrine regulator of ovulation. In mice, kisspeptin neurons located in the AVPV mediate the positive feedback of estradiol on the GnRH neuronal network and therefore the generation of the LH preovulatory pulse. In contrast, estrogens have an inhibitory effect on Kiss1 neurons in the ARC. Thus kisspeptin signaling appears to have both positive and negative feedback loops in the HPG axis. Whilst kisspeptin has been identified as a pivotal upstream regulator of GnRH neurons, whether kisspeptin is the key factor in triggering the onset of puberty remains unclear.¹⁸

Another excitatory neuropeptide, neurokinin B, has been implicated in the upstream control of GnRH secretion. Identification of this pathway was based on discovery of

loss-of-function mutations in *TAC3*, encoding neurokinin B, and its receptor *TACR3*, in patients with pubertal failure caused by normosmic HH.¹⁹ Kisspeptin neurons located in the ARC synthesize neurokinin B and dynorphin A and have been called *KNDy* (Kisspeptin, Neurokinin B, Dynorphin) neurons. Both *TAC3* and *KISS1* expression in the ARC is downregulated by estrogen. Therefore these neurons are considered as the relay of the negative feedback of steroid hormones on the gonadotropic axis. *KNDy* neurons also express the neurokinin B receptor, NK3R, suggesting that paracrine and autocrine loops control GnRH release. Dynorphin inhibits the release of GnRH, and together these peptides are currently believed to play a fundamental role in regulating the pulsatile GnRH release.

More recently, another RF-amide-related peptide (RFRP1, RFRP3), the mammalian orthologue of the avian peptide *gonadotrophin-inhibiting hormone (GnIH)*, was discovered as another inhibitory regulator of the gonadotropic axis by directly controlling GnRH neurons.²⁰ GnIH plays an essential role in the inhibition of the HPG axis in several species. In addition to neuropeptides, several neurotransmitters participate in the control of the GnRH network. Gamma aminobutyric acid (GABA) and glutamate control GnRH neuronal excitability in the ARC. In female rats, glutamine synthase is downregulated and glutamate dehydrogenase becomes more abundant in the hypothalamus at puberty, both leading to increased availability of glutamate, which acts through its n-methyl-D-aspartate (NMDA) and kainate receptors.²¹ The administration of glutamate agonists to prepubertal primates can stimulate the onset of puberty.

The neural network of GABA is quite complex because some of these neurons will have a direct effect on GnRH neurons, and others will act on interneurons. The inhibitory role of GABAergic neurotransmission in restraining the initiation of puberty has been clearly shown in primates but is more ambiguous in rodents. GABAergic signaling pathways are likely to be important in the stress-induced suppression of LH.

Some recent evidence highlights the importance in mice of micro ribonucleic acid (microRNAs) (particularly the miR-200/429 family and miR-155) in the epigenetic upregulation of GnRH transcription during the murine equivalent of the mini-puberty.²² Moreover, miR-7a2, has been demonstrated to be essential for normal pituitary development and HPG function, with deletion in mice leading to HH and infertility.

Corticotropin-releasing hormone (CRH) signaling has long been known to play an important role in the stress-induced suppression of the GnRH pulse generator in rodents. Intracerebroventricular (ICV) administration of CRH decreases LH pulse frequency in rats, whereas stress-induced suppression of LH pulses by insulin-induced hypoglycemia is blocked by ICV administration of a CRH antagonist. Juxtaposition of CRH immunoreactive fibers to GnRH neurons has been observed in the infundibular region of the hypothalamus in the human.

Finally, the synchronous pulsatile secretion of GnRH is also controlled through neuron-glia signaling pathways. Glial inputs appear to be predominantly facilitatory and they consist of growth factors and small diffusible molecules, including transforming growth factor (TGF) β 1, insulin-like growth factor 1 (IGF-1), and neuregulins, that directly or indirectly stimulate GnRH secretion.²³ Glial cells in the median eminence regulate GnRH secretion by production of growth factors, acting via receptors with tyrosine kinase activity. FGF signaling is required for GnRH neurons to reach their final destination in the hypothalamus, as well as for GnRH neuronal differentiation and survival. In addition, GnRH neuron secretory activity is facilitated by IGF-1 and by members of the epidermal growth factor family, such as neuregulin 1 β . Plastic rearrangements of glia-GnRH neuron adhesiveness, mediated by soluble molecules, such as

N-CAM and synaptic cell adhesion molecule (SynCAM), coordinate the controlled delivery of GnRH to the portal vasculature, a process that is also subject to sex steroid regulation.

Pituitary Gonadotropins

GnRH stimulates the production and secretion of LH and FSH from the gonadotrophs by binding to a cell-surface receptor, which triggers increased intracellular calcium concentration and phosphorylation of protein kinase C. LH is released from readily releasable pools, which lead to a rise in serum LH within minutes after a bolus of GnRH, but also from pools which take longer to mobilize. Whereas episodic stimulation by GnRH increases gonadotropin secretion, continuous infusion of GnRH decreases LH and FSH secretion and downregulates the pituitary receptors for GnRH—a phenomenon that is used in the medical treatment of central precocious puberty. Alterations in the GnRH receptor have other important roles in regulating gonadotroph function as estrogens increase and androgens decrease GnRH receptors.

FSH and LH are glycoproteins composed of two subunits, an α subunit that is common for all the glycoprotein hormones and distinct β subunits that confer receptor specificity. The β subunits are 115 amino acids long with two carbohydrate side chains. hCG produced by the placenta is almost identical in structure to LH except for an additional 32 amino acids and additional carbohydrate groups. Rare cases of mutations in the β subunit of gonadotropin molecules that cause pathological effects have been reported: a single case of an inactivating mutation of *LHB* caused absence of Leydig cells and lack of puberty in a male²⁴ and two cases of inactivating mutations of *FSHB* led to lack of follicular maturation and amenorrhea and, in two males, azoospermia.²⁵ In addition, a woman with a homozygous mutation in a 5' splice-donor site in the noncoding region of *LHB* displayed impaired LH secretion, normal pubertal development, secondary amenorrhea, and infertility.²⁶ What is conceptually important from these observations is that normal pubertal maturation in women, including breast development and menarche, can occur in a state of LH deficiency, whereas normal LH secretion appears obligatory for ovulation. This implies that although LH is essential for the normal maturation of Leydig cells and steroidogenesis in men, its primary role in women is to induce ovulation.

The same gonadotroph cell produces both LH and FSH in the pituitary. Gonadotroph cells that are not stimulated, for example, because of disease affecting GnRH secretion, are small in diameter, while the gonadotroph cells in primary hypogonadism, stimulated by large amounts of GnRH, are large in diameter and demonstrate prominent rough endoplasmic reticulum.

Gonadal Hormone Production

Sex Steroids

The LH stimulation of Leydig cells leads to increased testicular testosterone secretion, and typical Leydig cells become apparent on histology. Testosterone acts as a paracrine factor to induce Sertoli cell maturation. Pubertal maturation of the seminiferous tubules is characterized by cytoskeletal rearrangements, including development of tight junctions, Sertoli cell polarization, Sertoli cell proliferation, migration of spermatogonia toward the basement membrane, and decreased AMH secretion.²⁷

The Leydig cells of the testes synthesize testosterone through a series of enzymatic conversions for which cholesterol is the precursor. When LH binds to Leydig cell membrane receptors, the ligand-receptor complex stimulates membrane-bound

adenyl cyclase to increase cyclic adenosine monophosphate (cAMP), which then stimulates protein kinase A, which in turn causes the stimulation of the conversion of cholesterol to pregnenolone by P450_{sc} (side-chain cleavage enzyme), the first step in the production of testosterone. With substantial exposure to LH, the number of receptors for LH and the postreceptor pathway decrease their responsiveness to LH for at least 24 h. This explains the clinical finding of insensitivity to LH after daily injections of LH or hCG compared with every-other-day injections. When assessing the response of testes to LH, hCG or LH must be administered at 2- to 3-day intervals to avoid such downregulation.

When testosterone is secreted into the circulation, the majority is bound to sex-hormone-binding globulin. The remaining free testosterone (95% of which is bound to albumin with low affinity) is conventionally considered the active moiety. At the target cell, testosterone dissociates from the binding protein, diffuses into the cell, and may be converted by 5 α -reductase type 2 (a surface enzyme located on the genital skin and elsewhere and encoded by a gene on chromosome 2) to dihydrotestosterone or by aromatase (CYP19) to estrogen. Testosterone or dihydrotestosterone binds to the androgen receptor that is encoded by a gene on the X chromosome (Xq11-q12). The testosterone/dihydrotestosterone-receptor complex then attaches to the steroid-responsive region of genomic deoxyribonucleic acid (DNA) to initiate androgen-dependent transcription and translation, ultimately leading to virilization. However, androgens must be converted to estrogens to stimulate bone maturation at the epiphyseal plate.

FSH binds to specific receptors on the cell surface of Sertoli cells and causes a sequence of events that culminates in increased protein kinase A activity in a manner similar to the stimulatory effect of LH on Leydig cells. However, FSH causes an increase in the mass of seminiferous tubules, and in an undefined way supports the development of sperm. The increase in Sertoli cell number contributes to the increase in testicular volume that provides a physical marker of the onset of gonadarche in boys. As puberty progresses, the seminiferous tubules enlarge and develop a lumen. Although much variation in chronologic age and testicular volume has been described, spermathe (initial sperm production) precedes peak pubertal linear growth velocity and occurs at a median testicular volume of 10 to 12 mL, although sometimes as low as 4 mL.²⁸

Activin and Inhibin B

FSH stimulates Sertoli cells to secrete inhibin B, which from midpuberty onward serves as the major negative regulator of pituitary FSH secretion. Before puberty, inhibin B is germ cell independent. After puberty, inhibin B secretion becomes germ cell dependent and provides a marker of germ-cell integrity in adults.²⁹

Inhibin B is a heterodimeric glycoprotein member of the TGF- β family produced in males exclusively by the testis, primarily in the prepubertal testis by the Sertoli cells, and by the ovarian granulosa cells and the placenta in the female. Several studies show that serum inhibin-B levels in children change in concert with the secretion of gonadotropins. During the "minipuberty" serum inhibin-B levels increase to similar or higher levels to those observed in adolescent boys and adult men. This early inhibin-B secretion is sustained until the age of 18 to 24 months; thereafter, serum concentrations decline to lower but readily measurable levels. Early in puberty, between Tanner stages G1 and G2, serum inhibin-B concentrations again increase and reach peak levels at Tanner stage G2, but then the levels plateau. Inhibin suppresses FSH secretion from the pituitary gland and provides another explanation

for different serum concentrations of LH and FSH with only one hypothalamic peptide (GnRH) stimulating them. Activins are homodimers of the β subunit of inhibin and have the opposite effect, stimulating the secretion of FSH from the pituitary gland. Absence of inhibin B because of gonadal failure causes a greater rise in serum FSH than LH in pubertal and adult subjects.

Anti-Mullerian Hormone. AMH belongs to the same TGF- β family as inhibin and is produced from the Sertoli cells of the testes from the time of testicular differentiation to puberty, and in females by the granulosa cells from birth until menopause. In normal males, AMH is high in the fetus and newborn with peak levels around 2 months of age and then decreases by the age of 1 year. Patients with dysgenetic testes have decreased serum AMH, whereas values are elevated in Sertoli cell tumors and females with granulosa-cell tumors. Undetectable AMH and inhibin B are characteristic of congenital anorchia but may also be seen in severe hypogonadotropic hypogonadism in males. In infant girls, a similar pattern in AMH levels during the first months of life has been reported, but the levels in girls are significantly lower. AMH decreases during puberty as a sign of androgen action. Beginning in early puberty, testosterone represses AMH secretion by Sertoli cells, and testosterone and AMH begin to show an inverse relationship. Intratesticular testosterone and signaling through androgen receptors in Sertoli cells are essential for the decline in AMH concentrations, meiosis, and spermatogenesis.¹² Hence, AMH concentrations provide an indication of Sertoli cell function and androgen action in the testes.

Putting It All Together: the Pubertal Reactivation of the Gonadotropic Axis

Hormonal Changes

The gonadotropic axis undergoes complex cycles of activation and inhibition from fetal life to puberty (Fig. 18.3).

Fetal testosterone secretion early in pregnancy is caused by placental hCG stimulation. The fetal hypothalamus contains GnRH-containing neurons by 14 weeks of gestation, and the fetal pituitary gland contains LH and FSH by 20 weeks. The hypothalamopituitary portal system develops by 20 weeks of gestation, allowing hypothalamic GnRH to reach the pituitary gonadotrophs. At midgestation, there is a striking rise of circulating gonadotropin levels in both male and female fetuses, which reaches its peak at 34 to 38 weeks in the male fetus, and then falls to low levels at birth. This change in gonadotropin secretion results from the development of a negative feedback system through sex steroids, as well as from the development of inhibiting influences from the CNS on GnRH neurons.

LH and FSH secretion rise during the first month after birth, probably because the negative feedback effect of placental estrogens is withdrawn. LH is secreted in pulses during this postnatal period, often termed the *minipuberty*. After this postnatal activity, the HPG axis becomes relatively dormant in children between the age of 2 and 8 to 9 years.

The transition from the relative prepubertal quiescence to the adolescent pattern of GnRH secretion is a gradual rather than abrupt process. LH and FSH pulsatility has been detected in normal children as young as 4 years of age.³⁰⁻³³ Throughout childhood, GnRH secretion appears to undergo small but progressive increases until the onset of puberty, when GnRH secretion increases, first at night and eventually throughout the day.^{34,35} Because of the episodic nature of gonadotrophin secretion, a single gonadotrophin determination is not informative regarding the secretory dynamics of these hormones. However, modern

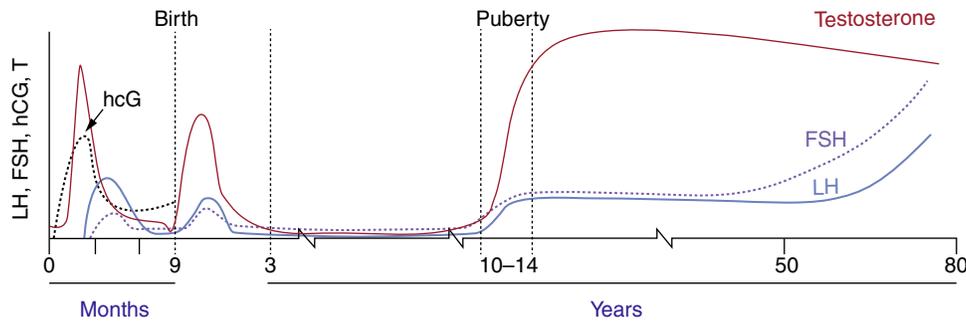


Fig. 18.3 Circulating concentrations of gonadotropins (hCG, FSH, LH) and testosterone during the life span of a male. The function of the reproductive endocrine axis is dynamic throughout life. Here levels are shown for the in utero, neonatal, prepubertal, pubertal, and adult periods. The “minipuberty” shown in the figure between birth and 3 years occurs at approximately 6 to 10 weeks of life. *hCG*, Human chorionic gonadotropin; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone. (Modified from Huhtaniemi, I., Howard, S., Dunkel, L., Anderson, R.A. (2017). The gonadal axis: a life perspective. In: Pfaff, D.W., Joels, M., editors. *Hormones, Brain, and Behavior*. 3rd ed: Oxford, Academic Press.³¹¹)

third-generation assays are sufficiently sensitive to indicate the onset of puberty in single basal unstimulated samples.

The first biological change demonstrating that the HPG axis is being reactivated at puberty is the augmentation of nocturnal LH pulses in children; this reactivation is subclinical and begins before clinical development of Tanner genital stage 2. This period may thus be seen as the hormonal onset of puberty. The differences between daytime and nighttime levels of LH remain until late stages of puberty but disappear by early adulthood. During this reactivation of the axis, there is a gradual development of a dynamic interplay between the central production of GnRH and gonadotropins and gonadal sex-steroid production. A progressive maturation of negative-feedback loops occurs but so too the prepubertal suppressant drive from the CNS gradually abates, and transient intensifying positive feedback results from increasing gonadal sex-steroid production.

During the period of relative quiescence, testosterone and estrogen are also measurable in the circulation using sensitive assays, demonstrating low but definite activity of the prepubertal gonads. However, gonadal contribution to the inhibition of the hypothalamic-pituitary system occurs later, becoming operative only at midpuberty, and eventually becomes dominant over the central inhibitory feedback drive (Fig. 18.4). Both mean LH and FSH levels increase through pubertal development, although LH rises to a greater extent, probably because of differences in feedback mechanisms for these two hormones. These rises are the result of both an increase in basal levels of LH and FSH, and a greater number and amplitude of LH peaks.

In boys during puberty, plasma testosterone levels increase dramatically. The pubertal increase in testis size results primarily from an increased number of proliferating and differentiating germ cells and, to a lesser extent, an increase in Sertoli cells. In early and midpuberty, there is a pronounced diurnal rhythm with a morning peak in measurable testosterone, but this is less pronounced in later puberty and declines gradually with age, probably because of decreased day-night ratios of gonadotropins.

The biological reactivation of the gonadotropic axis occurs earlier in girls than boys, and the dynamic of the reactivation of the gonadotropic axis is not identical in the two sexes. The secretion of testosterone increases shortly after the increase in the plasma concentration of LH and FSH. In girls, estradiol increases together with increasing LH and FSH. Then, a hormonal dialogue between gonads, the hypothalamus, and pituitary contributes to the progressive activation of the gonadotropic axis, until the occurrence of the LH ovulatory pulse in females. The basis for this sexual dimorphism is unclear and may be related to differences in gonadal hormone production and could also be a feature of the sexual dimorphism of the brain.

There are also some data suggesting that although most genes regulate the timing of puberty similarly in boys and girls, there is a small subset that may exert sex-specific effects.³⁶

Physiological Mechanisms. Our understanding of the activation of the gonadotropic axis at the end of the prepuberty remains incomplete but has advanced in some areas (see discussion later). GnRH neuronal activity is under the control of several neurotransmitters and neuropeptides, as described earlier, and the onset of puberty is triggered by a decline in these inhibitory signals and amplification of the excitatory inputs, leading to increased frequency and amplitude of GnRH pulses. The neuroendocrine mechanisms that control the activation of the gonadotropic axis in the fetus, as well as during the “minipuberty” are less well understood. Likewise, the exact nature of the brake that functions during prepuberty remains unknown.

One change at puberty is a shift in the balance of GABA-glutamate signaling in the brain. Another is an increase in dendritic spine density and a simplification of the dendritic architecture of GnRH neurons. A third is an increase in kisspeptin signaling in the hypothalamus, which is caused by an increase of kisspeptin synthesis, as well as an increased responsiveness of GnRH neurons to kisspeptin stimulation. Although mainly described in mice, this paradigm, which is well conserved in evolution, is probably true in monkeys as well in humans.³⁷

The mechanisms responsible for the increased biosynthesis of kisspeptins in the hypothalamus at the end of the juvenile period remain unknown. Data pointing to hypothalamic regulation via a hierarchical network of genes (see Fig. 18.2) have mainly come from a systems biology approach³⁸ and animal models,³⁹ with little data from human subjects to date; however, integration of findings from human genome-wide association studies will likely enrich these models. Candidate transcriptional regulators that have been identified via these approaches include octamer transcription factor 2 (*Oct-2*), thyroid transcription factor-1 (*TTF-1*), and enhanced at puberty 1 (*EAP1*). *TTF-1* is a homeobox gene that enhances GnRH expression. *TTF-1* expression is increased in pubertal rhesus monkeys. *Oct-2* is a transcriptional regulator of the POU-domain family of homeobox-containing genes. *Oct-2* messenger RNA (mRNA) is upregulated in the hypothalamus in juvenile rodents; blockage of *Oct-2* synthesis delays age at first ovulation; and hypothalamic lesions, which induce precocious puberty (e.g., hypothalamic hamartomas), activate *Oct-2* expression. *EAP1* mRNA levels also increase in the hypothalamus of primates and rodents during puberty, *EAP1* transactivates the *GnRH* promoter, and *EAP1* knockdown with small interfering RNA (siRNA) causes delayed puberty (DP) and disrupts estrous cyclicity in rodents.

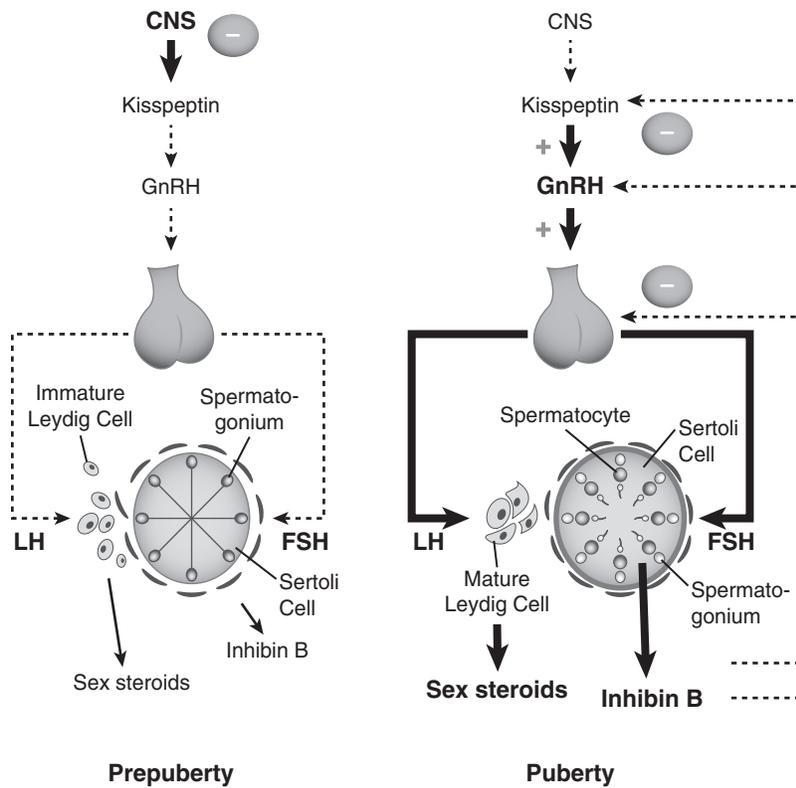


Fig. 18.4 Overview of the hypothalamic-pituitary-gonadal (HPG) axis prepuberty versus postpuberty. Although testicular products, such as inhibins, play a small role in regulation of the HPG axis before puberty, most of the dampening of the HPG axis after infancy and until the onset of puberty (*left panel*) derives from central inhibition. The basis for that inhibition is not fully understood nor is the basis for its diminution, which leads to emergence of central activation (*right panel*), increased gonadotropin-releasing hormone (GnRH) secretion, and the onset of puberty. As pubertal development progresses, inhibin B secreted from Sertoli cells of the mature testes (*right panel*) has a more substantial role in regulating/inhibiting hypothalamic-pituitary activity. The weight of lines (from dotted to thin solid to thick solid) indicates increasing strength of the particular signal. LH, luteinizing hormone; FSH, follicle-stimulating hormone.

Recent data have highlighted the importance of a genetic program that controls the expression of *Kiss1*. The intervention of the polycomb complex proteins, EED and Cbx7, in the transcriptional repression of *Kiss1* has recently been revealed.⁴⁰ The expression of these genes in the prepubertal period progressively decreases with increasing methylation of their promoters. Thus the binding of EED on the *Kiss1* promoter decreases at puberty. The inhibition of the repression of *Kiss1* is also correlated with a decrease in the expression of transcription factors with zinc-finger motifs. In addition, a microRNA switch was proposed to regulate the rise of *GnRH1* synthesis, which occurs during the juvenile period in GnRH neurons. The initiation of puberty from the hypothalamus therefore results from a complex network of transcription factors mainly acting as repressors of *Kiss1* and *GnRH1* transcription.

The concept that puberty results from the disappearance of gonadotropic axis repression is also supported by the description of loss-of-function mutations of *MKRN3* and *DLK1* in familial central precocious puberty (CPP, discussed in detail in "Precocious Puberty" later).⁴¹ Where these factors function in the hierarchical network of genes controlling kisspeptin has yet to be determined.

Somatic Changes

In boys, the first physical finding that marks the onset of puberty is the change from Tanner genital stage G1 to stage G2, including enlargement of the testes (i.e., achievement of volume ≥ 4 mL or testicular length ≥ 25 mm). Originally

Marshall and Tanner reported the mean (standard deviation [SD]) onset of puberty in boys to be 11.64 (1.07) years.^{42,43} These pubertal stages (Fig. 18.5) were based on longitudinal photographic observations of genital development of a relatively small sample of 228 boys living in a children's home. Despite the probably poor representative nature of this sample, studies in Switzerland,⁴⁴ the United States,⁴⁵ and Denmark⁴⁶ have reported roughly similar mean ages of puberty onset.

Although the mean age of onset may be fairly uniform across populations, the range of ages for onset of puberty in normal, healthy adolescents varies widely. Several pathologic states may further influence the timing of puberty either directly or indirectly and may contribute to this splay, but the great majority of the variation in pubertal timing cannot be attributed to clinical disorders. Some 95% of boys experience the onset of genital development between 9.5 and 13.5 years,^{47,48} and these data have led to the traditional definition of sexual precocity in boys as development of secondary sexual characteristics before age 9 years and DP as lack of testicular enlargement by age 14 years.

Development of secondary sexual characteristics results from both gonadarche and adrenarche. Adrenarche refers to the maturation of the zona reticularis of the adrenal gland, resulting in increased production of the adrenal androgens dehydroepiandrosterone (DHEA) and androstenedione, as well as the relatively inactive metabolite DHEA sulfate (DHEA-S). These adrenal androgens, along with testicular androgens, contribute to secondary sexual characteristics, such

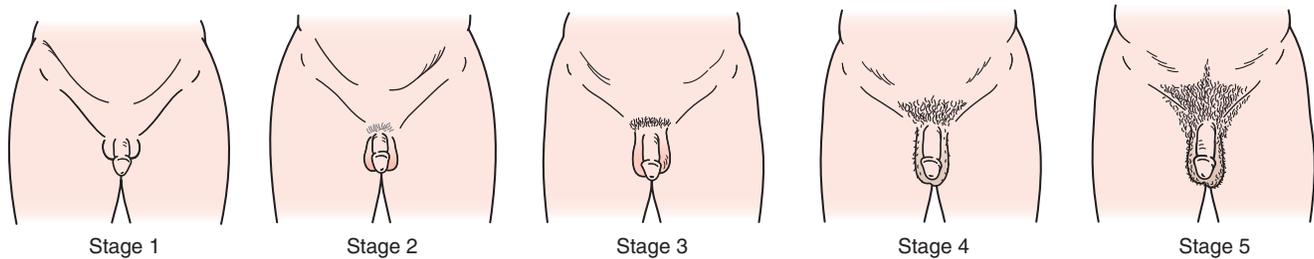


Fig. 18.5 Tanner stages in the male. In boys, genital development is rated from 1 (preadolescent) to 5 (adult); stage 2 marks the onset of pubertal development and is characterized by an enlargement of the scrotum and testis and by a change in the texture and a reddening of the scrotal skin. Pubic hair stages are rated from 1 (preadolescent, no pubic hair) to 5 (adult), and stage 2 marks the onset of pubic hair development. Although pubic hair and genital development are represented as synchronous in the illustration, they do not necessarily track together and should be scored separately. (Modified from Halpern, B., Blackburn, T., Incremona, B., et al. (1996). Preparticipation sports physicals. In: Zachazewski, J.E., Magee, D.J., Quillen, W.S., editors: *Athletic Injuries and Rehabilitation*, Philadelphia, WB Saunders, p. 855.)

as pubic hair (pubarche), axillary hair, apocrine body odor, and acne. Like gonadarche, the onset of adrenarche appears to be a gradual, progressive maturational process that begins in early childhood and is marked by the further increases of production of adrenal androgens around the time of puberty.⁴⁹ Although adrenarche and gonadarche typically overlap, they are separate processes that are independently regulated.^{50,51} The triggers for adrenarche remain unknown; however, alterations in body weight and body mass index (BMI), as well as in utero and neonatal physiology, likely modulate this developmental process, perhaps along with intraadrenal cortisol production.⁵²⁻⁵⁴

REGULATION OF THE TIMING OF PUBERTY

Secular Trends in the Timing of Puberty

The mean age of menarche in mid-nineteenth century Europe is reported to be between 17 and 18 years. Starting from the late nineteenth century to the mid-twentieth century, a gradual decline in age at puberty has been reported, after which this trend appears to have slowed. Much of this change in the timing in puberty has likely been the result of better hygiene and nutrition related to increased socioeconomic stability.

More recent declines in the age at puberty are more convincingly demonstrated among girls than in boys.^{55,56} In the mid-1990s, data from the Third National Health and Nutrition Examination Survey (NHANES III), where genital ratings were performed by visual inspection, reported earlier age at puberty in both boys and girls,⁵⁷⁻⁶⁰ than what previously had been reported from the United States.^{47,59,61} Using the traditional cutoff of 9 years, these data suggest that an increased number of boys would be classified as having precocious puberty. However, because of lack of data on pubertal onset in the previous population-based study (Third National Health Examination Survey [NHES III]),⁴⁷ some controversy remained as how to interpret the NHANES III findings.⁶²

Furthermore, questions have been raised regarding the criteria used for genital staging in NHANES III.⁶³ A subsequent secular trend analysis between NHES III (which lacked data from the early pubertal stages) and NHANES III did not find clear evidence supporting earlier age at puberty, although some indications were present in non-Hispanic white boys.⁵⁹ These data were also reviewed by an expert panel, which concluded that the available data are insufficient in quality and quantity to confirm a change in pubertal timing in US boys.⁵⁶ At the same time in Europe, in comparison with NHANES III studies, some data even reported suggesting older ages at pubertal onset in boys.^{44,64-66}

Secular trends in the timing have been assessed in a few European studies within specific populations. Earlier studies

do not support a substantial enough change in the age at pubertal onset in boys from the mid-1960s to the late 1990s to warrant a change in the age definitions for precocious and DP.^{46,65} However, the most recent data from Copenhagen, comparing the timing of puberty in boys assessed between 2006 and 2008 compared with a group assessed between 1991 and 1993, does report earlier mean age of onset of puberty (11.62 vs. 11.92 years).⁶⁶

In summary, data are conflicting but there is likely an overall suggestion that puberty may be occurring earlier in boys than in the past. However, the change is not as dramatic as in girls, and age cutoffs used to define disorders of puberty have not been uniformly changed.

Genetic Contributions to Variation in Normal Puberty

Although the precise mechanisms that trigger puberty remain unknown, it is well established that the timing of puberty is influenced by both genetic and environmental factors.^{55,67} Evidence for the genetic contribution is provided by correlations in timing within families and between monozygotic twins.^{55,62,68-75} It is important to note that this genetic component does not preclude a significant role for environmental influences; indeed, genetics alone cannot explain secular trends in the timing of puberty. However, the strong genetic component provides an opportunity to identify factors that modulate the timing of normal puberty in the general population.⁷⁶ Approaches used to identify specific genetic factors include candidate-gene studies and genome-wide association (GWA) studies.

Candidate Gene-Based Studies

One approach for identifying the variants that affect complex traits, such as the timing of puberty in the general population, has been candidate gene-based association studies, which seek to demonstrate a higher prevalence of genetic variants in one or more prespecified genes in cases than in controls.

In an association study that tested for associations between common variants in 10 HH-related genes (*GNRH1*, *GNRHR*, *KISS1R/GPR54*, *KISS1*, *LEP*, *LEPR*, *FGFR1*, *KAL1*, *PROK2*, and *PROKR2*) and age at menarche, only nominally significant associations between single nucleotide polymorphisms (SNPs) in several of the genes and age at menarche were identified.⁷⁷

Other work similarly did not identify associations between SNPs in *GNRH1* and *GNRHR* or *LEP* and *LEPR* and alterations in pubertal timing. However, large-scale GWA studies (see later) have demonstrated that variants in some of these genes do in fact influence pubertal timing and have revealed relatively

small effect sizes of these variants, such that earlier candidate-gene studies likely had insufficient power to detect these small effect sizes.

Genome-Wide Association Studies

In large-scale GWA studies, the age of menarche has been the most commonly used marker of the timing of puberty. The first GWA studies on age of menarche involved between 17,000 and 25,000 women, all of European descent. Common variants in *LIN28B* were associated with age at menarche in four independent GWA studies and one metaanalysis.^{78–82} *LIN28B* is a human homologue of *lin-28*, which in *Caenorhabditis elegans* controls the rate of progression from larval stages to adult cuticle formation, indicating conservation of specific micro-RNA regulatory mechanisms involved in developmental timing.⁷⁹ In each case, age at menarche (AAM) was analyzed, but in one study,⁸² additional phenotypes (breast development in girls, voice breaking and pubic hair development in boys, and tempo of height growth in both boys and girls) were found to associate with variants in *LIN28B*, suggesting that *LIN28B* affects timing of puberty more generally, not just timing of menarche, and that control of pubertal timing in boys and girls shares some common elements, although subsequent studies in mice suggest that this pathway may also exert sex-specific effects.⁸³ Effect sizes in the GWA studies were estimated at approximately 1.2 months earlier menarche per effect allele for *LIN28B*.^{80,82} A second menarche locus was identified in two of the four studies at 9q31.2.^{78,80} The biology behind the locus at 9q31.2 remains unknown, but its effect size is similar to the locus in/near *LIN28B*.⁸⁰ The associated SNPs lie in an intergenic region with no obvious candidate genes nearby. The closest gene is *TMEM38B*, a transmembrane protein gene, which lies approximately 400 kb away from the signal at 9q31.2.⁸⁰ Although these studies were groundbreaking, the *LIN28B* and 9q31.2 loci together explain only 0.6% of the variance in age at menarche.⁷⁸

Subsequent GWA studies have used larger and larger sample sizes, resulting in increases in statistical power and identification of many more genetic loci that influence pubertal timing.^{79,84}

The most recent GWA study on age at menarche studied over 350,000 women of European descent and identified 389 independent, genome-wide significant loci that influence pubertal timing.⁸⁵ Collectively, these loci are estimated to account for 7.4% of the overall variation in timing of menarche. There was significant enrichment of expression of genes associated with these loci in the brain, consistent with the hypothesis that pubertal timing is mainly regulated by hypothalamic factors.

A GWA study has also been performed on age at voice-breaking in over 55,000 men and identified 11 genome-significant loci.⁸⁶ Ten of these 11 loci have also been shown to be significantly associated with age at menarche, and the eleventh (*ANOS1* on the X chromosome, which is also associated with X-linked Kallmann syndrome, discussed further later) nearly reached genome-wide significance. Furthermore, analyzing all common variants across the genome, nearly all loci had similar effects on both age at voice breaking and age at menarche. Thus the mechanisms regulating pubertal timing in girls and boys appear to be largely, although not completely,³⁶ overlapping.

GWA studies are designed to assess the contribution of common genetic variants to a particular phenotype. However, it is likely that other forms of inheritance also underlie constitutional delay of growth and puberty (CDGP), including rarer variants (frequency <5% in the general population) with large or even small phenotypic effects; interactions (epistasis) between variants within a single gene or in multiple genes (oligogenicity); structural variation, such as copy number variants;

and epigenetics. Indeed, some of these mechanisms have been identified as causes of hypothalamic amenorrhea.⁸⁷

Other Factors that Modulate the Timing of Puberty

Although these genetic advances are exciting, genetic factors alone cannot explain the reported secular changes in pubertal timing that have occurred since the late twentieth century. Clearly, changes in environmental factors must be involved as well, whether as independent regulators or through gene-environment (G X E) interactions. Variables, such as increased adiposity, insulin resistance, physical inactivity, psychological factors, changed dietary habits, and endocrine-disrupting chemicals have all been implicated as environmental factors that influence pubertal timing.^{55,56,88}

Effect of Body Mass Index on Pubertal Timing

Among white American children aged 6 to 11 years, rates of obesity increased from approximately 5% between 1963 and 1965 to 12% in 1999 to 2000.⁸⁹ The possibility that the increasing rates of obesity contributed to the secular trend toward early puberty onset was originally highlighted in 1997.⁹⁰ However, despite strong evidence in girls in support of a link between increased adiposity and early onset of pubertal markers,⁹¹ data in boys have remained somewhat ambiguous, with increased BMI being associated with earlier puberty in most studies, but with some studies showing obesity being associated with no change in pubertal timing or even with DP.^{88,92–96}

For example, in a study of 463 Danish choir boys, a significant downward trend in age at voice break was found over a 10-year period (from 14 years to 13.7 years).^{89,96} Age at voice break was significantly different between boys in the different prepubertal BMI quartiles, and a trend toward earlier voice break was associated with increasing BMI SDS. Boys in the heaviest quartile at 8 years of age had an increased likelihood of earlier voice break compared with those in the thinnest quartile, suggesting a relationship between prepubertal BMI and the timing of puberty in boys.^{96,97}

In the Copenhagen study discussed earlier, secular trends in pubertal onset over a 15-year period were assessed as were their relationship to BMI in boys.^{66,98} In a total of 1528 boys, onset of puberty, defined as age at attainment of a testicular volume greater than 3 mL, occurred 3 months earlier in 2006 to 2008 than in 1991 to 1993. BMI SD scores increased significantly from 1991 to 1993 to 2006 to 2008 as well. Interestingly, age of pubertal onset was no longer significantly different between study periods after adjustment for BMI indicating that the observed decline in age at onset of puberty was associated at least partly with an increase in BMI.⁶⁶

In a study from Jamaica, the effect body composition on the timing of onset of puberty were assessed in both boys and girls.^{90,99} Elevated fat mass at 8 years of age was associated with advanced puberty in both sexes. These data support the hypothesis that fast growth throughout childhood, especially fat-mass accretion, is associated with advanced pubertal development.^{99,100}

But not all studies agree. For example, in one study from Germany, body weight, height, peak height velocity, and pubertal stages were evaluated in 1421 peripubertal children.¹⁰¹ In contrast to the findings from studies earlier, the researchers found no significant differences in mean pubic hair stage in girls and boys with obesity when compared with either lean or normal-weight children. When analysis was restricted to children at pubic hair stage 2, age at this stage was found not to differ significantly between normal-weight and obese individuals. In boys, testicular volume at a given age was also similar

across all weight groups.¹⁰² In the United States, a cohort of obese boys with DP has also been described.⁹²

In summary, research to date highlights inconsistencies in how obesity affects pubertal timing in boys. Recent data from a study in the United States may shed light on a reason for some of the inconsistency among the studies.¹⁰³ Some 3872 boys were divided based on BMI into three categories (normal weight, overweight, and obese). Based on genital development, puberty among white and African American boys occurred earlier in the overweight compared with normal weight group but later in the obese compared with overweight category. This relationship was not observed among Hispanic youth. These data suggest that the relationship between earlier puberty and body weight may not be linear in boys and may differ by racial/ethnic population group.

Effect of Endocrine-Disrupting Chemicals

Some evidence supports associations between human pubertal timing and exposure to environmental modifiers, although much of the data relate to girls.¹⁰⁴ For example, earlier menarche and pubarche have been associated with exposure to polybrominated biphenyls (PBBs) and dichlorodiphenyl dichloroethene (DDT), whereas delayed breast and pubic hair development, as well as delayed menarche have been associated with lead exposure.^{105,106} In addition, elevated serum levels of a mycotoxin (zearalenone) have been reported in girls with precocious puberty.¹⁰⁷

Some studies among boys have also been reported. Among the strongest studies is a longitudinal assessment of growth and pubertal timing among a cohort of around 500 boys in

Russia.¹⁰⁸ The results from this study have recently been summarized, with data indicating that prepubertal exposures to nondioxin-like polychlorinated biphenyls accelerate puberty, whereas levels of insecticides, dioxin-like compounds, organochlorine pesticides, and lead delay puberty.¹⁰⁹ Other studies indicate that greater exposure to pesticides is associated with cryptorchidism or hypospadias.¹¹⁰

The extent to which these are associations versus cause-and-effect relationships is not fully known, nor are underlying mechanisms. What effects these exposures have in the general population as opposed to isolated examples of associated abnormalities is also not clear. Certainly more research in this area is needed.¹¹¹⁻¹¹³

PRECOCIOUS PUBERTY

Traditionally, the onset of secondary sexual characteristics in a boy before age 9 years has been defined as precocious, whereas lack of testicular enlargement by age 14 years has been classified as DP. In clinical settings, boys present less often with precocious puberty than with DP, which is discussed more extensively later in the chapter. For clinical assessment, in addition to these dichotomous age cutoffs, a recent online tool allows for assessment of the stage of development (pubic hair, testicular volume, genital staging) relative to population norms. The progression of puberty can be monitored by using the puberty plots presented as stage line diagrams (Fig. 18.6).¹¹⁴ These plots provide probability curves for an individual being at a certain developmental stage at certain age. The centile value on the y-axis is a quantitative assessment of pubertal timing for the individual being assessed on the

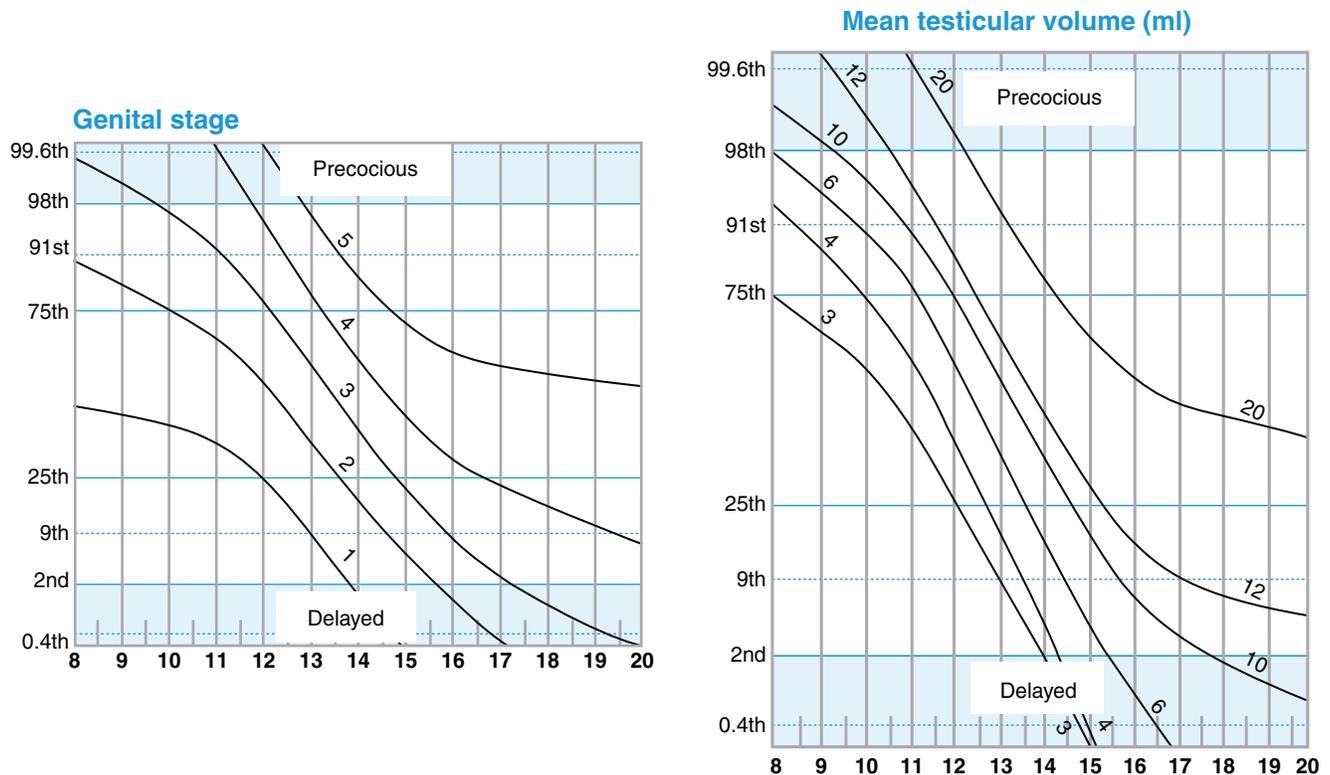


Fig. 18.6 The distribution of Tanner genital (G) stages and testis volume in healthy boys. In these stage line diagrams, lines 1–5 correspond to Tanner stages 1–5 (left panel) or testis volume in mL (right panel). X-axis values are age in years and the y-axis displays gender-appropriate centiles for development. Individuals can have their Tanner stage and testis volume plotted to determine precocity, delay in development, or appropriate progression to determine if development has plateaued or arrested. (From van Buuren, S. (2014). Growth charts of human development. *Stat Methods Med Res*, 23, 346–368.¹¹⁴)

plots. Such diagrams assist in identifying early and delayed development, as well as finding differences in the progression of puberty.

Gonadotropin-Releasing Hormone-Dependent Forms of Precocious Puberty

The most common form of precocious puberty is caused by the early activation of pulsatile GnRH secretion, termed *CPP* or *gonadotropin-dependent precocious puberty*. CPP may result from hypothalamic tumors or CNS lesions (neurogenic CPP) but in most cases remains unexplained (idiopathic CPP) (Table 18.1).^{115,116}

Hypothalamic hamartomas are an example of a cause of neurogenic CPP. The hamartomas are congenital malformations characterized by heterotopic gray matter, neurons, and glial cells, and they are generally located on the floor of the third ventricle or attached to the tuber cinereum. On magnetic resonance imaging (MRI), hamartomas exhibit an isodense fullness. Histologic examination has shown immunoreactivity for GnRH and for astroglial factors, such as TGF- α . Postulated mechanisms include GnRH secretion directly from these neurons, secretion of TGF- α that in turn stimulates GnRH secretion from GnRH neurons, and electrical activation of adjacent neural centers. Most hypothalamic hamartomas are sporadic, but they may occur in association with Pallister-Hall syndrome, which is caused by mutations in the *GLI3* gene. Optic gliomas, which can be associated with neurofibromatosis 1, can also cause neurogenic GnRH-dependent precocious puberty.¹¹⁷ Other etiologies of neurogenic CPP include pineal tumors, suprasellar cysts, head trauma, CNS radiation, and static encephalopathy.¹¹⁸

Loss-of-function mutations of the *MKRN3* gene, located at chromosome 15q11-q13, have been associated with GnRH-dependent precocious puberty. *MKRN3* encodes makorin RING-finger protein 3, which may function as an RNA-binding and/or a ubiquitin ligase. All clinically affected individuals inherited the mutated allele from their fathers, which can be explained because this gene is maternally imprinted and expressed only from the paternal allele. Both boys and girls are affected, although the degree of precocity appears to be greater in girls than boys. About 30% of familial cases and 3% of sporadic cases of CPP are associated with mutations in

MKRN3 (OMIM ID 603856). The mutations in *MKRN3* associated with CPP include frameshift mutations that are likely to cause loss of function; this suggests that *MKRN3* normally acts to delay the onset of puberty and calls attention to the importance of inhibitory factors in the regulation of pubertal timing.⁴¹

There is compelling evidence that mutations disrupting the *DLK1* gene can also cause CPP.¹¹⁹ Deletions and imprinting defects in the region on chromosome 14 around *DLK1* cause Temple syndrome, which is associated with precocious puberty. A smaller deletion that appears to affect *DLK1* alone was identified in four individuals with CPP from the same family, suggesting that disruption of *DLK1* is responsible for the precocious puberty seen in Temple syndrome. The association of *DLK1* with precocious puberty was confirmed by a subsequent report of frameshift mutations in *DLK1* in individuals with precocious puberty from three additional unrelated families.¹²⁰ Like *MKRN3*, *DLK1* is imprinted, and CPP is observed only if the paternal allele is disrupted. *DLK1* encodes a non-canonical ligand for the Notch family of receptors, and its precise function is unknown.

Gonadotropin-Releasing Hormone-Independent Forms of Precocity

Precocious development of secondary sexual characteristics may also be caused by mechanisms that do not involve activation of pulsatile GnRH secretion. These forms of precocity are called *gonadotropin-independent* or *peripheral precocity* and include gonadal and adrenal tumors, inappropriate activation of LH receptor because of gene mutations or signaling by tumors producing hCG, and exposure to exogenous sex steroids (see Table 18.1). Familial male-limited precocious puberty (FMPP, OMIM ID: 176410), formerly known as *testotoxicosis*, is a rare, dominant form of gonadotropin-independent precocity caused by constitutively activating mutations of the LH/hCG receptor (*LHCGR*).¹²¹ This disorder usually presents by age 1 to 4 years with rapid virilization, growth acceleration, skeletal (bone age) advancement, and elevated testosterone levels despite prepubertal levels of LH.^{122,123} McCune-Albright syndrome (MAS, OMIM ID: 174800) is another rare genetic cause of male sexual precocity. It is caused by postzygotic somatic activating mutations in *GNAS1*, the gene that encodes the α subunit of the heterotrimeric G_s protein. G_s transduces the signal from multiple G-protein-coupled receptors, including the LH receptor, and the constitutive activation of G_s signaling leads to autonomous cell proliferation and scattered hyperfunction in endocrine organs, with a wide phenotypic spectrum that depends on which organs carry the mutation.^{124,125} The classical features of MAS include the clinical triad of bone fibrous dysplasia (BFD), café-au-lait skin spots, and precocious development of secondary sexual characteristics. In addition, hyperthyroidism caused by activation of the thyroid-stimulating hormone (TSH) receptor, hypercortisolism caused by constitutive activation of the CRH and/or adrenocorticotropin hormone (ACTH) receptor, kidney phosphate wasting, cholestasis, and hypertrophic heart disease can be present.^{124,126} For reasons that are not clear, MAS leads to sexual precocity more often in girls than in boys. Many boys may have unrecognized testicular pathology, but the predominant finding is Leydig cell hyperplasia, which may not warrant active intervention.¹²⁷

In 1960 Van Wyk and Grumbach first described a syndrome characterized in girls by breast development, uterine bleeding, and multicystic ovaries in the presence of long-standing primary hypothyroidism.¹²⁸ A unique diagnostic feature of the Van Wyk-Grumbach syndrome is the combination of delayed bone age with apparent sexual precocity. Boys with this syndrome have macroorchidism without significant virilization.

TABLE 18.1 Common Etiologies of Sexual Precocity in Boys

Central (GnRH Dependent)	Peripheral (GnRH Independent)
Idiopathic	Congenital adrenal hyperplasia
Central nervous system (CNS) masses	McCune-Albright syndrome
– Hamartomas	Testosterone producing tumors
– Astrocytomas	– Adrenal carcinoma or adenoma
– Adenomas	– Leydig cell tumor
– Gliomas	hCG-producing tumors
– Germinomas	– Choriocarcinoma
CNS infection	– Dysgerminoma
Head trauma	– Hepatoblastoma
Iatrogenic	– Chorioepithelioma
– Low-dose CNS radiation	– Teratoma
– Chemotherapy	– Gonadoblastoma
– Surgical	Exogenous exposure to androgen
Malformations of CNS	Familial male limited precocious puberty
– Arachnoid or suprasellar cysts	Hypothyroidism (Van Wyk-Grumbach syndrome)
– Hydrocephalus	

hCG, Human chorionic gonadotropin.

(Modified from Nathan BM, Palmert MR (2005). Regulation and disorders of pubertal timing. *Endocrinol Metab Clin North Am*, 34:617–641, ix.)

Testicular histology shows enlargement of the seminiferous tubules without an increase in Leydig cell number.^{129,130} If performed, GnRH stimulation shows a prepubertal response with suppressed LH, confirming GnRH-independent precocity. Most cases appear to originate from autoimmune thyroid disease, but there are some case reports where the syndrome is secondary to unrecognized congenital hypothyroidism.¹³¹ The pathophysiology of Van Wyk–Grumbach syndrome involves a complex mechanism, which is mediated at least in part by the direct action of TSH on FSH receptors. Recombinant human TSH (Rec-hTSH) elicits a dose-dependent cAMP response in cells expressing the human FSH receptor *in vitro*; however, the concentration of rec-hTSH required for half-maximal stimulation is several logs greater than that of hFSH.¹²⁹ Early recognition and initiation of thyroid hormone replacement can lead not only to resolution of symptoms and improvement in adult height but also to avoidance of further diagnostic procedures.

GnRH-independent precocity can also be caused by the virilizing forms of congenital adrenal hyperplasia (CAH). These are autosomal recessive disorders of cortisol synthesis that present with premature pubic hair development, axillary hair development, accelerated linear growth, and phallic enlargement in the absence of testicular enlargement. The most common form is 21-hydroxylase deficiency because of loss-of-function mutations in *CYP21A2*. Mutations in 11 β -hydroxylase (*CYP11B1*) and 3 β -hydroxysteroid dehydrogenase type 2 (*HSD3B2*) account for 5% to 10% of cases. Androgen-secreting tumors are another rare cause of GnRH-independent precocity in boys. Leydig cell tumors secrete testosterone. Because these tumors are usually unilateral, testicular volume may be asymmetric. The majority of Leydig cell tumors are benign. Ultrasound may be useful because the tumor may be too small to palpate. Mutations in the *LHCGR* gene have been identified in some testicular adenomas.¹³² Tumors secreting hCG also stimulate testosterone secretion in boys and can resemble familial male limited precocious puberty (testotoxicosis). These tumors are generally hepatic in origin (of note, activating mutations of the LH receptor and hCG-secreting tumors do not cause precocious puberty in girls, as estradiol production requires activation of both the FSH and LH receptors). Adrenal tumors in children may secrete androgens, and virilization is a common presentation.

Whether precocious puberty is gonadotropin dependent or gonadotropin independent not always clear. Both forms can sometimes be present because peripheral forms of sexual precocity, such as CAH or testicular or adrenal tumors, can eventually lead to superimposed central precocious puberty through mechanisms that are unknown. In contrast, in some cases of precocious pubertal development among girls, pubertal manifestations will stop progressing or regress, making treatment unnecessary; such cases are not as commonly seen in boys.^{133,134} The mechanisms responsible for these nonprogressive forms of precocious puberty are not known, but there is evidence that in some cases the HPG axis is intermittently but not fully activated.^{134,135}

Premature Adrenarche

Another form of early development of secondary sexual characteristics occurs when the zona reticularis of the adrenal cortex matures early, causing premature adrenarche. Premature adrenarche, more common among girls, is not associated with progressive pubertal development and is manifested by pubic and axillary hair with modest elevation of DHEA-S. Usually, there is no substantial advancement in bone age; however, even in the subset with more advanced bone ages there appears to be little to no compromise of adult height, although admittedly, most of these data derive from studies among girls.^{136,137} As a result premature adrenarche does not require treatment.

Consequences of Precocious Puberty

In cases of progressive central precocious puberty or peripheral precocity, the concerns include adverse psychosocial outcomes and short adult stature because of early epiphyseal fusion.^{115,138} Several studies have assessed adult height in subjects with a history of precocious puberty, demonstrating that loss in adult height can be substantial.¹³⁹ Height loss is inversely correlated with the age at the onset of puberty.¹³⁹ It is especially important to assess carefully the growth rate and skeletal maturation of individuals with central precocious puberty because of CNS lesions (e.g., tumors, cerebral malformations or injury from trauma or radiation) because these lesions may be associated with concomitant GH deficiency, which can be masked by sex steroid-driven growth that may deceptively appear adequate. In such cases, the combination of undiagnosed and untreated GH deficiency and rapid skeletal maturation driven by sex steroids may result in severely compromised adult height.

Although available data derive mostly from study of girls with pubertal onset at the earlier end of the normal range, it has been suggested that a higher proportion of early-maturing adolescents within the general population engage in exploratory risky behaviors (sexual intercourse and substance use) at an earlier age than adolescents with average or late timing of maturation.^{140,141} However, available data regarding potential adverse psychosocial outcomes specific to patients with precocious puberty are limited, and it is not clear that data obtained from individuals with maturation at the early end of the normal spectrum are fully applicable to precocious puberty.

Epidemiological studies have associated earlier pubertal timing with increased risk for a number of negative adult health outcomes, including obesity; reproductive cancers, such as breast cancer in women and prostate cancer in men; type 2 diabetes; and cardiovascular disease.^{86,142} Most of these studies were conducted in women because recalled age at menarche is a fairly reliable indicator of pubertal timing, and a comparable marker is lacking in men, although recalled age at voice breaking has been used in some studies. Also most of these studies examined pubertal timing more generally rather than precocious puberty specifically, but many of the observed associations extended into the precocious range.

Early association studies were unable to establish causality, that is, whether DP causes the increased risk directly or whether some common upstream factor affects pubertal timing and adult-disease risk independently. More recently, human genetic approaches have been used to address this issue. One approach is to compare the effects of common genetic variants on two traits to determine whether the traits share a common genetic basis; this approach has been used to demonstrate that pubertal timing and BMI have shared genetic determinants.⁸⁶ Another technique called *Mendelian randomization* assesses whether (after adjustment for pleiotropy) the genetic determinants of one trait also influence a second trait, with the logical inference that any such influence is mediated by the first trait influencing the second trait. This technique has been used to suggest that it is pubertal timing itself that influences risk of reproductive cancers, including breast, ovarian, and endometrial cancers in women and prostate cancer in men.⁸⁵ Mendelian randomization also indicates that earlier pubertal timing leads to higher blood pressure in adulthood.¹⁴³ With regard to BMI, pubertal timing has been shown to influence adult BMI,¹⁴⁴ and childhood obesity has been shown to affect pubertal timing¹⁴⁵; thus more than one mechanism underlies the relationship between pubertal timing and BMI.

Pathologic entities leading to sexual precocity warrant treatment, but whether and at what ages one should initiate

treatment for idiopathic central precocious puberty is less clear, particularly if adult height does not appear to be compromised. Currently, robust data regarding the short-term and long-term psychosocial sequelae of CPP and data regarding whether treating CPP with GnRH analogues (GnRHa) alter these outcomes are lacking.¹⁴⁶ Thus one needs to be cautious when using psychosocial outcomes as a rationale for pharmacologic intervention to halt pubertal progression, especially among boys with the onset of puberty close to the normal range.

Evaluation of the Boy With Precocious Development of Secondary Sexual Characteristics

Boys with sexual precocity require careful evaluation because many have underlying disorders.¹¹⁵ Those with premature pubarche (early development of pubic hair or body odor) need to be assessed for peripheral causes of precocity before determining that the premature pubarche stems simply from premature adrenarche. Premature adrenarche may be distinguished from true precocity over time by lack of rapid progression. Conversely, progressive precocity is marked by significant bone age advancement (>2 SD for age), growth acceleration, and rapid progression of secondary sexual characteristics on physical examination.

The evaluation of a boy with sexual precocity is outlined in [Box 18.1](#). Considerations include first verifying that the pubertal development is occurring outside the range of normal development before initiating an evaluation. It is also important to note that not all tests are appropriate in each case and that the diagnostic yields of various tests are not known. Hence it is important to allow history and physical examination to guide the evaluation. The child with both testicles enlarged to 8 mL or more, for example, is most likely to have central precocious puberty, with FSH having led to the expansion of seminiferous tubule volume (whereas cases of testotoxicosis, and hCG-secreting tumors can also present with modest bilateral

testicular enlargement, volumes in these conditions are usually no more than 6 mL). Conversely, the child with bilateral prepubertal-sized testicles is more likely to have peripheral precocity; the child with unilateral testicular enlargement may have a testicular tumor. Testing should be directed accordingly.

Peripheral causes of sexual precocity are characterized by suppressed LH and FSH values in the setting of elevated sex steroid levels. Testosterone levels will be elevated in instances of isosexual precocity (secondary sexual characteristics consistent with male sex), whereas estradiol levels may be elevated in the rare instances of contrasexual precocity (secondary sexual characteristics inconsistent with sex, such as marked breast development as the presenting sign in a boy). Determining the underlying mechanism for progressive peripheral precocity is important because all instances will result from pathologic conditions or exogenous exposures.¹⁴⁷ DHEA-S is often used as a screen for adrenal tumors or adrenal pathology. Determination of the 17-hydroxyprogesterone concentration is used to screen for congenital adrenal hyperplasia because of 21-hydroxylase deficiency. Congenital adrenal hyperplasia and hormone-secreting tumors of the adrenal gland are discussed in detail in [Chapter 14](#). Evaluation of skeletal maturation with a bone age is an important adjunct, as it allows for prediction of adult height.

Historically, the gold standard for the diagnosis of central precocious puberty was GnRH stimulation testing and the demonstration of pubertal gonadotropin responses that derive from the gonadotropes having been primed by endogenous GnRH secretion. However, with the advent of ultrasensitive gonadotropin assays,¹⁴⁸ the recognition that these assays can identify individuals with CPP using unstimulated random samples,¹⁴⁹ and the unavailability of GnRH for testing has led many to forego stimulation testing. GnRH agonists can be used as an alternative to GnRH for stimulation testing, but again the diagnosis can often be made through the combination of clinical features and a baseline LH value in the pubertal range. Although determining the diagnostic cutoff for the basal LH level is difficult because of a lack of normative data and variability among assays, a value of 0.3 IU/L or more, using an ultrasensitive assay with a detection limit near 0.1 IU/L, is commonly cited.¹⁴⁶ If stimulation testing is done, a diagnostic cutoff of 5 IU/L for peak value has been suggested. LH values are more useful than FSH values in the diagnostic evaluation of precocious puberty, but stimulated LH/FSH values can help to identify patients with slowly progressive precocious puberty because these children tend to have FSH-predominant responses.^{134,150} Idiopathic central precocious puberty is a more common cause of precocious puberty among girls than among boys in endocrinology clinics.^{151,152} Hence all boys with CPP should have brain MRI to exclude underlying pathology.¹⁴⁶

In summary, it is important for physicians evaluating patients with suspected precocious puberty to address these questions: Is pubertal development occurring outside the normal range of development? What is the underlying mechanism, and is that mechanism associated with a risk of a serious condition, such as an intracranial lesion? Is the pubertal development likely to progress, and could this impair the child's normal physical and psychosocial development?

Treatment of the Child With Precocious Puberty

Unless there is underlying pathology that requires intervention, whether the precocious pubertal development is likely to impair the boy's normal physical or psychosocial development is often the feature that determines if treatment is needed.

BOX 18.1 Outline of the Evaluation of Sexual Precocity in Boys

COMMON INITIAL SCREENING TESTS

- Careful history, physical examination, and assessment of growth velocity
- Bone age
- LH, FSH
- Testosterone (and in some cases estradiol)
- DHEA-S
- 17-hydroxyprogesterone (to screen for congenital adrenal hyperplasia)
- TSH, T⁴

SECONDARY TESTS TO CONSIDER

- MRI of pituitary and brain (to screen for a central nervous system lesion)
- GnRH agonist stimulation test (can distinguish central precocious puberty from peripheral precocity)
- ACTH stimulation test (to diagnose congenital adrenal hyperplasia)

ACTH, Adrenocorticotropic hormone; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; MRI, magnetic resonance imaging; T⁴, thyroxine; TSH, thyroid-stimulating hormone.

(Modified from Nathan, B. M., Palmert, M. R. (2005). Regulation and disorders of pubertal timing. *Endocrinol Metab Clin North Am*, 34, 617–641, ix.)

Central Precocious Puberty

Because nonprogressive forms of precocious puberty do not require intervention, a prerequisite for the consideration of therapy for CPP is the presence of documented, progressive pubertal development over a 3- to 6-month period, although this period of observation may not be necessary if the child is at or beyond Tanner stage 3 genital development.¹⁴⁶ Epiphyseal fusion is an estrogen-dependent process, and early and progressive production of sex steroids can cause rapid advancement of skeletal maturation and result in compromised adult height. Thus preservation of adult stature is one of the main reasons to consider treatment of CPP with GnRH agonists, which downregulate the pituitary-gonadal axis and limit pubertal progression. Although the data derive primarily from studies among girls, it appears that the risk of short stature is most pronounced in those children with earlier onset of symptoms.¹⁵³ An advanced bone age also contributes to poorer height outcomes.¹⁵³ Available data suggest that the greatest height gain (preservation) occurs with treatment of girls with onset of puberty before 6 years of age, with more modest benefit being seen with onset between 6 and 8 years.¹⁴⁶ However, insufficient data exist to draw similar conclusions regarding age of onset and height outcomes among boys^{154,155}; consequently, a consensus conference recommended considering initiation of GnRHa therapy for all boys with onset of CPP before age 9 years who have compromised height potential.¹⁴⁶

The second main reason to consider therapy in CPP is to mitigate potential psychosocial effects of precocious puberty. However, as noted earlier, additional studies are needed to determine the effects of precocious puberty on quality of life and psychosocial functioning and to evaluate whether treatment with GnRHa therapies affects these outcomes. Thus the consensus conference concluded that the use of GnRHa therapies solely to influence psychosocial consequences of precocious puberty needs to be considered carefully, given the absence of convincing data.¹⁴⁶ These cautions are even more pertinent for boys because data regarding males are even more scarce.

Finally, although the associations with pubertal timing and later life health outcomes mentioned earlier are intriguing, there are no data regarding whether treatment of precocious (or delayed) puberty affects the risk of such outcomes. This question represents an important area of further study because the ability to mitigate risks for cancer or cardiovascular disease would be an important consideration when deciding for or against treatment of precocious puberty.

Various formulations (intramuscular, subcutaneous, and intranasal) of short- (daily) and long-acting GnRH agonists are available to treat CPP. The depot formulations are preferred, as they are easier for patients and families to administer regularly over a long treatment period. The initial depot medications were 1-month formulations, which have been shown to be well tolerated and effective.^{156,157} Subsequently, 3-month and 6-month depot formulations were developed,^{146,158,159} and a 1-year implant (histrelin-acetate) has also become available.¹⁶⁰⁻¹⁶² Which GnRHa to use depends on patient and provider preference, local regulatory approvals, and reimbursement systems.¹⁴⁶

GnRH agonists are generally well tolerated. Occasionally, an initial, temporary flare of GnRH activity can occur and result in transient advancement of secondary sexual characteristics. Local reactions (sterile abscesses) can occur in 10% to 15% of patients using injected depot formulations of GnRH analogs. Other considerations include long-term effects of GnRH agonists on bone mineral density (BMD) and reproductive function. These outcomes have largely been studied among girls, and the available data are reassuring.¹⁴⁶ Whether GnRHa

use is associated with increased obesity is less clear, and data among boys with CPP are sparse. However, most studies in girls are reassuring and indicate that this concern is not great enough to argue against use of GnRHa in cases of CPP, especially because risk factors for obesity may be associated with CPP itself and not necessarily the use of GnRHa.¹⁴⁶

Monitoring of GnRH agonist therapy should consist of assessment of Tanner stage and growth velocity every 3 to 6 months, with assessment of skeletal maturation every 6 to 12 months. Progression of pubertal development or of rapid skeletal maturation indicates a lack of efficacy, poor adherence, or a misdiagnosis. Such patients warrant reevaluation, including potential measurement of baseline or stimulated LH levels. Whether it is helpful to routinely measure gonadotropins to monitor suppression during GnRHa treatment is controversial.^{116,146} When to discontinue GnRH agonist therapy must be evaluated for each individual patient. Data among boys are limited, but examination of clinical characteristics at the discontinuation of therapy—such as treatment duration, height, growth velocity, bone age, and chronologic age—has failed to identify clear predictors of adult height that might guide decisions around when to discontinue GnRH agonist therapy. In the absence of this data, it seems reasonable to time discontinuation based on patient and family preference, typically with the intent of having pubertal development resume at an age that allows for development to occur in parallel with the patient's peers.¹⁴⁶

Peripheral Precocity

In the past, treatment of GnRH-independent causes of sexual precocity, such as MAS or familial male-limited precocious puberty, included using inhibitors of steroidogenesis (ketokonazole), weak antiandrogen agents (spironolactone), and first-generation aromatase inhibitors (AIs) (testolactone).¹⁶³⁻¹⁶⁷ Although these therapies were partially effective in slowing growth velocity and reducing virilization,¹⁶⁸⁻¹⁷¹ the risk of hepatotoxicity and adrenal insufficiency with ketoconazole, as well as the requirement of multiple daily dosing, were obstacles to achieving a favorable therapeutic outcome.¹⁷² More recently, short-term combination therapy with a potent antiandrogen agent, bicalutamide, and third-generation AIs, anastrozole and letrozole, appears to be effective in reducing growth rate and virilization and improving predicted adult height.¹⁷³⁻¹⁷⁶ This combination therapy provides a more convenient once-daily dosing regimen, although the therapy is more expensive than previous treatments and can be associated with gynecostasia. Full endorsement of these regimens awaits further controlled clinical trials, including the evaluation of the long-term effects of such therapy on adult height, fertility, metabolic parameters, cognitive functions,¹⁷⁷ and bone health.¹⁷⁸ If superimposed CPP develops in a patient with peripheral precocity, treatment with a GnRH agonist may be warranted.

DELAYED PUBERTY

The etiologies, evaluation, and treatment of DP have previously been reviewed by the authors.¹⁷⁹ DP is defined as the absence of testicular enlargement 2 to 2.5 SDs later than the population mean (traditionally age 14 years in boys). As noted previously, because of the downward trend in pubertal timing in some but not all reports from the United States,^{58,180,181} and other countries,^{66,182} some advocate for younger age cutoffs for the general population or perhaps for particular countries or ethnic groups. However, the secular change in the onset of puberty has not been as notable in late developing boys⁶⁶ and hence the need to readjust age definitions for DP in males may not be necessary. Of note, because pubarche may result from

maturation of the adrenal glands (adrenarche) independent of HPG axis activation, the presence of adrenarchal signs (such as pubic hair) does not exclude a diagnosis of DP.

DP is often quite concerning to patients and families.¹⁸³ It can affect psychosocial well-being, peer relationships, and sports participation, and these issues are common reasons for initiating therapy. However, as with precocious puberty, further studies are needed to assess fully the psychosocial distress experienced by individuals with DP, whether this distress has long-term sequelae, and what impact sex-steroid supplementation has on these outcomes.¹⁷⁹ Patients, families, and practitioners are also often worried that DP may affect adult stature, and many patients present with relative familial short stature, along with DP and lack of a pubertal growth spurt, which accentuates concerns about both current and adult stature. Adult height can indeed be affected by DP, but on average it is only slightly below the genetic target.¹⁸⁴ It remains unclear whether adult bone mass is adversely affected by pubertal delay¹⁸⁵ and whether concerns related to bone health represent a medical reason to initiate therapy. Finally, as with early puberty, older age at onset of puberty has been associated with later life outcomes,^{86,142} but it is not known if treatment of DP affects these associations.

Etiologies of Delayed Puberty

The most common cause of DP in boys is self-limited DP or CDGP, which refers to an extreme of the normal spectrum of pubertal timing. In two large series, approximately between 65% to 80% of boys and 30% to 50% of girls with DP had self-limited DP^{186,187} (Fig. 18.7). However, because the data derive from referral centers, these percentages may underestimate the frequency of self-limited DP encountered by primary care providers.

Although self-limited DP/CDGP represents the single most common cause of DP in boys, it is a diagnosis of exclusion, and potential pathologic causes of delay should be considered. Other causes of DP can be divided into three main categories^{179,186,187} (see Fig. 18.7 and Table 18.2): persistent HH, which represents approximately 10% of cases among boys and is characterized by low LH and FSH levels caused by

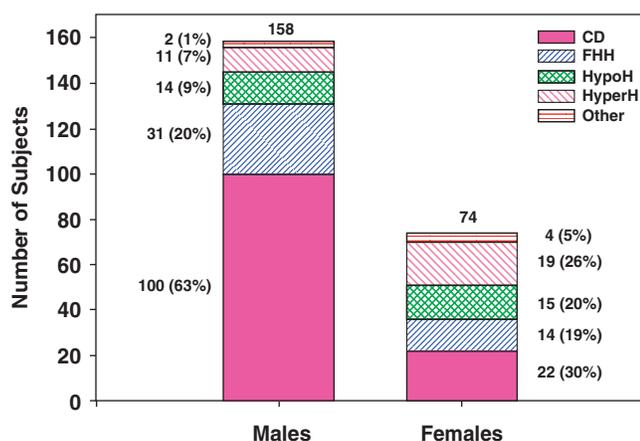


Fig. 18.7 Distribution of diagnostic categories among males and females with delayed puberty. CD, Constitutional delay of growth and puberty; FHH, functional hypogonadotropic hypogonadism; HypoH, persistent hypogonadotropic hypogonadism; HyperH, permanent hypergonadotropic hypogonadism. (From Sedlmeyer, I. L., & Palmert, M. R. [2002]. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab*, 87, 1613–1620. With permission.)¹⁸⁶

hypothalamic or pituitary disorders; functional HH, which represents approximately 10% to 20% of cases and where pubertal delay is transient and secondary to underlying conditions; and hypergonadotropic hypogonadism caused by primary gonadal insufficiency, which represents approximately 5% to 10% of cases and is characterized by elevated LH and FSH levels because of lack of negative feedback from the gonads.

Persistent Hypogonadotropic Hypogonadism

Genetic defects are a major cause of HH, and the study of genetic disorders that cause GnRH deficiency has significantly increased our understanding of development and function of the HPG axis.^{74,189–196} GnRH deficiency is more common in boys than girls, affecting one in 7500 males but only one in 70,000 females; the reason for this sex difference is unclear.¹⁹⁶ Inheritance patterns include X-linked, autosomal dominant, and autosomal recessive. However, sporadic cases are more common than familial forms. HH has been subclassified into three major categories: (1) HH associated with anosmia, called *Kallmann syndrome (KS)*; (2) isolated HH (IHH) without anosmia; and (3) acquired HH. However, there is likely to be overlap among these categories, as the same mutation may cause heterogeneous phenotypes even within families.

Genes Involved Gonadotropin-Releasing Hormone Neuronal Development

The association of HH with an abnormal sense of smell seen in KS arises from the developmental origins of GnRH neurons in the olfactory placode and migration of GnRH neurons along olfactory pathways. Several genes critical to HPG axis function and olfactory development have been identified through investigation of KS.^{58,60} The classic form of KS is characterized by isolated gonadotropin deficiency, anosmia, and X-linked inheritance. This disorder is caused by mutations in anosmin 1 encoded by the *ANOS1/KALI* gene, resulting in failure of GnRH neurons to migrate to the hypothalamus.^{70,71,197,198} MRI can confirm reduced size or complete absence of the olfactory bulbs. Nonreproductive features may include unilateral renal agenesis and synkinesia (“mirror movements,” with movements on one side of the body causing involuntary movements on the contralateral side). *ANOS1* mutations are also associated with a relatively high incidence of cryptorchidism, microphallus, and decreased inhibin B concentrations.

The list of genes associated with HH is expanding, and nonreproductive phenotypes implicate the causative gene in some cases. To date, numerous mutations have been identified in the *FGFR1* gene, the most common autosomal dominant cause of KS; associated features can include skeletal anomalies, hearing loss, and synkinesia.^{199,200} Mutations in *FGF8*, the ligand for *FGFR1*, have been associated with holoprosencephaly, septooptic dysplasia, and Moebius syndrome.²⁰¹ Nonreproductive clinical features of patients with mutations *PROK2* and *PROK2R*, which encode prokineticin 2 and its G-protein-coupled receptor, respectively, include fibrous dysplasia, obesity, synkinesia, and epilepsy.^{194,200,202,203} One study reported a patient heterozygous for both a *PROK2R* mutation and an *ANOS1* mutation, suggesting a possible digenic mode of inheritance in some cases.^{200,202} Mutations in *CHD7* can be associated with other features of CHARGE (coloboma, heart defects, atresia choanae [also known as choanal atresia], growth retardation, genital abnormalities, and ear abnormalities) syndrome. Both normosmia and anosmia have been reported for all of these genes.

The NMDA receptor synaptonuclear signaling and neuronal migration factor (NSMF, also known as *nasal embryonic LHRH factor, NELF*) is also implicated in KS. NSMF appears to have an

TABLE 18.2 Causes of Delayed Puberty Other Than Constitutional Delay of Growth and Puberty

Hypergonadotropic Hypogonadism	Persistent Hypogonadotropic Hypogonadism	Functional Hypogonadotropic Hypogonadism
Genetic syndromes — Noonan syndrome and related disorders Gonadal dysgenesis Testicular regression syndrome Trauma/testicular torsion Chemotherapy/radiation therapy Gonadal infection — Mumps, Coxsackie Autoimmune orchitis Defects in steroidogenesis — 5-alpha reductase deficiency (SR5A2) — 17, 20 lyase deficiency (CYP17A1) — Congenital lipoid adrenal hyperplasia (StAR) — 17-hydroxysteroid dehydrogenase deficiency (HSD17B3) Androgen insensitivity Sertoli cell only syndrome (Del Castillo syndrome)	CNS tumors/infiltrative diseases — Astrocytoma — Germinoma — Glioma — Craniopharyngioma — Prolactinoma — Langerhans cell histiocytosis Rathke's pouch cyst Genetic defects ^a — Kallmann syndrome (ANOS1/KAL1, FGFR1, PROK2, PROKR2, FGF8, HS6ST1, and CHD7) — Isolated hypogonadotropic hypogonadism (<i>GNRHR</i> , <i>GNRH1</i> , <i>GPR54</i> , <i>FGFR1</i> , <i>FGF8</i> , <i>PROK2</i> , <i>PROKR2</i> , <i>TAC3</i> , <i>TACR3</i> , <i>HS6ST1</i> , <i>NSMF/NELF</i>) — HPG axis development (<i>NROB1/DAX1</i> , <i>NR5A1/SF-1</i> , <i>HESX-1</i> , <i>LHX3</i> , and <i>PROP-1</i>) — Obesity and hypogonadotropic hypogonadism (<i>LEP</i> , <i>LEPR</i> , and <i>PCSK1</i>) syndromes — Prader-Willi — Bardet-Biedl — CHARGE Gaucher disease Prior CNS infection Midline defects — Septo-optic dysplasia — Congenital hypopituitarism Chemotherapy/radiation therapy Trauma	Systemic illness/conditions — Cystic fibrosis — Asthma — Inflammatory bowel disease — Celiac disease — Juvenile rheumatoid arthritis — Anorexia nervosa/bulimia — Sickle cell disease — Hemosiderosis — Thalassemia — Chronic renal disease — AIDS Endocrinopathies — Diabetes mellitus — Hypothyroidism — Hyperprolactinemia — Growth hormone deficiency — Cushing syndrome Excessive exercise Malnutrition

^aSome genes have been identified as causes of both Kallmann syndrome and isolated hypogonadotropic hypogonadism (IHH). *CHARGE*, Coloboma, heart defects, atresia choanae [also known as choanal atresia], growth retardation, genital abnormalities, and ear abnormalities; CNS, central nervous system; *HPG*, hypothalamic-pituitary-gonadal. (Modified from Palmert, M. R., Dunkel, L. (2012). Clinical practice. Delayed puberty. *N Engl J Med*, 366, 443–453.)

important role as a common guidance molecule for olfactory axon projections and subsequently, either directly or indirectly in the migration of GnRH neurons.^{204,205} A heterozygous deletion in *NSMF* was initially reported as a component of digenic KS, along with *FGFR1*,²⁰⁶ but it has since been reported that *NSMF* can lead to HH or KS via apparently monogenic inheritance as well.^{5,207}

Mutations in *HS6ST1*, *SEMA3A*, and *WDR11*, have also been identified in patients with HH.^{207–210} In families carrying mutations in these genes, phenotypic heterogeneity for anosmia occurs, and HH often segregates as a complex trait found in association with mutations in other genes. Thus it remains to be determined whether mutations in these genes alone are sufficient to lead to GnRH deficiency. Mutations in genes encoding proteins, such as *FGF17* and *IL17RD* that modulate the signaling efficiency of FGF8 through FGFR1, have been identified, suggesting the existence of a FGF8 synexpression group that may form an oligogenic basis for HH.²¹¹

Genes Involved in Gonadotropin-Releasing Hormone Neuronal Function

Conditions that affect GnRH neuronal function but not GnRH neuronal development would be expected to cause the normosmic variety of IHH, as would conditions that cause resistance to GnRH. Indeed, mutations in *GNRHR*, which encodes the GnRH receptor, account for 3.5% to 10.4% cases of normosmic HH.^{191,212,213} Interestingly, a case of CDGP has also been associated with a homozygous partial loss-of-function mutation in *GNRHR*,^{204,214} and pedigrees of probands with HH can include individuals with delayed but otherwise normal puberty.

Mutations in *GNRH1* have also been identified in patients with normosmic HH but only in rare case reports.^{192,193,215,216}

Mutations in *KISS1* and *KISS1R*, which encode kisspeptin and its receptor, respectively, are rare causes of IHH. Mutations in the genes encoding neurokinin B and its receptor, *TAC3* and *TACR3*, respectively, have been identified in IHH patients.²¹⁷ As noted previously, these genes are highly expressed in the same neurons that express kisspeptin, emphasizing the role of kisspeptin in the regulation of pubertal timing. The gene for prohormone convertase-1 (*PCSK1*) has been associated with obesity, HH, insulin-deficient diabetes mellitus, and ACTH deficiency, with HH presumably, as a result of defective processing of neuropeptides and prohormones that are components of GnRH secretion.^{193,218} Curiously, patients have been reported to have reversible HH, with achievement of normal reproductive endocrine activity in adulthood, despite carrying loss-of-function mutations in *FGFR1*, *CHD7*, *TAC3*, or *TACR3* genes. The biologic mechanism for this reversible gonadotropin deficiency remains unclear, and it remains possible that some cases of HH may represent severe versions of CDGP, further blurring the distinction between HH and CDGP.^{98,220–222}

Genes That Modulate Gonadotropin-Releasing Hormone Neuronal Function

The adipocyte hormone leptin appears to act as a permissive factor in pubertal maturation, highlighting the importance of adequate nutrition in modulating maturation and function of the HPG axis.⁶⁸ HH accompanied by obesity can result from defects in leptin signaling either because of mutations in the leptin (*LEP*) or the leptin receptor (*LEPR*) genes. Furthermore,

GWA studies have demonstrated that common genetic variants near *LEPR* influence pubertal timing, underscoring the role of leptin in regulating GnRH neuronal activity. The neuronal targets for leptin's action are incompletely explained because leptin receptors are not expressed by GnRH neurons, suggesting that the site of leptin action may be upstream of the GnRH neurons.

Genes Involved in Pituitary Development

Mutations in the GnRH receptor (*GNRHR*) gene are one of the more common causes of normosmic IHH. The phenotype ranges from neonatal presentation with micropHallus and cryptorchidism to adolescent presentation of DP.²²² Mutations in the genes encoding FSH-beta and LH-beta have also been described. Developmental anomalies of pituitary development, such as midline defects or septooptic dysplasia, can be associated with gonadotropin and other pituitary hormone deficiencies. Genes associated with developmental anomalies of the pituitary include *HESX1*, *SOX2*, *PITX2*, *LHX3*, and *PROP1*, as well as some of the genes known to cause HH, such as *FGFR1*, *FGF8*, and *PROKR2*.

Conditions that Affect Both the Hypothalamus and Pituitary Gland

Adrenal hypoplasia congenita is an X-linked disorder caused by mutations in *NROB1/DAX1* and is characterized by primary adrenal insufficiency and a complex pattern of hypogonadism. In this disorder, the fetal adrenal cortex develops normally, but the adult zone of the adrenal cortex fails to develop. Affected males may present with adrenal insufficiency at 6 to 8 weeks of age. If assessed, the minipuberty of infancy may be present,²²³ but patients then exhibit HH in adolescence, and some may present for the first time because of DP. Decreased hypothalamic GnRH secretion or decreased pituitary responsiveness can occur leading to decreased LH, FSH, and testosterone concentrations. Interestingly, patients may also have primary gonadal insufficiency that is masked by the HH. Mutations in this gene may be part of a contiguous gene-deletion syndrome that also causes Duchenne muscular dystrophy and glycerol kinase deficiency.²²⁴ Mutations in *NR5A1/SF-1* may also affect both hypothalamic and pituitary development.

Nongenetic etiologies can also cause HH. Nongenetic factors, such as maternal cocaine abuse, valproate toxicity, and intrauterine vascular disruptive events may cause CNS developmental anomalies and lead to HH. Intracranial tumors can also lead to HH. The most common neoplasm is a craniopharyngioma, which is thought to arise from remnants of Rathke's pouch. Other symptoms can include decreased growth velocity, headache, polyuria/polydipsia, and visual disturbances. Suprasellar or intracellular calcifications may be detected on computed tomography (CT) scans. Other tumors include germ cell tumors, epidermoid and dermoid cysts, prolactinomas, and optic gliomas. Histiocytosis X is characterized by infiltration of lipid-laden histiocytes into skin, bone, or visceral organs. Although diabetes insipidus is the most common endocrine manifestation of histiocytosis X, other anterior pituitary hormone deficiencies may occur.

Hyperprolactinemia can also lead to HH, as prolactin suppresses pulsatile GnRH secretion. Hyperprolactinemia can be caused by a prolactin-secreting tumor, including pituitary microadenomas (adenomas <10 mm) and macroadenomas (>10 mm) and can also be caused by disruption of the pituitary stalk, as hypothalamic dopamine release inhibits prolactin secretion. MRI provides greater anatomic detail than CT scans. Hyperprolactinemia may also be caused by primary hypothyroidism (because thyrotropin-releasing hormone also induces

prolactin secretion) or psychotropic medications with antido-paminergic properties.

Finally, several syndromes are associated with HH. These syndromes include Prader-Willi, Bardet-Biedl, Alström, and Bloom syndromes. Hereditary hemochromatosis leads to iron overload and iron deposition in endocrine organs leading to HH, impaired glucose tolerance, and diabetes mellitus.

Self-limited Delayed Puberty or Constitutional Delay of Growth and Puberty

Self-limited DP or CDGP is a condition in which puberty is late to start but, once started, progresses to full sexual maturity.¹⁸³ Delays may also be seen in other aspects of growth and physical development. Review of growth records often shows that linear growth during the first few years of life decelerates, followed by growth velocity tracking along lower percentiles than expected based on parents' heights during childhood. Skeletal maturation is usually delayed as well. The etiology of self-limited DP is unknown. Suggested causes include increased total energy expenditure²²⁵ and increased insulin sensitivity,²²⁶ but no definitive etiology has been identified.

Self-limited DP does have a strong genetic basis. Fifty percent to 80% of variation of the timing of puberty in humans is caused by genetic factors,⁷⁶ and 50% to 75% of individuals with self-limited DP have a family history of DP.^{227,228} The inheritance patterns observed among pedigrees with self-limited DP are variable but are most often consistent with an autosomal dominant pattern, with or without complete penetrance. Inheritance of self-limited DP is not sex specific and is characterized by family members having either relatively delayed development (e.g., the average age at menarche among mothers of individuals with self-limited DP is 14.3 years compared with a mean of 12.7 among controls³³) or evidence of pubertal timing that would have met clinical criteria for DP.

As discussed earlier, the investigation of etiologies of persistent HH—KS and IHH—has led to the identification of genes that play critical roles in development and regulation of the HPG axis. Some, but not all, studies suggest that mutations in the genes identified to date can also cause self-limited DP, but most cases of self-limited DP are not associated with mutations in IHH/KS genes.^{77,189,229} Loci that are associated with the age of menarche in the general population,^{79–82,231} have also been associated with variation in the timing of puberty in boys,⁸⁶ with some slight sex differences having been noted²³⁰; however, the neuroendocrine pathophysiology and its genetic basis remain unclear in the majority of patients with self-limited DP. The phenotype of self-limited DP represents the tail of a normally distributed trait within the population. Therefore the genetic analyses of families with self-limited DP is complicated by the fact that gene variants that govern the inheritance of this condition can also be found in the general population at a low level, confounding interpretation of data from sequencing studies.

A few studies have specifically examined the contribution of mutations in genes underlying KS/IHH to the phenotype of self-limited DP. In a direct sequencing study that assessed variation in *FGFR1*, *GNRHR*, *TAC3*, and *TACR3* in 146 Finnish subjects, variants in the coding regions of these genes were not identified as likely causes of CDGP in the general population.^{19,229} Similarly, genetic variation in neither *GNRH1* nor *GNRHR* nor other HH-related genes is a common cause of late puberty in the general population.^{77,205,231} Variation in *FGFR1* also does not appear to be a major cause of CDGP,^{77,232} although loss-of-function mutations in *FGFR1* can cause DP in members of HH pedigrees.^{208,213,234–237}

In contrast, another study did identify mutations in *FGFR1*, as well as *HS6ST1* and *Klotho Beta (KLB)*, in some of the kindreds of KS/IHH patients and their relatives with DP phenotype that is not associated with persistent GnRH deficiency.^{208,236,237} In addition, a limited number of variants in KS/IHH genes including *GNRHR*, *TAC3*, *TACR3*, *IL17RD*, *SEMA3* have been discovered by whole exome sequencing in some cases of self-limited DP.²³⁸ However, many of these variants have not been tested in vitro or in vivo for pathogenicity, or investigated for segregation with trait within pedigrees, and thus the finding may be an overestimation of how often KS/IHH genes are found within self-limited DP. Most recently, a comparative study of the frequency of mutations in 24 KS/IHH genes between probands with self-limited DP and those with KS/IHH found a significantly higher proportion of mutations in the KS/IHH group (51% of KS/IHH probands vs. 7% of DP probands, $P = 7.6 \times 10^{-11}$).²³⁹ The same study also reported a higher proportion of oligogenicity in the KS/IHH group, indicating that the genetic architecture of the KS/IHH group was distinct from self-limited DP. In addition, mutations in KS genes, such as *ANOS1* and *NELF*, have not been identified in pedigrees with DP to date.

Thus although the data are at times contradictory, overall, the sum of the available data suggests that although there may be some overlap, either the genetic background of IHH/KS and DP is largely different or it is shared by genes yet to be discovered.²²⁹

Genetic variation in the GnRH receptor gene (*GNRHR*) is another example of partial overlap but mostly distinct pathogenesis. Loss-of-function mutations within the GnRH receptor gene are the most frequent cause of autosomal recessive normosmic IHH, accounting for 16% to 40% of patients.²⁴⁰ On the other hand, variation in self-limited DP is extremely limited. To date, a homozygous partial loss-of-function mutation in *GNRHR* was found in only two brothers, one with self-limited DP and one with normosmic IHH,²⁴¹ and a further heterozygous mutation found in one male with self-limited DP.²²⁹

Genes underlying self-limited DP have recently been reviewed (<https://www.ncbi.nlm.nih.gov/pubmed/?term=31220230>). Whole exome and targeted resequencing methods from a large Finnish cohort have implicated two pathogenic mutations in *immunoglobulin superfamily member 10 (IGSF10)* as the causal factor for self-limited DP in six unrelated families.²⁴² Functional studies indicate that mutations in *IGSF10* appear to cause a dysregulation of GnRH neuronal migration during embryonic development, which presents in adolescence as pure DP, without affecting the prepubertal linear growth rate. Pathogenic *IGSF10* mutations leading to disrupted IGSF10 signaling may result in reduced numbers or mistimed arrival of GnRH neurons at the hypothalamus, suggesting that intact GnRH neurosecretory network is necessary for the correct temporal pacing of puberty. A GnRH neuronal migration defect system might cause an increased “threshold” for the onset of puberty, with an ensuing delay in pubertal timing. *IGSF10* loss-of-function mutations were also discovered in patients with a hypothalamic amenorrhoea. Although loss-of-function mutations in *IGSF10* are also enriched in patients with KS/IHH, these mutations do not alone appear sufficient to cause the phenotype of full GnRH deficiency. These findings represent a new concept of fetal origin of self-limited DP.

Further analysis of individuals from the cohort in which *IGSF10* was identified also provides additional data in support of the partial overlap among KS/IHH and DP genes. A deleterious mutation in the KS/IHH gene *HS6ST1* was found as the causal factor for self-limited DP in one pedigree from the same large cohort of patients with familial DP.²⁴³ The variant was carried by six family members from three generations, all with typical features of self-limited DP. In the same report parallel studies in a murine model corroborated heterozygous *Hs6st1*

deficiency as a cause of delayed pubertal timing without compromised fertility. *Hs6st1*^{+/-} mice were born at normal Mendelian ratios without obvious defects in GnRH neuron or testes development, but they later exhibited delayed pubertal onset. GnRH deficiency was excluded by reproductive competence and by normal spermatogenesis in males. These findings suggest that perturbations in a single allele of a gene regulating the HPG axis are sufficient to cause self-limited DP. Furthermore, data from this example suggest that more deleterious alterations in the same gene, or in combination with additional genes, can cause more severe KS/IHH phenotypes.²⁰⁶

Mutations in genes implicated in body mass and timing of puberty in the general population may also underlie self-limited DP. In a recent study functionally annotated pathogenic, rare variants in *FTO* were found to cause familial self-limited DP in three families.²⁴³ The mutations were also associated with leanness in these subjects.

The neuroendocrine control of puberty is regulated by a hierarchically organized network of transcriptional factors acting upstream of GnRH (Fig. 18.2). These include enhanced at puberty 1 (*EAP1*), which contributes to the initiation of puberty through transactivation of the GnRH promoter. Recently, the first *EAP1* mutations leading to reduced GnRH transcriptional activity resulting in a phenotype of self-limited DP was reported.²⁴⁴

Functional Hypogonadotropic Hypogonadism

Transient causes of HH or functional HH, in which pubertal delay is secondary to effects of underlying conditions, are the cause in 10% to 20% of patients with DP, likely affecting more girls than boys (see Table 18.2).^{186,187} Examples of these conditions include anorexia nervosa, which is a chronic disorder characterized by decreased caloric intake, weight loss, and excessive physical activity. Although anorexia nervosa primarily affects females, approximately 10% of patients are male. Osteopenia and osteoporosis are potential consequences. In addition to low gonadotropin concentrations, cortisol, GH, and reverse T₃ concentrations may be elevated. Chronic disorders, such as sickle cell anemia, thalassemia, cystic fibrosis, inflammatory bowel disease, celiac disease, and chronic renal disease may also be associated with DP. Intensive physical activity and the need to “make weight” in wrestlers may disrupt pubertal development. For wrestlers, these changes generally reverse within months of the end of the wrestling season.

Hypergonadotropic Hypogonadism

Primary gonadal insufficiency leads to loss of negative feedback inhibition and hypergonadotropic hypogonadism. Although they represent only 5% to 10% of the causes of DP, etiologies within this category are broad and include gonadal injury and/or loss, aberrant gonadal differentiation, and disorders affecting steroid hormone synthesis and action. Some disorders, such as Klinefelter syndrome, Noonan syndrome, and Prader-Willi syndrome affect both Leydig and Sertoli cell function.

Chemotherapy, Radiation Therapy, and Cancer Survival

Treatment of childhood cancers can lead to gonadal insufficiency, DP, and infertility. As treatments have improved, quality-of-life concerns have become increasingly relevant because many young cancer patients become long-term cancer survivors. Multiple components of the HPG axis may be affected by chemotherapy and radiation therapy with specific consequences, depending on the drugs and fields of irradiation. The germ cells of the gonads are particularly vulnerable to radiation. Discussions regarding fertility preservation are crucial

for pediatric patients with cancer and their parents; however, detailed discussion of this important subject is beyond the scope of this chapter.

Androgen Insensitivity Syndrome and Gonadal Dysgenesis

Androgen insensitivity syndrome in 46,XY individuals; gonadal dysgenesis in 46,XY individuals or in individuals with mosaicism/chimerism affecting the Y chromosome; and testicular and ovotesticular disorders of sex development in 46,XX individuals can all result in a range of phenotypes. Although many cases present early because of genital ambiguity and/or discordance between genital appearance and a previously established karyotype, some cases may have no obvious external phenotype or a mild phenotype (e.g., isolated hypospadias). In these cases, the condition may not have been recognized as a difference in sex development (DSD), and patients may first present to medical care for evaluation of stalled pubertal development. These conditions, as well as other DSDs, such as those caused by steroidogenic defects that can cause lack of complete endogenous pubertal development, are discussed extensively in [Chapter 6](#).

Syndromes Associated With Delayed or Stalled Puberty

Klinefelter syndrome, which is associated with 47,XXY karyotype, is not a cause of DP per se but can be a cause of a prolonged or stalled pubertal course. The incidence is reported to be one in 667 when ascertained by prenatal cytogenetic analysis, but it is much lower when identified solely by clinical features. Thus incomplete ascertainment of affected individuals likely occurs.²⁴⁶

The typical clinical features of Klinefelter syndrome are small, firm testes; tall stature; small penis; and gynecomastia. Boys with mosaic karyotypes with one normal 46,XY cell line tend to have a milder phenotype. The tall stature may be caused by the extra copy of the short stature homeobox-containing (*SHOX*) gene in the pseudoautosomal region of the X chromosome.²⁴⁷ Both androgen secretion and spermatogenesis are impaired. Inhibin B concentrations are relatively normal during childhood but decline during puberty, consistent with the progressive testicular failure. AMH levels similarly follow the normal male pattern but progressively decline from midadolescence because of Sertoli cell dysfunction.

The decreased testicular volume is secondary to degeneration of the seminiferous tubules. The magnitude of testosterone deficiency and testicular failure varies among affected men, but most men require treatment with testosterone at some point. Treatment goals include development of secondary sexual characteristics, increased muscle mass, optimal bone density, and sexual function.

Apart from decreased number of germ cells, testicular architecture is relatively normal in fetal and prepubertal testes. Loss of germ cells beginning in utero, culminating in fibrosis and hyalinization of the seminiferous tubules, is the proximate cause of the infertility associated with Klinefelter syndrome.²⁴⁸ Fertility preservation is a relevant topic for the older adolescent boys. Some men with Klinefelter's have oligospermia and are able to father children without medical intervention. For others, paternity has been achieved with microdissection and testicular sperm extraction combined with intracytoplasmic sperm injection (microTESE-ICSI), and offspring do not appear to be at increased risk for sex chromosome aneuploidies.²⁴⁹

Nonreproductive features can include an increased incidence of type 2 diabetes, breast cancer, mediastinal tumors, and vascular disease.²⁵⁰ Academic and emotional difficulties are common, and some boys are detected through their learning disabilities. The cognitive phenotype is characterized by deficits in language and executive function. The executive

dysfunction is characterized by poor decision making, problem solving, reasoning, and planning issues. Difficulties with self-control, increased distractibility, and reduced fine- and gross-motor skills are common.²⁵¹

Although much is known,²⁵² there are also several unanswered questions about the management of boys with Klinefelter syndrome. These questions include: does sperm banking/extraction in early adolescence versus young adulthood result in higher success rates? Are behavioral and cognitive deficits improved by administration of testosterone in early childhood or even infancy? Should testosterone be administered in adolescence even if LH values remain in the normal range? These topics are areas of active investigation, and results will influence care of these individuals in the future.

Noonan syndrome is an autosomal dominant disorder characterized by short stature, ptosis, hypertelorism, mild intellectual disability, and congenital heart disease. Cryptorchidism is common in males. Approximately 50% of cases are caused by mutations in the protein-tyrosine phosphatase, nonreceptor type 11 protein (*PTPN11*) gene (also known as *SHP2*). Other genes associated with Noonan syndrome include *KRAS*, *SOS1*, *NRAS*, and *RAF1*. The proteins encoded by these genes are members of the RAS-MAPK signaling pathway. In one longitudinal study, LH, FSH, testosterone, AMH, and inhibin B concentrations were comparable to healthy boys before the onset of puberty. However, some individuals with Noonan syndrome have DP, and adult men with Noonan syndrome have higher LH, FSH, and testosterone concentrations and lower AMH and inhibin B concentrations compared with healthy controls.²⁵³

Prader-Willi syndrome can also be associated with DP. Cryptorchidism is common in boys with Prader-Willi syndrome. Inhibin B concentrations are generally normal in infants and prepubertal boys. As puberty progresses, inhibin B concentrations decline and FSH concentrations increase. Testicular histology is variable and is reported to range from normal to complete absence of spermatogonia (Sertoli cell only syndrome).²⁵⁴ Longitudinal data indicate that the hypogonadism among boys with Prader-Willi syndrome is likely caused by primary testicular dysfunction involving both Sertoli and Leydig cell compartments. Inadequate HPG axis function may also contribute to the hypogonadism.²⁵⁵

Consequences of Delayed Puberty

Sex steroids are essential for the acquisition and maintenance of BMD. Men with IHH have lower BMD, men with Klinefelter syndrome are at increased risk for osteopenia, and some studies have suggested that [this is](#) also true for men with a history of constitutional delay.^{256–258} Mendelian randomization studies have demonstrated that the association between late puberty and BMD is likely causal, that is, that late pubertal timing itself leads to decreased BMD in adulthood.²⁵⁹

Early epidemiological studies demonstrated that earlier pubertal timing is associated with increased risk for cardiovascular disease in adulthood. A larger study has now shown that this relationship is nonlinear in women, such that both early and late pubertal timing demonstrate this increased risk.²⁶⁰ However, data in men suggest that later pubertal timing is protective against cardiovascular outcomes.²⁶¹

Late voice breaking has also been associated with increased risk for psychiatric issues, such as anxiety and depression, as well as atopic conditions, including eczema and asthma.²⁶¹ For all of these associations, one wonders if timely intervention with sex-steroid treatment could lower these risks. One study has demonstrated that earlier age at initiating testosterone treatment was associated with higher BMD in men with IHH²⁵⁷; however, for most of these associations, direct evidence for benefits of sex-steroid treatment on adult disease risk is lacking.

EVALUATION OF THE YOUNG MAN WITH DELAYED PUBERTY

Initial Evaluation

The aim of the initial evaluation is to rule out causes of DP other than CDGP (Table 18.3 and Fig. 18.8). Eventual normal progression of puberty verifies a diagnosis of CDGP, whereas absent or slow development, or cessation of development after onset, is consistent with persistent hypogonadism. Whether caused by a referral bias or by differences in underlying biology,⁵⁵ more boys than girls are seen in endocrinology clinics for DP in general and CDGP in particular.¹⁸⁶

History

The medical history should inquire about chronic diseases, such as inflammatory bowel disease, sickle cell disease, celiac disease, asthma, chronic renal failure, cardiac disease; endocrine disorders, such as hypothyroidism and poorly controlled diabetes mellitus; and chronic therapy with glucocorticoids,²⁶² all of which may delay both linear growth and puberty. Inadequate nutritional intake, when associated with conditions, such as cystic fibrosis, anorexia nervosa, or excessive energy utilization²²⁵ because of vigorous exercise in athletes or ballet dancers, may result in pubertal delay. Successful intervention in such situations can be followed by catch-up growth and pubertal progression.

TABLE 18.3 Investigations for Delayed Puberty

FIRST-LINE INVESTIGATIONS		
Variable	Requirements and Limitations	Interpretation
Growth rate	Two or more height measurements, preferably 6–12 months apart.	In early adolescence in both sexes a growth rate less than 3 cm/year is suggestive of a disease specifically inhibiting growth (e.g., GH deficiency, hypercortisolism, hypothyroidism), but such rates can also be seen in CDGP. Boys with delayed puberty who are overweight tend to have height and predicted adult consistent with the genetic height potential. ^{9,22,62}
Tanner stages	Pubic hair stages and genital stages should be scored separately because they do not necessarily track together	Testicular volume of ≥ 4 mL is a more reliable indicator of the onset of puberty than Tanner stage 2 genital development.
Testis volume	Prader's orchidometer or a ruler	Testicular volume of ≥ 4 mL (≥ 2.5 cm in length) indicates central puberty. Most healthy boys with a testicular volume 4 mL or greater will have a further increase in testicular volume, pubic hair stage, or both, at repeated examination 6 months later. ⁶¹
Bone age	X-ray of left hand and wrist. Greulich and Pyle Atlas. Bone age is also used to predict adult height.	A bone age delay of more than 2 years has arbitrarily been used as a criterion for CDGP but is not always present and is also not specific to that condition. A bone age delay of 4 years has been associated with an average of 8 cm over prediction of adult height, whereas in short stature with no bone age delay, adult height is usually underestimated by the Bayley-Pinneau tables. ³¹²
Biochemistry	Evaluation varies but common tests include complete blood count, erythrocyte sedimentation rate, creatinine, electrolytes, thyrotropin, and free thyroxine.	To rule out chronic disorders evaluation should be directed based on history and physical examination because the yield of untargeted screening is low. Additional investigations may be necessary based on family history, symptoms, and signs, including screening for celiac disease and inflammatory bowel disease.
Serum luteinizing hormone (LH)	Morning sample. Use immunochemiluminometric (ICMA) or immunofluorometric (IFMA) assays with a lower limit of detection at or below 0.1 IU/L.	At low levels, values obtained on ICMA are at least 50% lower than those by IFMA. ²⁷¹ Values < 0.1 IU/L are not specific for hypogonadotropic hypogonadism. Value > 0.6 (IFMA) or 0.2 (ICMA) IU/L is specific but not sensitive for the initiation of central puberty, because in early puberty some adolescents have lower values. ²⁷¹ In delayed puberty, elevated values suggest primary hypogonadism.
Serum follicle-stimulating hormone (FSH)	Morning sample. Use ICMA or IFMA assays with a lower limit of detection at or below 0.1 IU/L if possible.	At low levels, values obtained on ICMA are $\sim 50\%$ lower than those by IFMA. Values < 0.2 (ICMA) or < 1 (IFMA) IU/L suggest hypogonadotropic hypogonadism but are not diagnostic. ^{270,271} In delayed puberty, a value above the upper limit of normal for the assay is a marker of inhibin B deficiency and of primary gonadal insufficiency with high sensitivity and specificity.
Serum insulin-like growth factor 1 (IGF-1)	Blood samples should be processed within 2 h to avoid an artifactual increase in results. Only assays that recognize IGF-1 without any interference from IGF binding proteins provide reliable results.	IGF-1 level is used to screen for GH deficiency. An increase in the levels during follow-up or during or after treatment with sex steroids makes the diagnosis of GH deficiency less likely. The IGF-1 concentration shows the greatest change during childhood and puberty and then changes more slowly with advancing age. Therefore normal values for children and adolescents should include narrow age ranges and Tanner stages. GH provocation tests are needed to diagnose GH deficiency.
Serum testosterone	Morning sample is ideal. Use an assay with a lower limit of detection at or below 10 ng/dL (0.35 nmol/L) if possible. Concentrations show diurnal variation.	An 8 am serum testosterone value of ≥ 20 ng/dL (0.7 nmol/L) often predicts the appearance of pubertal signs within 12 to 15 months. ³¹³
SECOND-LINE INVESTIGATIONS		
Variable	Requirements and Limitations	Interpretation
Gonadotropin-releasing hormone (GnRH) test ^a	Assay requirements are the same as for basal LH and FSH. Test can be performed any time of day. LH and FSH values vary according to assay used and with the stimulating agent (GnRH or GnRH agonist)	A predominant LH over FSH response after GnRH stimulation or peak LH levels of 5 to 8 IU/L (depending on assay) suggests onset of central puberty. There is an overlap between prepubertal and early pubertal post-GnRH values. ²⁷¹ A prepubertal response is seen in some patients with CDGP as well as in hypogonadotropic hypogonadism, but a post-GnRH LH value < 0.8 IU (IFMA) and FSH value < 1.1 IU/L (IFMA) may be more consistent with hypogonadotropic hypogonadism in boys. ²⁷¹

Continued

TABLE 18.3 Investigations for Delayed Puberty—cont'd

Human chorionic gonadotropin (hCG) test ^a	IM or SC injections on several days. There are number of different protocols available. Results vary by the protocol.	Peak testosterone concentrations to both 3-d and 19-d hCG tests have been reported to be significantly lower in patients with hypogonadotropic hypogonadism compared with CDGP. A combination of the GnRH test and hCG test (peak LH cutoff, 2.8 U/L; peak 19-d testosterone cutoff, 275 ng/dL [9.5 nmol/L]) gave a sensitivity and a specificity of 100% in a small study of CDGP and hypogonadotropic hypogonadism. ³¹⁴
Serum inhibin B ^a	Can be measured any time of day. Has diagnostic value only in boys.	Measurement is used in differentiation of hypogonadotropic hypogonadism from CDGP. Boys with higher baseline inhibin B levels had a higher likelihood of CDGP in one study. ²⁷² In prepubertal boys, sensitivity and specificity of 100% was obtained at concentration of >35 pg/mL. With Tanner stage 2 genitalia, sensitivities were 86% and 80%, and specificities 92% and 88%, respectively, for CDGP with an inhibin B value of >65 pg/mL. In boys, unmeasurable inhibin B indicates testicular insufficiency.
Serum prolactin	Measurement is indicated only in some cases. Physiologic states including stress, exercise, and sleep can increase prolactin levels, as can hypothyroidism and some medications.	Elevated levels may indicate either a prolactin-secreting tumor or disruption of the pituitary stalk, in which case additional pituitary-hormone deficiencies may be present. Macroprolactin (physiologically inactive form of prolactin) measurement is recommended as additional test in patients with hyperprolactinemia.
Brain magnetic resonance imaging (MRI)	Indicated in any suspicion of CNS pathology (e.g., headache, changes in vision, changes in behavior).	Imaging is primarily performed to rule out lesions of the hypothalamic-pituitary region. Other findings may include aplasia or hypoplasia of the olfactory bulb and sulcus in patients with Kallmann syndrome, which may help differentiate the Kallmann syndrome from isolated hypogonadotropic hypogonadism in patients with an apparently normal or difficult-to-evaluate sense of smell. In hypogonadotropic hypogonadism there is a moderate agreement between the MRI of the olfactory bulbs and formal smell testing (overall Kappa 0.5), but in the presence of aplastic bulbs and anosmia, there is good agreement (Kappa 0.9).
Olfactory function test	One test, UPSIT, ³¹⁵ uses microencapsulated odorants, which are released by scratching standardized odor-impregnated test booklets.	Used to assess for hyposmia and anosmia as part of evaluation for Kallmann syndrome.
Genetic testing	In more than 60% of patients with Kallmann syndrome or isolated hypogonadotropic hypogonadism no specific gene defect is found.	Genotyping for known monogenic causes is currently a research procedure and not warranted in routine clinical practice. It may be warranted when there is a positive family history or the patient has phenotypic signs suggestive of a specific mutation. If performed, genetic testing should be accompanied by genetic counseling.
GH testing	Various protocols both for testing and priming are available. The choice of GH stimuli to be used is variable.	Response in GH provocation test is greater after the administration of exogenous (aromatizable) androgens or estrogens (priming). Reliable assay performance and appropriate normative data are critical for appropriate use of GH and IGF-1 measurements.
Karyotype		Diagnostic of Klinefelter syndrome

^aThese tests are used to try to differentiate CDGP and IHH. However, validation in larger, independent studies is needed before they can be endorsed for routine clinical use.^{268,269} Clinical follow-up is often needed to confirm the diagnosis; no endogenous puberty by age 18 years is diagnostic of IHH. CDGP, Constitutional delay of growth and puberty; CNS, central nervous system; GH, growth hormone; IM, intramuscular; SC, subcutaneous; UPSIT, University of Pennsylvania smell identification test. (Modified from Palmert, M. R., Dunkel, L. (2012). Clinical practice. Delayed puberty. *N Engl J Med*, 366, 443–453.)

Delayed cognitive development associated with obesity or dysmorphic features may suggest an underlying genetic syndrome. Cryptorchidism or small penis at birth, hyposmia, or anosmia may suggest HH. A history of chemo- or radiotherapy may indicate primary gonadal failure. However, some patients receiving treatment for malignancy may have elevated gonadotropin levels during and shortly after therapy, but subsequently, the gonadotropin levels may decrease as these patients can experience varying degrees of gonadal recovery. As noted previously, although Klinefelter syndrome is a type of hypergonadotropic hypogonadism, males with this condition commonly present with unusually small testes, lack of pubertal progression, or infertility rather than pubertal delay.

A family history—including childhood growth patterns, age at pubertal onset of the parents, and infertility—should be obtained. DP in a parent or sibling, regardless of gender, followed by spontaneous onset of puberty suggests CDGP. However, if pubertal development was induced by sex steroids in family members, IHH is also possible, as reversal of hypogonadism is noted after discontinuation of sex steroids in 10% or more of IHH cases.^{219,220}

Physical Examination

Previous height and weight measurements should be obtained and plotted so that longitudinal growth can be carefully assessed. Height, weight, and body proportions should be determined. Compared with previous data, height and weight can be used to calculate annual growth rates, preferably based on a 12-month interval, as intervals less than 4 to 6 months can be inadequate. DP is often associated with short stature and slow growth for age and for midparental target height, although height and growth rate are usually within the normal prepubertal range. Individuals who are underweight for height have a higher likelihood of an underlying condition delaying HPG axis activation. Conversely, in boys, unlike girls, being obese can be associated with later pubertal development as discussed previously.^{92,263} Upper-to-lower segment ratio can be determined by measuring sitting height or lower segment (from top of pubic symphysis to floor) and compared with normal for age: ratio decreases with age to 1 or lower depending on racial group. Arm span (fingertip to fingertip) should be within 5 cm of the standing height. Longer limbs suggest hypogonadism.

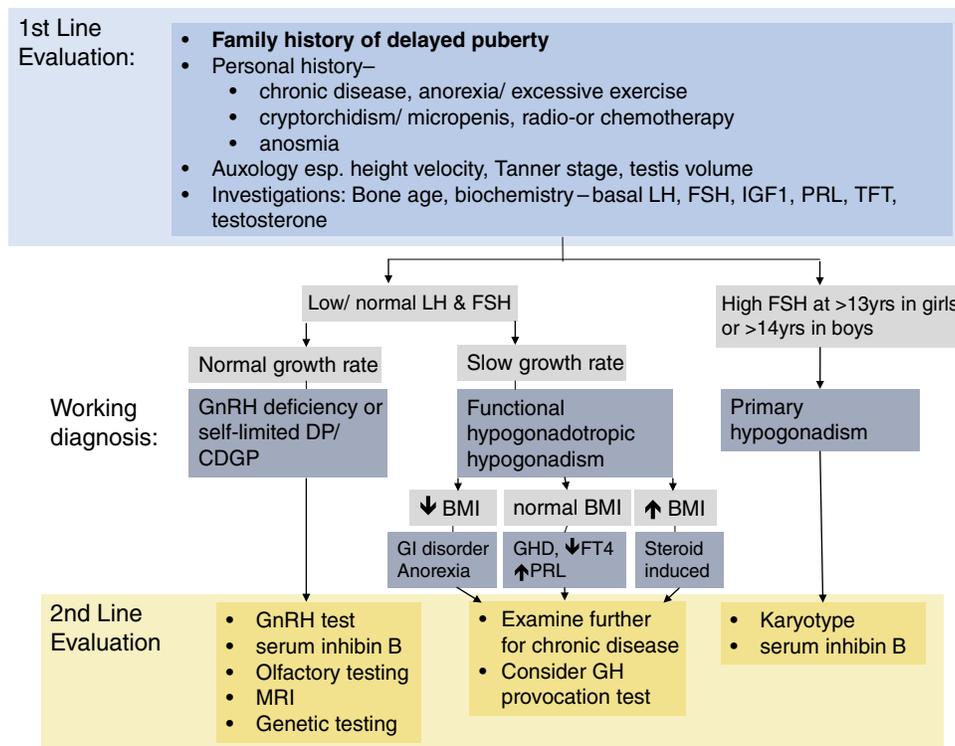


Fig. 18.8 Flowchart for the evaluation of a patient with delayed puberty. *BMI*, body mass index; *CDGP*, constitutional delay of growth and puberty; *DP*, delayed puberty; *FSH*, follicle-stimulating hormone; *GH*, growth hormone; *GHD*, growth hormone deficiency; *GI*, gastrointestinal; *GnRH*, gonadotropin-releasing hormone; *IGF-1*, insulin-like growth factor 1; *LH*, luteinizing hormone; *MRI*, magnetic resonance imaging; *PRL*, prolactin; *TFT*, thyroid function tests. (Modified from Palmert, M. R., & Dunkel, L. [2012]. Clinical practice: delayed puberty. *N Engl J Med*, 366, 443–453.)¹⁷⁹

The physical examination should also concentrate on identifying markers of chronic disease, malnutrition, neurologic abnormalities, thyroid and other endocrinopathies, and syndromic features. In boys, in addition to determining height, weight, and skeletal proportions, documentation should include assessment of penile size, using a stretched length if necessary, position of urethral opening, testicular size, and whether both testes are present in a normally developed scrotum. Cryptorchidism, bifid scrotum, micropenis, or perineoscrotal hypospadias may indicate a disorder of sex development because of defects in gonadal development or gonadal steroidogenesis. Small testes, eunuchoid habitus, and gynecomastia suggest Klinefelter syndrome. The presence of an abnormal neurological examination and/or visual field deficits raises concern for a space-occupying lesion of the hypothalamic-pituitary region. Obesity and DP may suggest defects in the prohormone convertase 1 (*PCSK1*), leptin, or leptin receptor genes. When obesity is associated with DP and dysmorphic features, it suggests Prader-Willi, Bardet-Biedl, or other genetic syndromes.

In boys, Tanner stage 2 genitalia mark the onset of pubertal development and are characterized by enlargement of scrotum and testes and by a change in the texture and color of the scrotal skin (see Fig. 18.5). Pubic hair stage and testicular volume should also be measured; a testicular volume of 4 mL or more indicates the initiation of central puberty. As with assessment of precocious puberty, some find use of “puberty plots” that assess stages of development compared with population data useful (see Fig. 18.6). In patients with CDGP, both adrenarche and hormonal activation of the gonads often occur later than average, but in isolated HH adrenarche usually occurs at a normal age.^{186,264}

Testing

The history and physical examination should direct the laboratory and radiologic studies so that a parsimonious, cost-effective evaluation is performed. **Certainly**, not all tests need to be performed in all individuals.^{265,266}

An individual who is experienced in interpreting bone ages should review these x-rays. A delay in bone age is characteristic of CDGP but is not diagnostic, as a delayed bone age may also occur in individuals with chronic illness, HH, or gonadal failure. Adult height prediction is an important part of counseling if short stature is a component of the presentation, and practitioners need to be aware that the Bayley-Pinneau tables overestimate adult height in patients with CDGP if bone age is delayed by more than 2 years (see Table 18.3).

Pubertal onset is characterized by accentuation of diurnal gonadotropin and testosterone secretion before apparent phenotypic changes. Basal LH and FSH levels are low in CDGP and in HH, whereas they are usually elevated in gonadal failure. In general, LH is a better marker of pubertal initiation than FSH, and FSH is a better marker of gonadal failure than LH. Serum IGF-1 concentrations can help in the evaluation of GH deficiency but must be interpreted carefully because levels are often low for chronologic age but within normal range for bone age in patients with DP. Thyroid function tests are often obtained but may not be necessary in the absence of any signs or symptoms of thyroid disease other than DP.

Brain and pituitary MRI may be indicated when there are signs or symptoms to suggest a CNS lesion. Otherwise, although some clinicians obtain brain imaging routinely, a reasonable strategy is to defer this test until age 15 years,

at which point many patients with CDGP will have spontaneously begun puberty and will require no further evaluation. Full neuroendocrine testing is warranted in patients with hypothalamic-pituitary tumors causing HH, as affected individuals may have additional pituitary hormone deficiencies. Assessment of olfactory bulbs on MRI may be helpful in patients with HH and anosmia, although there have been reports of patients with normal reproductive endocrine function despite anosmia and absence of olfactory bulbs.²⁶⁷

Additional Evaluation

Most boys will not have an apparent alternative cause for DP on initial evaluation, suggesting CDGP as the likely diagnosis. However, no current test can reliably distinguish CDGP from IHH, so the diagnosis of CDGP cannot be made with certainty.^{268,269} Observation usually resolves this conundrum; IHH is diagnosed if endogenous puberty has not begun by age 18 years. Several tests have been proposed to distinguish CDGP from IHH (see Table 18.3). If basal gonadotropin levels are inconclusive, some have suggested that stimulation by GnRH or a GnRH agonist may be helpful.^{270,271} Stimulated LH levels in the pubertal range indicate reactivation of the HPG axis and suggest that secondary sexual development is likely to occur within 1 year. However, the GnRH test alone often cannot differentiate CDGP from HH because prepubertal values may be observed both in HH and in individuals with CDGP who have not yet activated the HPG axis. Furthermore, individuals with HH experiencing stalled pubertal development will exhibit pubertal responses to GnRH. Data suggest that baseline inhibin B levels may facilitate discrimination between these conditions.^{272,273} Low inhibin B levels may predict IHH, but some adults with HH have normal inhibin B levels. Interestingly, recent data indicate that testicular volume may be as good as stimulated LH and inhibin B levels at distinguishing CDGP from HH.^{186,187} Thus further experience is needed before these additional biochemical tests can be adopted routinely.

GH secretion in the basal state, as well as after provocative testing, may be decreased in individuals with CDGP compared with pubertal peers. If concerns about growth are sufficient to warrant GH stimulation testing, sex-steroid priming with estrogen or testosterone is necessary for reliable results in patients with DP; estrogen stimulates endogenous GH secretion, and sex-steroid priming facilitates separation of true GH deficiency from physiologic low GH secretion that stems from low circulating sex steroids. Another strategy of assessing for true GH deficiency is to remeasure IGF-1 levels after the initiation of testosterone therapy. If IGF-1 increases appropriately with administration of testosterone, then true GH deficiency is less likely. If a patient has normal growth rate, GH provocation tests are not necessary, whereas low IGF-1 levels together with reduced growth velocity warrant testing.

TREATMENT OF DELAYED PUBERTY

The main options for management of CDGP include expectant observation or therapy with low-dose testosterone (Table 18.4 and recent reviews^{4,179,274,275}). If puberty has started, clinically or biochemically, and stature is not a major concern, reassurance with realistic adult height prediction is frequently all that is needed. If therapy is initiated, it is usually to assuage psychosocial difficulties that may derive from negative interactions with peers, decreased self-esteem, and anxiety about growth rate or body habitus.¹⁷⁹ Therapy is usually not initiated solely for medical reasons, such as accrual of bone mass. However,

conflicting data regarding the effect of pubertal timing on bone mass suggest that additional data are needed to determine definitively whether medical reasons to initiate therapy should be given more consideration.¹⁸⁵

Since the 1980s, there have been numerous studies of treatment of CDGP in boys, mostly involving treatment with short courses of low-dose androgens. There are limited randomized controlled trials, most with small numbers of subjects, but data from these and other studies strongly suggest that treatment leads to increased growth velocity and sexual maturation and positively affects psychosocial well-being without significant side effects, rapid advancement of bone age, or reduction of adult height.^{276–279} For boys who elect to be treated, we initiate supplementation with 50 mg testosterone enanthate or cypionate intramuscular (IM) each month for 3 to 6 months, which can be repeated for another 3 to 6 months with dose escalation (see Table 18.4). Although administration of IM testosterone esters is the mainstay of treatment, IM injections are painful and may require frequent healthcare visits for administration; moreover, serum testosterone levels lack diurnal rhythm and are characterized by nonphysiologic peaks and troughs. Hence there is rationale for alternative approaches, including subcutaneous testosterone injections²⁸⁰; oral androgens, such as testosterone undecanoate²⁸¹; and transdermal testosterone gels.^{282–284} The use of testosterone gels during the initiation of secondary sexual characteristics is somewhat limited by the requirement for low doses that can be delivered reproducibly. Novel therapies, such as synthetic kisspeptin,^{285,286} are also being explored. Although promising, additional data and experience are needed before these alternatives can be recommended for routine management in place of testosterone esters.

If spontaneous puberty has not occurred after 1 year, other diagnoses, such as persistent HH, should be reconsidered, and an MRI of the brain may be indicated. With lack of endogenous puberty, doses of testosterone can be gradually advanced toward adult levels, while still assessing (examination of testicular size, measurement of LH, FSH and testosterone) every 6 months for evidence of spontaneous puberty.

For a subset of patients with CDGP, short stature can be more concerning than DP, and indeed in idiopathic short stature (ISS) there is a component of pubertal delay in many of the subjects.²⁸⁷ Although the US Food and Drug Administration (FDA) has approved the use of GH for the treatment of ISS and height SDS 2.25 (or less) for age, this therapy has at best a modest effect on adult height in adolescents with CDGP, and its routine use in CDGP is not recommended.²⁸⁸ Nor do we use anabolic steroids, such as oxandrolone, for treatment of DP. Its use for treatment of ISS was not endorsed by the Consensus Statement,²⁸⁷ and it is rare that boys after age 14 years are not concerned about the lack of pubertal changes, which cannot be induced by oxandrolone because of its weak androgenic effects.

In boys with CDGP and short stature, another potential therapeutic approach is aromatase inhibition.^{289,290} AIs inhibit conversion of androgens to estrogens. Because estrogen is the predominant hormone needed for epiphyseal closure, AIs could prolong linear growth and potentially increase adult height.²⁹¹ In controlled trials in boys with short stature or DP,^{289,290} or GH deficiency,²⁹² AIs have been shown to delay bone maturation and increase predicted adult height. However, adult heights have not been reported from all studies, and characteristics of patients who respond and those who do not as well as the optimal timing, dose, and duration of AI treatment remain unresolved.²⁹³

A complete profile of the potential side effects associated with the use of AIs has not been established.²⁹⁴ Testosterone levels are elevated during AI therapy, and erythrocytosis has been observed in pubertal boys treated with AIs and in men

TABLE 18.4 Medications Used for the Treatment of Constitutional Delay of Growth and Puberty and Persistent Hypogonadism

Drug and Formulation	Treatment of Boys Recommended Dose		Side Effects and Cautions
	CDGP	HYPOGONADISM	
Testosterone ^a			Erythrocytosis, weight gain, prostate hyperplasia. High doses can cause premature epiphyseal closure. Not for use in boys with bone age < 10 years
Testosterone enanthate, cypionate, and propionate Testosterone enanthate has longer duration of effect than testosterone propionate. Intramuscular injection.	Not initiated until diagnosis of delayed puberty is made (~age 13.5–14 yrs). Initial dose 50–100 mg every 4 weeks for 3 to 6 months. If no signs of endogenous puberty after one course, consider repeat treatment with 25–50 mg increment in dose (not exceeding 100 mg).	Can initiate at age to allow puberty to occur in concert with peers at 50 mg every 4 weeks. Increase with 50-mg increments every 6 to 12 months. After reaching 100–150 mg monthly, decrease interval to every 2 weeks. Adult dose ~100–200 mg every 2 weeks IM or 80 mg SC weekly.	All intramuscular preparations: local side effects (pain, erythema, inflammatory reaction and sterile abscess). Priapism can occur in patients with sickle cell disease.
Testosterone undecanoate Intramuscular injection	No data available	Adult dose is 1000 mg every 10–14 weeks	
Testosterone gel Transdermal preparations, applied topically at bedtime.	No data available	Can be started when ~50% adult dose with intramuscular testosterone has been achieved. Adult dose 50–80 mg daily.	Local irritation. After applying, avoid close skin contact with others, and gel must be completely dry before putting on clothes.
Aromatase Inhibitors			Not yet approved for this indication. After onset of puberty, may increase gonadotropin secretion and circulating T levels. ³¹⁶
Letrozole PO	2.5 mg daily	Not recommended	Decreased level of high-density lipoprotein cholesterol, erythrocytosis, vertebral deformities have been reported. ¹⁷⁸
Anastrozole PO	1 mg daily	Not recommended	Less potent than letrozole
Pulsatile GnRH Subcutaneous pump ^b	Not recommended routinely	Initial: 5–25 ng/kg/pulse every 90–120 min; increase to 25–600 ng/kg/pulse	Requires extensive experience. Most physiologic form of replacement.
hCG plus recombinant FSH Subcutaneous or intramuscular hCG injections Subcutaneous rhFSH injections ^b	Not recommended routinely	hCG: dose 500 to 3000 IU twice weekly, increased to every 2 days. Dose adjusted based on serum T levels rhFSH: dose 75 to 225 IU 2–3 times weekly.	hCG: inflammation locally in the testis, may induce apoptosis of germ cells. In hypogonadotropic hypogonadism with prepubertal onset it is necessary to add FSH to induce testicular growth and spermatogenesis. No data on effects on future fertility.

^aTestosterone undecanoate tablets and anabolic steroids are not recommended for the induction of secondary sexual characteristics.

^bInduction of fertility may be less successful in men who have lower baseline testicular volumes, have received previously testosterone treatment,²⁹⁹ and have not previously received treatment with GnRH,^{296,297} or gonadotropins.²⁹⁶

CDGP, Constitutional delay of growth and puberty; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; IM, intramuscular; PO, orally; rhFSH, recombinant human follicle-stimulating hormone; SC, subcutaneous.

(Modified from Palmert, M. R., Dunkel, L. (2012). Clinical practice. Delayed puberty. *N Engl J Med*, 366, 443–453.)

with aromatase deficiency. Theoretical risks include a decline in adiponectin and subsequent development of nonalcoholic hepatic steatosis. Letrozole has no apparent adverse effects on BMD in adolescents. However, estrogen deficiency and letrozole treatment have been associated with impaired trabecular bone development in boys with ISS, and letrozole treatment during prepuberty or early puberty has also been associated with increased risk of vertebral body deformities.¹⁷⁸ Thus the use of AIs, even in adolescents with compromised predicted adult height, requires further careful study.²⁹⁵

In boys with persistent hypogonadism, the initial sex-steroid therapy is the same as for CDGP except that in cases of known defects therapy can be initiated at younger ages. Testosterone doses are gradually increased to full adult replacement levels over approximately 3 years (see Table 18.4). During the last year of dose escalation, the interval of

testosterone ester administration is decreased from once each month to once every 2 weeks, with a typical regimen for adult replacement being 100 to 200 mg IM every 2 weeks or 80 mg subcutaneously weekly. Transdermal preparations can be initiated during the final stages of dose escalation, if preferred. In HH, exogenous testosterone does not induce testicular growth or spermatogenesis. Thus if male patients with persistent HH wish to father a child or achieve increased testicular volume, induction of testicular growth and fertility can be accomplished through treatment with exogenous gonadotropins;²⁹⁶ in hypothalamic disorders, pulsatile GnRH can be also be used if available,^{296–299} (see Table 18.4). It remains an area of uncertainty whether fertility outcomes would be improved if GnRH and/or gonadotropins were used to induce puberty instead of waiting to administer these agents in adulthood.^{4,274,275,300–302}

In some cases of hypergonadotropic hypogonadism, individuals may still be able to father children using assisted reproductive technologies, such as intracytoplasmic sperm injection (ICSI), although this technology may be associated with a slightly increased risk of birth defects.³⁰³ In cases where ICSI is not desired or not feasible, adoption and in vitro fertilization using donor sperm provide additional options for those wanting to have children. As noted earlier, fertility preservation is an important topic for boys with Klinefelter syndrome and those with malignancies.

GYNECOMASTIA

Gynecomastia deserves discussion, although it is not necessarily associated with alterations in pubertal timing. Physical examination is extremely important in the evaluation of gynecomastia to distinguish among true gynecomastia, pseudogynecomastia, and pathologic gynecomastia. With the patient lying supine with his hands clasped beneath his head, the examiner can slowly compress the breast area between forefinger and thumb beginning from the sides of the breast. In true gynecomastia, the breast tissue is located concentrically under the nipple-areolar complex, feels rubbery or firm, and is often bilateral. During the early phase, ductal and epithelial proliferation occurs. This early proliferation is associated with periductal inflammation and edema, which correspond to the tenderness noted by the patient.²⁵⁴

The differential diagnosis includes lipomastia or pseudogynecomastia that is characterized by breast fullness and the absence of a nipple-areolar complex mound, such that the rib cage can be palpated under the areolae. Lipomas and neurofibromas can occur in the breast. Although extremely rare in adolescents, breast cancer feels firm or hard and is often located outside the nipple-areolar complex. Nipple discharge, skin dimpling, and nipple retraction do not occur in physiologic gynecomastia.

Pubertal or physiologic gynecomastia is a common midpubertal finding noted at Tanner stage 3 to stage 4 for pubic hair.³⁰⁴ Pubertal gynecomastia has been attributed to a relative and transient imbalance in the testosterone to estradiol ratio. With the progression of puberty and rising testosterone concentrations, the gynecomastia typically resolves or at least stabilizes.

Medications, exposures, and rare hormone disorders are associated with pathologic gynecomastia. Medications associated with gynecomastia include spironolactone, cimetidine, ketoconazole, estrogens, antiandrogens, GH, GnRH analogs, and 5 α -reductase inhibitors.³⁰⁵ Exposures to estrogen-containing substances, such as lavender and tea tree oils, and phytoestrogens have been considered to be etiologies. Other drugs associated with gynecomastia include tricyclic antidepressants, chemotherapeutic agents, and cardiovascular medications (e.g., digitalis). Drugs of abuse associated with gynecomastia include marijuana, ethanol, heroin, and amphetamines.

Estrogen excess can cause pathologic gynecomastia. Autosomal dominant mutations in the aromatase (*CYP19A1*) gene result in constitutively increased gene transcription and overexpression of the aromatase enzyme.³⁰⁶ Feminizing tumors can directly secrete estrogen. Peutz-Jeghers syndrome (PJS) is characterized by mucocutaneous pigmentation, multiple gastrointestinal polyps, and a variety of neoplasms. Gynecomastia has been described in prepubertal boys secondary to large cell calcifying Sertoli cell tumors associated with PJS or Carney complex.³⁰⁷ Gynecomastia can occur in other conditions including hyperthyroidism, hypogonadism, Klinefelter syndrome, androgen insensitivity, and ovotesticular disorder.³⁰⁸ Additional associations include obesity and liver disease.

Gynecomastia in a prepubertal boy is pathologic and warrants investigation. In contrast, gynecomastia occurring in a

midpubertal boy who is otherwise healthy most likely represents physiologic gynecomastia for which an extensive laboratory evaluation is unwarranted. Physiologic gynecomastia is typically self-limited. If present, treatment of the underlying disorder or removal of environmental causes is appropriate and may lead to regression of the breast tissue. AIs and estrogen receptor blockers have been used. An open label study concluded that anastrozole reduced breast size.³⁰⁹ However, the single randomized double-blind, placebo-controlled pediatric trial noted that anastrozole was no more effective than placebo to reduce gynecomastia, indicating that its routine use cannot be recommended.³¹⁰ If the gynecomastia is persistent and particularly troubling to the youth, surgical reduction can be pursued.

CONCLUSION

The foundations for the pubertal process are laid during gestation with the differentiation and development of the components of the HPG axis. The tools of molecular biology and molecular genetics have brought research into the factors governing the timing and tempo of puberty to the forefront of reproductive endocrinology. The identification of novel genes associated with pubertal disorders has generated more questions regarding the elusive critical components governing GnRH and gonadotropin secretion. Additional new discoveries about HPG axis regulation will further enhance our understanding of normal variation in pubertal timing and provide further insight into the etiology and treatment of pubertal disorders.

REFERENCES

- Patton GC, Viner R. Pubertal transitions in health. *Lancet*. 2007;369(9567):1130–1139.
- Cariboni A, Maggi R, Parnavelas JG. From nose to fertility: the long migratory journey of gonadotropin-releasing hormone neurons. *Trends Neurosci*. 2007;30(12):638–644.
- Gill JC, Tsai PS. Expression of a dominant negative FGF receptor in developing GNRH1 neurons disrupts axon outgrowth and targeting to the median eminence. *Biol Reprod*. 2006;74(3):463–472.
- Boehm U, Bouloux PM, Dattani MT, de Roux N, Dode C, Dunkel L, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment *Nat Rev Endocrinol*. 2015;11(9):547–564.
- Beate K, Joseph N, Nicolas de R, Wolfram K. Genetics of isolated hypogonadotropic hypogonadism: role of GnRH receptor and other genes. *Int J Endocrinol*. 2012;2012: 147893.
- Bancalari RE, Gregory LC, McCabe MJ, Dattani MT. Pituitary gland development: an update. *Endocr Dev*. 2012;23:1–15.
- Larkin S, Ansorge O. Development and microscopic anatomy of the pituitary gland. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., eds. *South Dartmouth (MA): Endotext*; 2000.
- Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev*. 2007;87(1):1–28.
- Valeri C, Scheingart HF, Rey RA. The prepubertal testis: biomarkers and functions. *Curr Opin Endocrinol Diabetes Obes*. 2013;20(3):224–233.
- Bay K, Main KM, Toppari J, Skakkebaek NE. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol*. 2011;8(4):187–196.
- Kuiri-Hanninen T, Seuri R, Tyrvaainen E, Turpeinen U, Hamalainen E, Stenman UH, et al. Increased activity of the hypothalamic-pituitary-testicular axis in infancy results in increased androgen action in premature boys. *J Clin Endocrinol Metab*. 2011;96(1):98–105.
- Rey RA, Grinspon RP, Gottlieb S, Pasqualini T, Knoblovits P, Aszpis S, et al. Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. *Andrology*. 2013;1(1):3–16.

13. Ojeda SR, Lomniczi A, Mastronardi C, Heger S, Roth C, Parent AS, et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? *Endocrinology*. 2006;147(3):1166–1174.
14. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KISS1-derived peptide receptor GPR54. *Proc Natl Acad Sci U S A*. 2003;100(19):10972–10976.
15. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Aciermo Jr JS, Shagoury JK, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med*. 2003;349(17):1614–1627.
16. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, et al. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med*. 2008;358(7):709–715.
17. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, et al. Mutations of the KISS1 gene in disorders of puberty. *J Clin Endocrinol Metab*. 2010;95(5):2276–2280.
18. Tena-Sempere M. Kisspeptin signaling in the brain: recent developments and future challenges. *Mol Cell Endocrinol*. 2010;314(2):164–169.
19. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*. 2009;41(3):354–358.
20. Pineda R, Garcia-Galiano D, Roseweir A, Romero M, Sanchez-Garrido MA, Ruiz-Pino F, et al. Critical roles of kisspeptins in female puberty and preovulatory gonadotropin surges as revealed by a novel antagonist. *Endocrinology*. 2010;151(2):722–730.
21. Song W, Li K, Sun C, Xue J. Kisspeptin permits the sexual development of female rats with normal and precocious puberty but is not a trigger for it. *Neuro Endocrinol Lett*. 2017;38(6):422–428.
22. Messina A, Langlet F, Chachlaki K, Roa J, Rasika S, Jouy N, et al. A microRNA switch regulates the rise in hypothalamic GnRH production before puberty. *Nat Neurosci*. 2016;19(6):835–844.
23. Ojeda SR, Prevot V, Heger S, Lomniczi A, Dziedzic B, Mungenast A. Glia-to-neuron signaling and the neuroendocrine control of female puberty. *Ann Med*. 2003;35(4):244–255.
24. Potorac I, Rivero-Muller A, Trehan A, Kielbus M, Jozwiak K, Pralong F, et al. A vital region for human glycoprotein hormone trafficking revealed by an LHB mutation. *J Endocrinol*. 2016;231(3):197–207.
25. Layman LC, Lee EJ, Peak DB, Namnoum AB, Vu KV, van Lingem BL, et al. Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene. *N Engl J Med*. 1997;337(9):607–611.
26. Lofrano-Porto A, Barra GB, Giacomini IA, Nascimento PP, Latronico AC, Casulari LA, et al. Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med*. 2007;357(9):897–904.
27. Josso N, Picard JY, Rey R, di Clemente N. Testicular anti-Mullerian hormone: history, genetics, regulation and clinical applications. *Pediatr Endocrinol Rev*. 2006;3(4):347–358.
28. Nielsen CT, Skakkebaek NE, Richardson DW, Darling JA, Hunter WM, Jorgensen M, et al. Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. *J Clin Endocrinol Metab*. 1986;62(3):532–535.
29. Jensen TK, Andersson AM, Hjollund NH, Scheike T, Kolstad H, Giwercman A, et al. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab*. 1997;82(12):4059–4063.
30. Dunkel L, Alfthan H, Stenman UH, Perheentupa J. Gonadal control of pulsatile secretion of luteinizing hormone and follicle-stimulating hormone in prepubertal boys evaluated by ultrasensitive time-resolved immunofluorometric assays. *J Clin Endocrinol Metab*. 1990;70(1):107–114.
31. Dunkel L, Alfthan H, Stenman UH, Tapanainen P, Perheentupa J. Pulsatile secretion of LH and FSH in prepubertal and early pubertal boys revealed by ultrasensitive time-resolved immunofluorometric assays. *Pediatr Res*. 1990;27(3):215–219.
32. Apter D, Butzow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab*. 1993;76(4):940–949.
33. Wu FC, Butler GE, Kelnar CJ, Huhtaniemi I, Veldhuis JD. Ontogeny of pulsatile gonadotropin releasing hormone secretion from midchildhood, through puberty, to adulthood in the human male: a study using deconvolution analysis and an ultrasensitive immunofluorometric assay. *J Clin Endocrinol Metab*. 1996;81(5):1798–1805.
34. Dunkel L, Alfthan H, Stenman UH, Selstam G, Rosberg S, Albertsson-Wikland K. Developmental changes in 24-hour profiles of luteinizing hormone and follicle-stimulating hormone from prepuberty to midstages of puberty in boys. *J Clin Endocrinol Metab*. 1992;74(4):890–897.
35. Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E. Twenty-four-hour profiles of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol levels: a semilongitudinal study throughout puberty in healthy boys. *J Clin Endocrinol Metab*. 1997;82(2):541–549.
36. Cousminer DL, Widen E, Palmert MR. The genetics of pubertal timing in the general population: recent advances and evidence for sex-specificity. *Curr Opin Endocrinol Diabetes Obes*. 2016;23(1):57–65.
37. Guerriero KA, Keen KL, Millar RP, Terasawa E. Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (*Macaca mulatta*): implication for the mechanism of puberty. *Endocrinology*. 2012;153(2):825–836.
38. Ojeda SR, Dubay C, Lomniczi A, Kaidar G, Matagne V, Sandau US, et al. Gene networks and the neuroendocrine regulation of puberty. *Mol Cell Endocrinol*. 2010;324(1–2):3–11.
39. Plant TM. Neuroendocrine control of the onset of puberty. *Front Neuroendocrinol*. 2015;38:73–88.
40. Toro CA, Aylwin CF, Lomniczi A. Hypothalamic epigenetics driving female puberty. *J Neuroendocrinol*. 2018;30(7). e12589.
41. Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, et al. Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med*. 2013;368(26):2467–2475.
42. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44(235):291–303.
43. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;45(239):13–23.
44. Largo RH, Prader A. Pubertal development in Swiss boys. *Helvetica Paediatr Acta*. 1983;38(3):211–228.
45. Roche AF, Wellens R, Attie KM, Siervogel RM. The timing of sexual maturation in a group of US white youths. *JPEM*. 1995;8(1):11–18.
46. Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, et al. Pubertal development in Danish children: comparison of recent European and US data. *Int J Androl*. 2006;29(1):247–255; discussion 86–90.
47. Harlan WR, Grillo GP, Cornoni-Huntley J, Leaverton PE. Secondary sex characteristics of boys 12 to 17 years of age: the U.S. Health Examination Survey. *J Pediatr*. 1979;95(2):293–297 1979.
48. Harlan WR, Harlan EA, Grillo GP. Secondary sex characteristics of girls 12 to 17 years of age: the U.S. Health Examination Survey. *J Pediatr*. 1980;96(6):1074–1078.
49. Palmert MR, Hayden DL, Mansfield MJ, Crigler Jr JF, Crowley Jr WF, Chandler DW, et al. The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. *J Clin Endocrinol Metab*. 2001;86(9):4536–4542.
50. Sklar CA, Kaplan SL, Grumbach MM. Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab*. 1980;51(3):548–556.
51. Wierman ME, Beardsworth DE, Crawford JD, Crigler Jr JF, Mansfield MJ, Bode HH, et al. Adrenarche and skeletal maturation during luteinizing hormone releasing hormone analogue suppression of gonadarche. *J Clin Invest*. 1986;77(1):121–126.
52. Remer T, Manz F. Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab*. 1999;84(11):3936–3944.
53. Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, et al. Opposing influences of prenatal and postnatal weight gain on adrenarche in normal boys and girls. *J Clin Endocrinol Metab*. 2004;89(6):2647–2651.

54. Topor LS, Asai M, Dunn J, Majzoub JA. Cortisol stimulates secretion of dehydroepiandrosterone in human adrenocortical cells through inhibition of 3betaHSD2. *J Clin Endocrinol Metab.* 2011;96(1):E31–E39.
55. Parent AS, Teilmann G, Juul A, Skakkebaek NE, Toppari J, Bourguignon JP. The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration. *Endocr Rev.* 2003;24(5):668–693.
56. Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sorensen TI, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics.* 2008;121(Suppl 3):S172–S191.
57. Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP. Stature and pubertal stage assessment in American boys: the 1988–1994 Third National Health and Nutrition Examination Survey. *J Adolesc Health.* 2002;30(3):205–212.
58. Sun SS, Schubert CM, Chumlea WC, Roche AF, Kulin HE, Lee PA, et al. National estimates of the timing of sexual maturation and racial differences among US children. *Pediatrics.* 2002;110(5):911–919.
59. Sun SS, Schubert CM, Liang R, Roche AF, Kulin HE, Lee PA, et al. Is sexual maturity occurring earlier among U.S. children? *J Adolesc Health.* 2005;37(5):345–355.
60. Herman-Giddens ME, Wang L, Koch G. Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey III, 1988–1994. *Arch Pediatr Adolesc Med.* 2001;155(9):1022–1028.
61. Biro FM, Lucky AW, Huster GA, Morrison JA. Pubertal staging in boys. *J Pediatr.* 1995;127(1):100–102.
62. Morris DH, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. Familial concordance for age at menarche: analyses from the Breakthrough Generations Study. *Paediatr Perinat Epidemiol.* 2011;25(3):306–311.
63. Lee PA, Guo SS, Kulin HE. Age of puberty: data from the United States of America. *Apmis.* 2001;109(2):81–88.
64. Sorensen K, Nielsen J, Busch P, et al. Measurement of testicular volume with Prader's orchidometer in 1,389 boys 5–15 1/2 years old. *Ugeskrift Laeger.* 1979;141:915–918.
65. Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965–1997. *Pediatr Res.* 2001;50(4):479–486.
66. Sorensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab.* 2010;95(1):263–270.
67. Gajdos ZK, Hirschhorn JN, Palmert MR. What controls the timing of puberty? An update on progress from genetic investigation. *Curr Opin Endocrinol Diabetes Obes.* 2009;16(1):16–24.
68. Plant TM, Barker-Gibb ML. Neurobiological mechanisms of puberty in higher primates. *Hum Reprod update.* 2004;10(1):67–77.
69. Fischbein S. Onset of puberty in MX and DZ twins. *Acta Genet Med Gemellol.* 1995;26(2):151–158.
70. Sklad M. The rate of growth and maturing of twins. *Acta Genet Med Gemellol.* 1977;26(3–4):221–237.
71. Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M, Rose RJ. Common genetic influences on BMI and age at menarche. *Hum Biol.* 1995;67(5):739–753.
72. van den Berg SM, Boomsma DI. The familial clustering of age at menarche in extended twin families. *Behav Genet.* 2007;37(5):661–667.
73. Hodges CA, Palmert MR. Genetic regulation of the variation in pubertal timing. In: Pescovitz OH, Walvoord EC, eds. *When Puberty is Precocious: Scientific and Clinical Aspects.* Totowa, NJ: Humana Press; 2007:83–102.
74. Palmert MR, Hirschhorn JN. Genetic approaches to stature, pubertal timing, and other complex traits. *Mol Genet Metab.* 2003;80(1–2), 1–10.
75. Towne B, Czerwinski SA, Demerath EW, Blangero J, Roche AF, Siervogel RM. Heritability of age at menarche in girls from the Fels Longitudinal Study. *Am J Phys Anthropol.* 2005;128(1):210–219.
76. Gajdos ZK, Henderson KD, Hirschhorn JN, Palmert MR. Genetic determinants of pubertal timing in the general population. *Mol Cell Endocrinol.* 2010;324(1–2):21–29.
77. Gajdos ZK, Butler JL, Henderson KD, He C, Supelak PJ, Egyud M, et al. Association studies of common variants in 10 hypogonadotropic hypogonadism genes with age at menarche. *J Clin Endocrinol Metab.* 2008;93(11):4290–4298.
78. He C, Kraft P, Chen C, Buring JE, Pare G, Hankinson SE, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet.* 2009;41(6):724–728.
79. Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet.* 2010;42(12):1077–1085.
80. Perry JR, Stolk L, Franceschini N, Lunetta KL, Zhai G, McArdle PF, et al. Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat Genet.* 2009;41(6):648–650.
81. Sulem P, Gudbjartsson DF, Rafnar T, Holm H, Olafsdottir EJ, Olafsdottir GH, et al. Genome-wide association study identifies sequence variants on 6q21 associated with age at menarche. *Nat Genet.* 2009;41(6):734–738.
82. Ong KK, Elks CE, Li S, Zhao JH, Luan J, Andersen LB, et al. Genetic variation in LIN28B is associated with the timing of puberty. *Nat Genet.* 2009;41(6):729–733.
83. Corre C, Shinoda G, Zhu H, Cousminer DL, Crossman C, Bellissimo C, et al. Sex-specific regulation of weight and puberty by the Lin28/let-7 axis. *J Endocrinol.* 2016;228(3):179–191.
84. Hirschhorn JN. Genomewide association studies—illuminating biologic pathways. *N Engl J Med.* 2009;360(17):1699–1701.
85. Day FR, Thompson DJ, Helgason H, Chasman DI, Finucane H, Sulem P, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet.* 2017;49(6):834–841.
86. Day FR, Bulik-Sullivan B, Hinds DA, Finucane HK, Murabito JM, Tung JY, et al. Shared genetic aetiology of puberty timing between sexes and with health-related outcomes. *Nat Commun.* 2015;6:8842.
87. Caronia LM, Martin C, Welt CK, Sykiotis GP, Quinton R, Thambundit A, et al. A genetic basis for functional hypothalamic amenorrhea. *N Engl J Med.* 2011;364(3):215–225.
88. Biro FM, Khoury P, Morrison JA. Influence of obesity on timing of puberty. *Int J Androl.* 2006;29(1):272–277; discussion 86–90.
89. Kaplowitz PB. Link between body fat and the timing of puberty. *Pediatrics.* 2008;121(Suppl 3):S208–S217.
90. Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics.* 1997;99(4):505–512.
91. Foster TA, Voors AW, Webber LS, Frerichs RR, Berenson GS. Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community—the Bogalusa Heart Study. *Am J Clin Nutr.* 1977;30(4):582–591.
92. Nathan BM, Sedlmeyer IL, Palmert MR. Impact of body mass index on growth in boys with delayed puberty. *J Pediatr Endocrinol Metab.* 2006;19(8):971–977.
93. Kaplowitz P. Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr.* 1998;133(6):745–749.
94. Sandhu J, Ben-Shlomo Y, Cole TJ, Holly J, Davey Smith G. The impact of childhood body mass index on timing of puberty, adult stature and obesity: a follow-up study based on adolescent anthropometry recorded at Christ's Hospital (1936–1964). *Int J Obes.* 2006;30(1):14–22 (2005).
95. He Q, Karlberg J. BMI in childhood and its association with height gain, timing of puberty, and final height. *Pediatr Res.* 2001;49(2):244–251.
96. Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE. Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. *Int J Androl.* 2007;30(6):537–542.
97. Juul A, Magnusdottir S, Scheike T, Prytz S, Skakkebaek NE. Age at voice break in Danish boys: effects of pre-pubertal body mass index and secular trend. *Int J Androl.* 2007;30(6):537–542.
98. Perry JR, Stolk L, Franceschini N, Lunetta KL, Zhai G, McArdle PF, et al. Meta-analysis of genome-wide association data identifies two loci influencing age at menarche. *Nat Genet.* 2009;41(6):648–650.

99. Boyne MS, Osmond C, Fraser RA, Reid M, Taylor-Bryan C, Soares-Wynter S, et al. Developmental origins of cardiovascular risk in Jamaican children: the Vulnerable Windows Cohort study. *Br J Nutr*. 2010;104(7):1026–1033.
100. Boyne MS, Thame M, Osmond C, Fraser RA, Gabay L, Reid M, et al. Growth, body composition, and the onset of puberty: longitudinal observations in Afro-Caribbean children. *J Clin Endocrinol Metab*. 2010;95(7):3194–3200.
101. Heger S, Korner A, Meigen C, Gausche R, Keller A, Keller E, et al. Impact of weight status on the onset and parameters of puberty: analysis of three representative cohorts from central Europe. *JPEM*. 2008;21(9):865–877.
102. Meigen C, Keller A, Gausche R, Kromeyer-Hauschild K, Bluher S, Kiess W, et al. Secular trends in body mass index in German children and adolescents: a cross-sectional data analysis via CrescNet between 1999 and 2006. *Metab Clin Exp*. 2008;57(7):934–939.
103. Lee JM, Wasserman R, Kaciroti N, Gebremariam A, Steffes J, Dowshen S, et al. Timing of puberty in overweight versus obese boys. *Pediatrics*, 137(2), e20150164.
104. Bourguignon JP, Juul A, Franssen D, Fudvoye J, Pinson A, Parent AS. Contribution of the endocrine perspective in the evaluation of endocrine disrupting chemical effects: the case study of pubertal timing. *Horm Res Paediatr*. 2016;86(4):221–232.
105. Mouritsen A, Aksglaede L, Sorensen K, Mogensen SS, Leffers H, Main KM, et al. Hypothesis: exposure to endocrine-disrupting chemicals may interfere with timing of puberty. *Int J Androl*. 2010;33(2):346–359.
106. Toppari J, Juul A. Trends in puberty timing in humans and environmental modifiers. *Mol Cell Endocrinol*. 2010;324(1-2):39–44.
107. Massart F, Saggese G. Oestrogenic mycotoxin exposures and precocious pubertal development. *Int J Androl*. 2010;33(2):369–376.
108. Williams PL, Sergeyev O, Lee MM, Korrick SA, Burns JS, Humblet O, et al. Blood lead levels and delayed onset of puberty in a longitudinal study of Russian boys. *Pediatrics*. 2010;125(5):e1088–e1096.
109. Greenspan LC, Lee MM. Endocrine disrupters and pubertal timing. *Curr Opin Endocrinol Diabetes Obes*. 2018;25(1):49–54.
110. Toppari J, Virtanen HE, Main KM, Skakkebaek NE. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection. *Birth Defects Res A Clin Mol Teratol*. 2010;88(10):910–919.
111. Skakkebaek NE, Toppari J, Soder O, Gordon CM, Divall S, Draznin M. The exposure of fetuses and children to endocrine disrupting chemicals: a European Society for Paediatric Endocrinology (ESPE) and Pediatric Endocrine Society (PES) call to action statement. *J Clin Endocrinol Metab*. 2011;96(10):3056–3058.
112. Sharpe RM. Pediatrics: endocrine disruption and human health effects—a call to action. *Nat Rev Endocrinol*. 2011;7(11):633–634.
113. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. Executive Summary to EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocr Rev*. 2015;36(6):593–602.
114. van Buuren S. Growth charts of human development. *Stat Methods Med Res*. 2014;23(4):346–368.
115. Nathan BM, Palmert MR. Regulation and disorders of pubertal timing. *Endocrinol Metab Clin North Am*. 2005;34(3):617–641, ix.
116. Carel JC, Leger J. Clinical practice. Precocious puberty. *N Engl J Med*. 2008;358(22):2366–2377.
117. Virdis R, Street ME, Bandello MA, Tripodi C, Donadio A, Villani AR, et al. Growth and pubertal disorders in neurofibromatosis type 1. *J Pediatr Endocrinol Metab*. 2003;16(Suppl 2):289–292.
118. Kaulfers AM, Backeljauw PF, Reifschneider K, Blum S, Michaud L, Weiss M, et al. Endocrine dysfunction following traumatic brain injury in children. *J Pediatr*. 2010;157(6):894–899.
119. Dauber A, Cunha-Silva M, Macedo DB, Brito VN, Abreu AP, Roberts SA, et al. Paternally inherited DLK1 deletion associated with familial central precocious puberty. *J Clin Endocrinol Metab*. 2017;102(5):1557–1567.
120. Gomes LG, Cunha-Silva M, Crespo RP, Ramos CO, Montenegro LR, Canton A, et al. DLK1 is a novel link between reproduction and metabolism. *J Clin Endocrinol Metab*. 2019;104(6):2112–2120.
121. Shenker A, Laue L, Kosugi S, Merendino Jr JJ, Minegishi T, Cutler Jr GB. A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature*. 1993;365(6447):652–654.
122. Schedewie HK, Reiter EO, Beitins IZ, Seyed S, Wooten VD, Jimenez JF, et al. Testicular leydig cell hyperplasia as a cause of familial sexual precocity. *J Clin Endocrinol Metab*. 1981;52(2):271–278.
123. Latronico AC, Segaloff DL. Naturally occurring mutations of the luteinizing-hormone receptor: lessons learned about reproductive physiology and G protein-coupled receptors. *Am J Hum Genet*. 1999;65(4):949–958.
124. Shenker A, Weinstein LS, Moran A, Pescovitz OH, Charest NJ, Boney CM, et al. Severe endocrine and nonendocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein GS. *J Pediatr*. 1993;123(4):509–518.
125. Mamkin I, Philibert P, Anhalt H, Ten S, Sultan C. Unusual phenotypical variations in a boy with McCune-Albright syndrome. *Horm Res Paediatr*. 2010;73(3):215–222.
126. Lala R, Matarazzo P, Andreo M, Defilippi C, de Sanctis C. Impact of endocrine hyperfunction and phosphate wasting on bone in McCune-Albright syndrome. *JPEM*. 2002;15(Suppl 3):913–920.
127. Boyce AM, Chong WH, Shawker TH, Pinto PA, Linehan WM, Bhattacharyya N, et al. Characterization and management of testicular pathology in McCune-Albright syndrome. *J Clin Endocrinol Metab*. 2012;97(9):E1782–E1790.
128. Van Wyk JJ, Grumbach MM. Syndrome of precocious menstruation and galactorrhoea in juvenile hypothyroidism: an example of hormonal overlap in pituitary feedback. *J Pediatr*. 1960;57:416–435.
129. Anasti JN, Flack MR, Froehlich J, Nelson LM, Nisula BC. A potential novel mechanism for precocious puberty in juvenile hypothyroidism. *J Clin Endocrinol Metab*. 1995;80(1):276–279.
130. Bruder JM, Samuels MH, Bremner WJ, Ridgway EC, Wierman ME. Hypothyroidism-induced macroorchidism: use of a gonadotropin-releasing hormone agonist to understand its mechanism and augment adult stature. *J Clin Endocrinol Metab*. 1995;80(1):11–16.
131. Durbin KL, Diaz-Montes T, Loveless MB, Van Wyk and Grumbach syndrome: an unusual case and review of the literature. *J Pediatr Adolesc Gynecol*. 2011;24(4):e93–e96.
132. Boot AM, Lumbroso S, Verhoef-Post M, Richter-Unruh A, Looijenga LH, Funaro A, et al. Mutation analysis of the LH receptor gene in Leydig cell adenoma and hyperplasia and functional and biochemical studies of activating mutations of the LH receptor gene. *J Clin Endocrinol Metab*. 2011;96(7):E1197–E1205.
133. Kaplowitz P. Clinical characteristics of 104 children referred for evaluation of precocious puberty. *J Clin Endocrinol Metab*. 2004;89(8):3644–3650.
134. Palmert MR, Malin HV, Boepple PA. Unsustained or slowly progressive puberty in young girls: initial presentation and long-term follow-up of 20 untreated patients. *J Clin Endocrinol Metab*. 1999;84(2):415–423.
135. Bordini B, Littlejohn E, Rosenfield RL. LH dynamics in overweight girls with premature adrenarche and slowly progressive sexual precocity. *Int J Pediatr Endocrinol*. 2010;2010. 724996.
136. DeSalvo DJ, Mehra R, Vaidyanathan P, Kaplowitz PB. In children with premature adrenarche, bone age advancement by 2 or more years is common and generally benign. *J Pediatr Endocrinol Metab*. 2013;26(3-4):215–221.
137. Oron T, Lebenthal Y, de Vries L, Yackobovitch-Gavan M, Phillip M, Lazar L. Interrelationship of extent of precocious adrenarche in appropriate for gestational age girls with clinical outcome. *J Pediatr*. 2012;160(2):308–313.
138. Klein KO. Precocious puberty: who has it? Who should be treated? *J Clin Endocrinol Metab*. 1999;84(2):411–414.
139. Carel JC, Lahlou N, Roger M, Chaussain JL. Precocious puberty and statural growth. *Hum Reprod update*. 2004;10(2):135–147.
140. Tremblay L, Frigon JY. Precocious puberty in adolescent girls: a biomarker of later psychosocial adjustment problems. *Child Psychiatry Hum Dev*. 2005;36(1):73–94.
141. Michaud PA, Suris JC, Deppen A. Gender-related psychological and behavioural correlates of pubertal timing in a national sample of Swiss adolescents. *Mol Cell Endocrinol*. 2006;254–255:172–178.
142. Day FR, Elks CE, Murray A, Ong KK, Perry JR. Puberty timing associated with diabetes, cardiovascular disease and also diverse

- health outcomes in men and women: the UK Biobank study. *Sci Rep.* 2015;5: 11208.
143. Bell JA, Carslake D, Wade KH, Richmond RC, Langdon RJ, Vincent EE, et al. Influence of puberty timing on adiposity and cardiometabolic traits: A Mendelian randomisation study. *PLoS Med.* 2018;15(8). e1002641.
 144. Gill D, Brewer CF, Del Greco MF, Sivakumaran P, Bowden J, Sheehan NA, et al. Age at menarche and adult body mass index: a Mendelian randomization study. *Int J Obes (Lond).* 2018;42(9):1574–1581.
 145. Mumby HS, Elks CE, Li S, Sharp SJ, Khaw KT, Luben RN, et al. Mendelian randomisation study of childhood BMI and early menarche. *J Obes.* 2011;2011: 180729.
 146. Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, Antoniazzi F, et al. Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics.* 2009;123(4):e752–e762.
 147. Henley DV, Lipson N, Korach KS, Bloch CA. Prepubertal gynaecomastia linked to lavender and tea tree oils. *N Engl J Med.* 2007;356(5):479–485.
 148. Neely EK, Hintz RL, Wilson DM, Lee PA, Gautier T, Argente J, et al. Normal ranges for immunochemiluminometric gonadotropin assays. *J Pediatr.* 1995;127(1):40–46.
 149. Neely EK, Wilson DM, Lee PA, Stene M, Hintz RL. Spontaneous serum gonadotropin concentrations in the evaluation of precocious puberty. *J Pediatr.* 1995;127(1):47–52.
 150. Pescovitz OH, Hench KD, Barnes KM, Loriaux DL, Cutler Jr GB. Premature thelarche and central precocious puberty: the relationship between clinical presentation and the gonadotropin response to luteinizing hormone-releasing hormone. *J Clin Endocrinol Metab.* 1988;67(3):474–479.
 151. De Sanctis V, Corrias A, Rizzo V, Bertelloni S, Urso L, Galluzzi F, et al. Etiology of central precocious puberty in males: the results of the Italian Study Group for Physiopathology of Puberty. *J Pediatr Endocrinol Metab.* 2000;13(Suppl 1):687–693.
 152. Cisternino M, Arrigo T, Pasquino AM, Tinelli C, Antoniazzi F, Beduschi L, et al. Etiology and age incidence of precocious puberty in girls: a multicentric study. *J Pediatr Endocrinol Metab.* 2000;13(Suppl 1):695–701.
 153. Bar A, Linder B, Sobel EH, Saenger P, DiMartino-Nardi J, Bayley-Pinneau method of height prediction in girls with central precocious puberty: correlation with adult height. *J Pediatr.* 1995;126(6):955–958.
 154. Mul D, Bertelloni S, Carel JC, Saggese G, Chaussain JL, Oostdijk W. Effect of gonadotropin-releasing hormone agonist treatment in boys with central precocious puberty: final height results. *Horm Res.* 2002;58(1):1–7.
 155. Bertelloni S, Mul D. Treatment of central precocious puberty by GnRH analogs: long-term outcome in men. *Asian J Androl.* 2008;10(4):525–534.
 156. Neely EK, Lee PA, Bloch CA, Larsen L, Yang D, Mattia-Goldberg C, et al. Leuprolide acetate 1-month depot for central precocious puberty: hormonal suppression and recovery. *Int J Pediatr Endocrinol.* 2010;2010: 398639.
 157. Lee PA, Neely EK, Fuqua J, Yang D, Larsen LM, Mattia-Goldberg C, et al. Efficacy of Leuprolide Acetate 1-month depot for central precocious puberty (CPP): growth outcomes during a prospective, longitudinal study. *Int J Pediatr Endocrinol.* 2011;2011(1):7.
 158. Trueman JA, Tillmann V, Cusick CF, Foster P, Patel L, Hall CM, et al. Suppression of puberty with long-acting goserelin (Zoladex-LA): effect on gonadotrophin response to GnRH in the first treatment cycle. *Clin Endocrinol (Oxf).* 2002;57(2):223–230.
 159. Lee PA, Klein K, Mauras N, Neely EK, Bloch CA, Larsen L, et al. Efficacy and safety of leuprolide acetate 3-month depot 11.25 milligrams or 30 milligrams for the treatment of central precocious puberty. *J Clin Endocrinol Metab.* 2012;97(5):1572–1580.
 160. Eugster EA, Clarke W, Kletter GB, Lee PA, Neely EK, Reiter EO, et al. Efficacy and safety of histrelin subdermal implant in children with central precocious puberty: a multicenter trial. *J Clin Endocrinol Metab.* 2007;92(5):1697–1704.
 161. Hirsch HJ, Gillis D, Strich D, Chertin B, Farkas A, Lindenberg T, et al. The histrelin implant: a novel treatment for central precocious puberty. *Pediatrics.* 2005;116(6):e798–e802.
 162. Rahhal S, Clarke WL, Kletter GB, Lee PA, Neely EK, Reiter EO, et al. Results of a second year of therapy with the 12-month histrelin implant for the treatment of central precocious puberty. *Int J Pediatr Endocrinol.* 2009;2009: 812517.
 163. Almeida MQ, Brito VN, Lins TS, Guerra-Junior G, de Castro M, Antonini SR, et al. Long-term treatment of familial male-limited precocious puberty (testotoxicosis) with cyproterone acetate or ketoconazole. *Clin Endocrinol (Oxf).* 2008;69(1):93–98.
 164. Babovic-Vuksanovic D, Donaldson MD, Gibson NA, Wallace AM. Hazards of ketoconazole therapy in testotoxicosis. *Acta Paediatr.* 1994;83(9):994–997.
 165. Holland FJ, Fishman L, Bailey JD, Fazekas AT. Ketoconazole in the management of precocious puberty not responsive to LHRH-analogue therapy. *N Engl J Med.* 1985;312(16):1023–1028.
 166. Rajfer J, Sikka SC, Rivera F, Handelsman DJ. Mechanism of inhibition of human testicular steroidogenesis by oral ketoconazole. *J Clin Endocrinol Metab.* 1986;63(5):1193–1198.
 167. Soriano-Guillen L, Lahlou N, Chauvet G, Roger M, Chaussain JL, Carel JC. Adult height after ketoconazole treatment in patients with familial male-limited precocious puberty. *J Clin Endocrinol Metab.* 2005;90(1):147–151.
 168. Laue L, Jones J, Barnes KM, Cutler Jr GB. Treatment of familial male precocious puberty with spironolactone, testolactone, and deslorelin. *J Clin Endocrinol Metab.* 1993;76(1):151–155.
 169. Laue L, Kenigsberg D, Pescovitz OH, Hench KD, Barnes KM, Loriaux DL, et al. Treatment of familial male precocious puberty with spironolactone and testolactone. *N Engl J Med.* 1989;320(8):496–502.
 170. Leschek EW, Jones J, Barnes KM, Hill SC, Cutler Jr GB. Six-year results of spironolactone and testolactone treatment of familial male-limited precocious puberty with addition of deslorelin after central puberty onset. *J Clin Endocrinol Metab.* 1999;84(1):175–178.
 171. Merke DP, Keil MF, Jones JV, Fields J, Hill S, Cutler Jr GB. Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation despite elevated androgen levels in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab.* 2000;85(3):1114–1120.
 172. Best TR, Jenkins JK, Murphy FY, Nicks SA, Bussell KL, Vesely DL. Persistent adrenal insufficiency secondary to low-dose ketoconazole therapy. *Am J Med.* 1987;82(3 Spec No):676–680.
 173. Eyssette-Guerreau S, Pinto G, Sultan A, Le Merrer M, Sultan C, Polak M. Effectiveness of anastrozole and cyproterone acetate in two brothers with familial male precocious puberty. *J Pediatr Endocrinol Metab.* 2008;21(10):995–1002.
 174. Hero M, Janne OA, Nanto-Salonen K, Dunkel L, Raivio T. Circulating antiandrogenic activity in children with congenital adrenal hyperplasia during peroral flutamide treatment. *J Clin Endocrinol Metab.* 2005;90(9):5141–5145.
 175. Kreher NC, Pescovitz OH, Delameter P, Tiulpakov A, Hochberg Z. Treatment of familial male-limited precocious puberty with bicalutamide and anastrozole. *J Pediatr.* 2006;149(3):416–420.
 176. Tessaris D, Matarazzo P, Mussa A, Tuli G, Verna F, Fiore L, et al. Combined treatment with bicalutamide and anastrozole in a young boy with peripheral precocious puberty due to McCune-Albright Syndrome. *Endocr J.* 2012;59(2):111–117.
 177. Hero M, Maury S, Luotoniemi E, Service E, Dunkel L. Cognitive effects of aromatase inhibitor therapy in peripubertal boys. *Eur J Endocrinol.* 2010;163(1):149–155.
 178. Hero M, Toivainen-Salo S, Wickman S, Makitie O, Dunkel L. Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J Bone Miner Res.* 2010;25(7):1536–1543.
 179. Palmert MR, Dunkel L. Clinical practice. Delayed puberty. *N Engl J Med.* 2012;366(5):443–453.
 180. Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988–1994. *Pediatrics.* 2002;110(4):752–757.
 181. Susman EJ, Houts RM, Steinberg L, Belsky J, Cauffman E, Dehart G, et al. Longitudinal development of secondary sexual characteristics in girls and boys between ages 9 1/2 and 15 1/2 years. *Arch Pediatr Adolesc Med.* 2010;164(2):166–173.

182. Aksglaede L, Sorensen K, Petersen JH, Skakkebaek NE, Juul A. Recent decline in age at breast development: the Copenhagen Puberty Study. *Pediatrics*. 2009;123(5):e932–e939.
183. Zhu J, Chan YM. Adult consequences of self-limited delayed puberty. *Pediatrics*. 2017;139(6).
184. Albanese A, Stanhope R. Predictive factors in the determination of final height in boys with constitutional delay of growth and puberty. *J Pediatr*. 1995;126(4):545–550.
185. Gilsanz V, Chalfant J, Kalkwarf H, Zemel B, Lappe J, Oberfield S, et al. Age at onset of puberty predicts bone mass in young adulthood. *J Pediatr*. 2011;158(1):100–105. 5 e1–e2.
186. Sedlmeyer IL, Palmert MR. Delayed puberty: analysis of a large case series from an academic center. *J Clin Endocrinol Metab*. 2002;87(4):1613–1620.
187. Varimo T, Miettinen PJ, Kansakoski J, Raivio T, Hero M. Congenital hypogonadotropic hypogonadism, functional hypogonadotropism or constitutional delay of growth and puberty? An analysis of a large patient series from a single tertiary center. *Hum Reprod*. 2017;32(1):147–153.
188. Palmert MR, Hirschhorn JN. Genetic approaches to stature, pubertal timing, and other complex traits. *Mol Genet Metab*. 2003;80:1–2), 1–10.
189. Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol*. 2009;5(10):569–576.
190. Bhangoo A, Jacobson-Dickman E. The genetics of idiopathic hypogonadotropic hypogonadism: unraveling the biology of human sexual development. *Pediatr Endocrinol Rev*. 2009;6(3):395–404.
191. Herbison AE. Genetics of puberty. *Horm Res*. 2007;68(Suppl 5): 75–79.
192. Kalantaridou SN, Chrousos GP. Clinical review 148: monogenic disorders of puberty. *J Clin Endocrinol Metab*. 2002;87(6): 2481–2494.
193. Silveira LF, MacColl GS, Bouloux PM. Hypogonadotropic hypogonadism. *Semin Reprod Med*. 2002;20(4):327–338.
194. Martin C, Balasubramanian R, Dwyer AA, Au MG, Sidis Y, Kaiser UB, et al. The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. *Endocr Rev*. 2011;32(2):225–246.
195. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci U S A*. 2010;107(34):15140–15144.
196. Hay C, Wu F. Genetics and hypogonadotropic hypogonadism. *Curr Opin Obstet Gynecol*. 2002;14(3):303–308.
197. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature*. 1991;353(6344):529–536.
198. Legouis R, Hardelin JP, Leveilliers J, Claverie JM, Compain S, Wunderle V, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell*. 1991;67(2):423–435.
199. Dode C, Leveilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*. 2003;33(4): 463–465.
200. Dode C, Teixeira L, Leveilliers J, Fouveaut C, Bouchard P, Kottler ML, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet*. 2006;2(10). e175.
201. McCabe MJ, Gaston-Massuet C, Tziaferi V, Gregory LC, Alatzoglou KS, Signore M, et al. Novel FGF8 mutations associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction. *J Clin Endocrinol Metab*. 2011;96(10):E1709–E1718.
202. Hodges CA, Palmert MR. Genetic regulation of the variation in pubertal timing. In: Pescovitz OH, Walvoord EC, eds. *When Puberty is Precocious: Scientific and Clinical Aspects*. Totowa: NJ, Humana Press; 2007:83–102.
203. Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, Pignatelli D, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotropin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab*. 2008;93(9):3551–3559.
204. Kramer PR, Wray S. Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. *Gene Dev*. 2000; 14(14):1824–1934.
205. Miura K, Acierio Jr JS, Seminara SB. Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). *J Hum Genet*. 2004;49(5):265–268.
206. Pitteloud N, Quinton R, Pearce S, Raivio T, Acierio J, Dwyer A, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest*. 2007;117(2):457–463.
207. Xu N, Kim HG, Bhagavath B, Cho SG, Lee JH, Ha K, et al. Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril*. 2011;95(5). 1613–20 e1–7.
208. Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA*. 2011;108(28):11524–11549.
209. Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, et al. SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. *PLoS Genet*. 2012;8(8). e1002896.
210. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, et al. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet*. 2010;87(4):465–479.
211. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet*. 2013;92(5):725–743.
212. Bhagavath B, Ozata M, Ozdemir IC, Bolu E, Bick DP, Sherins RJ, et al. The prevalence of gonadotropin-releasing hormone receptor mutations in a large cohort of patients with hypogonadotropic hypogonadism. *Fertil Steril*. 2005;84(4):951–957.
213. Beranova M, Oliveira LM, Bedecarrats GY, Schipani E, Vallejo M, Ammini AC, et al. Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2001;86(4):1580–1588.
214. Lin L, Conway GS, Hill NR, Dattani MT, Hindmarsh PC, Achermann JC. A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of growth and puberty with subsequent borderline oligospermia. *J Clin Endocrinol Metab*. 2006;91(12):5117–5121.
215. Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, et al. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci U S A*. 2009;106(28):11703–11708.
216. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med*. 2009;360(26): 2742–2748.
217. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet*. 2009;41(3): 354–358.
218. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. 1997;16(3):303–306.
219. Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, Hughes VA, et al. Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med*. 2007;357(9):863–873.
220. Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in

- neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab.* 2010;95(6):2857–2867.
221. Laitinen EM, Tommiska J, Sane T, Vaaralahti K, Toppari J, Raivio T. Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations. *PLoS One.* 2012;7(6): e39450.
 222. Bedecarrats GY, Kaiser UB. Mutations in the human gonadotropin-releasing hormone receptor: insights into receptor biology and function. *Semin Reprod Med.* 2007;25(5):368–378.
 223. Kaiserman KB, Nakamoto JM, Geffner ME, McCabe ER. Mini-puberty of infancy and adolescent pubertal function in adrenal hypoplasia congenita. *J Pediatr.* 1998;133(2):300–302.
 224. Peter M, Viemann M, Partsch CJ, Sippell WG. Congenital adrenal hypoplasia: clinical spectrum, experience with hormonal diagnosis, and report on new point mutations of the DAX-1 gene. *J Clin Endocrinol Metab.* 1998;83(8):2666–2674.
 225. Han JC, Balagopal P, Sweeten S, Darmaun D, Mauras N. Evidence for hypermetabolism in boys with constitutional delay of growth and maturation. *J Clin Endocrinol Metab.* 2006;91(6):2081–2086.
 226. Wilson DA, Hofman PL, Miles HL, Sato TA, Billett NE, Robinson EM, et al. Enhanced insulin sensitivity in prepubertal children with constitutional delay of growth and development. *J Pediatr.* 2010;156(2):308–312.
 227. Sedlmeyer IL, Hirschhorn JN, Palmert MR. Pedigree analysis of constitutional delay of growth and maturation: determination of familial aggregation and inheritance patterns. *J Clin Endocrinol Metab.* 2002;87(12):5581–5586.
 228. Wehkalampi K, Widen E, Laine T, Palotie A, Dunkel L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *J Clin Endocrinol Metab.* 2008;93(3):723–728.
 229. Vaaralahti K, Wehkalampi K, Tommiska J, Laitinen EM, Dunkel L, Raivio T. The role of gene defects underlying isolated hypogonadotropic hypogonadism in patients with constitutional delay of growth and puberty. *Fertil Steril.* 2011;95(8):2756–2758.
 230. Tommiska J, Wehkalampi K, Vaaralahti K, Laitinen EM, Raivio T, Dunkel L. LIN28B in constitutional delay of growth and puberty. *J Clin Endocrinol Metab.* 2010;95(6):3063–3066.
 231. Sedlmeyer IL, Pearce CL, Trueman JA, Butler JL, Bersaglieri T, Read AP, et al. Determination of sequence variation and haplotype structure for the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes: investigation of role in pubertal timing. *J Clin Endocrinol Metab.* 2005;90(2):1091–1099.
 232. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature.* 1991;353(6344):529–536.
 233. Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, et al. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *J Clin Invest.* 2008;118(8):2822–2831.
 234. Pitteloud N, Acierno Jr JS, Meysing AU, Dwyer AA, Hayes FJ, Crowley Jr WF. Reversible kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. *J Clin Endocrinol Metab.* 2005;90(3):1317–1322.
 235. Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, et al. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet.* 2008;83(4):511–519.
 236. Pitteloud N, Meysing A, Quinton R, Acierno Jr JS, Dwyer AA, Plummer L, et al. Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. *Mol Cell Endocrinol.* 2006;254–255:60–69.
 237. Xu C, Messina A, Somm E, Miraoui H, Kinnunen T, Acierno Jr J, et al. KLB, encoding beta-Klotho, is mutated in patients with congenital hypogonadotropic hypogonadism. *EMBO Mol Med.* 2017;9(10):1379–1397.
 238. Zhu J, Choa RE, Guo MH, Plummer L, Buck C, Palmert MR, et al. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2015;100(4):E646–E654.
 239. Cassatella D, Howard SR, Acierno JS, Xu C, Papadakis GE, Santoni FA, et al. Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures. *Eur J Endocrinol.* 2018;178(4):377–388.
 240. Chevrier L, Guimiot F, de Roux N. GnRH receptor mutations in isolated gonadotropic deficiency. *Mol Cell Endocrinol.* 2011;346(1–2):21–28.
 241. Lin L, Conway GS, Hill NR, Dattani MT, Hindmarsh PC, Achermann JC. A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of growth and puberty with subsequent borderline oligospermia. *J Clin Endocrinol Metab.* 2006;91(12):5117–5121.
 242. Howard SR, Guasti L, Ruiz-Babot G, Mancini A, David A, Storr HL, et al. IGSF10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty. *EMBO Mol Med.* 2016;8(6):626–642.
 243. Howard SR, Oleari R, Poliandri A, Chantzara V, Fantin A, Ruiz-Babot G, et al. HS6ST1 insufficiency causes self-limited delayed puberty in contrast with other GnRH deficiency genes. *J Clin Endocrinol Metab.* 2018;103(9):3420–3429.
 244. Howard SR, Guasti L, Poliandri A, David A, Cabrera CP, Barnes MR, et al. Contributions of function-altering variants in genes implicated in pubertal timing and body mass for self-limited delayed puberty. *J Clin Endocrinol Metab.* 2018;103(2):649–659.
 245. Mancini A, Howard SR, Cabrera CP, Barnes MR, David A, Wehkalampi K, et al. EAP1 regulation of GnRH promoter activity is important for human pubertal timing. *Hum Mol Genet.* 2019;28(8):1357–1368.
 246. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab.* 2003;88(2):622–626.
 247. Ottesen AM, Aksglaede L, Garn I, Tartaglia N, Tassone F, Gravholt CH, et al. Increased number of sex chromosomes affects height in a nonlinear fashion: a study of 305 patients with sex chromosome aneuploidy. *Am J Med Genet A.* 2010;152A(5):1206–1212.
 248. Aksglaede L, Juul A. Testicular function and fertility in men with Klinefelter syndrome: a review. *Eur J Endocrinol.* 2013;168(4):R67–R76.
 249. Oates RD. The natural history of endocrine function and spermatogenesis in Klinefelter syndrome: what the data show. *Fertil Steril.* 2012;98(2):266–273.
 250. Groth KA, Skakkebaek A, Host C, Gravholt CH, Bojesen A. Clinical review: Klinefelter syndrome—a clinical update. *J Clin Endocrinol Metab.* 2013;98(1):20–30.
 251. Sokol RZ. It's not all about the testes: medical issues in Klinefelter patients. *Fertil Steril.* 2012;98(2):261–265.
 252. Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, Skakkebaek A. Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev.* 2018;39(4):389–423.
 253. Ankarberg-Lindgren C, Westphal O, Dahlgren J. Testicular size development and reproductive hormones in boys and adult males with Noonan syndrome: a longitudinal study. *Eur J Endocrinol.* 2011;165(1):137–144.
 254. Vogels A, Moerman P, Frijns JP, Bogaert GA. Testicular histology in boys with Prader-Willi syndrome: fertile or infertile? *J Urol.* 2008;180(4 Suppl):1800–1804.
 255. Siemensa EP, van Wijngaarden de Lind R.F, Otten B.J., de Jong F.H., Hokken-Koelega A.C. Testicular failure in boys with Prader-Willi syndrome: longitudinal studies of reproductive hormones. *J Clin Endocrinol Metab.* 2012;97(3):E452–E459.
 256. Finkelstein JS, Klibanski A, Neer RM, Greenspan SL, Rosenthal DI, Crowley Jr WF. Osteoporosis in men with idiopathic hypogonadotropic hypogonadism. *Ann Intern Med.* 1987;106(3):354–361.
 257. De Rosa M, Paesano L, Nuzzo V, Zarrilli S, Del Puente A, Oriente P, et al. Bone mineral density and bone markers in hypogonadotropic and hypergonadotropic hypogonadal men after prolonged testosterone treatment. *J Endocrinol Invest.* 2001;24(4):246–252.
 258. Finkelstein JS, Neer RM, Biller BM, Crawford JD, Klibanski A. Osteopenia in men with a history of delayed puberty. *N Engl J Med.* 1992;326(9):600–604.

259. Cousminer DL, Mitchell JA, Chesi A, Roy SM, Kalkwarf HJ, Lappe JM, et al. Genetically determined later puberty impacts lowered bone mineral density in childhood and adulthood. *J Bone Miner Res*. 2018;33(3):430–436.
260. Canoy D, Beral V, Balkwill A, Wright FL, Kroll ME, Reeves GK, et al. Age at menarche and risks of coronary heart and other vascular diseases in a large UK cohort. *Circulation*. 2015;131(3):237–244.
261. Moriyama T. Analysis of the binding site of macroamylase (IgA-amylase complex). *Nihon Shokakibyo Gakkai Zasshi*. 1984;81(10):2605.
262. De Luca F, Argente J, Cavallo L, Crowne E, Delemarre-Van de Waal HA, De Sanctis C, et al. Management of puberty in constitutional delay of growth and puberty. *J Pediatr Endocrinol Metab*. 2001;14(Suppl 2):953–957.
263. Lee JM, Kaciroti N, Appugliese D, Corwyn RF, Bradley RH, Lumeng JC. Body mass index and timing of pubertal initiation in boys. *Arch Pediatr Adolesc Med*. 2010;164(2):139–144.
264. Counts DR, Pescovitz OH, Barnes KM, Hench KD, Chrousos GP, Sherins RJ, et al. Dissociation of adrenarche and gonadarche in precocious puberty and in isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 1987;64(6):1174–1178.
265. Harrington J, Palmert MR. Distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab*. 2012;97(9):3056–3067.
266. Abitbol L, Zborovski S, Palmert MR. Evaluation of delayed puberty: what diagnostic tests should be performed in the seemingly otherwise well adolescent? *Arch Dis Child*. 2016;101(8):767–771.
267. Moya-Plana A, Villanueva C, Laccourreye O, Bonfils P, de Roux N. PROKR2 and PROK2 mutations cause isolated congenital anosmia without gonadotropic deficiency. *Eur J Endocrinol*. 2013;168(1):31–37.
268. Harrington J, Palmert MR. Clinical review: distinguishing constitutional delay of growth and puberty from isolated hypogonadotropic hypogonadism: critical appraisal of available diagnostic tests. *J Clin Endocrinol Metab*. 2012;97(9):3056–3067.
269. Young J. Approach to the male patient with congenital hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2012;97(3):707–718.
270. Grinspon RP, Ropelato MG, Gottlieb S, Keselman A, Martinez A, Ballerini MG, et al. Basal follicle-stimulating hormone and peak gonadotropin levels after gonadotropin-releasing hormone infusion show high diagnostic accuracy in boys with suspicion of hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2010;95(6):2811–2818.
271. Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. *J Clin Endocrinol Metab*. 2007;92(4):1424–1429.
272. Coutant R, Biette-Demeneix E, Bouvattier C, Bouhours-Nouet N, Gatelais F, Dufresne S, et al. Baseline inhibin B and anti-Müllerian hormone measurements for diagnosis of hypogonadotropic hypogonadism (HH) in boys with delayed puberty. *J Clin Endocrinol Metab*. 2010;95(12):5225–5232.
273. Adan L, Lechevalier P, Couto-Silva AC, Boissan M, Trivin C, Brailly-Tabard S, et al. Plasma inhibin B and antimüllerian hormone concentrations in boys: discriminating between congenital hypogonadotropic hypogonadism and constitutional pubertal delay. *Med Sci Monit*, 16(11). In: CR511–517; 2010.
274. Howard S, Dunkel L. Sex steroid and gonadotropin treatment in male delayed puberty. *Endocr Dev*. 2016;29:185–197.
275. Wei C, Crowne EC. Recent advances in the understanding and management of delayed puberty. *Arch Dis Child*. 2016;101(5):481–488.
276. Giri D, Patil P, Blair J, Dharmaraj P, Ramakrishnan R, Das U, et al. Testosterone therapy improves the first year height velocity in adolescent boys with constitutional delay of growth and puberty. *Int J Endocrinol Metab*. 2017;15(2). e42311.
277. Richman RA, Kirsch LR. Testosterone treatment in adolescent boys with constitutional delay in growth and development. *N Engl J Med*. 1988;319(24):1563–1567.
278. Rosenfeld RG, Northcraft GB, Hintz RL. A prospective, randomized study of testosterone treatment of constitutional delay of growth and development in male adolescents. *Pediatrics*. 1982;69(6):681–687.
279. Soliman AT, Khadir MM, Asfour M. Testosterone treatment in adolescent boys with constitutional delay of growth and development. *Metab Clin Exp*. 1995;44(8):1013–1015.
280. Spratt, D.I., Stewart, I., Savage, C., Craig, W., Spack, N.P., Chandler, D.W., et al. Subcutaneous injection of testosterone is an effective and preferred alternative to intramuscular injection: demonstration in female-to-male transgender patients. *J Clin Endocrinol Metab*, 102(7), 2349–2355.
281. Lawaetz JG, Hagen CP, Mieritz MG, Blomberg Jensen M, Petersen JH, Juul A. Evaluation of 451 Danish boys with delayed puberty: diagnostic use of a new puberty nomogram and effects of oral testosterone therapy. *J Clin Endocrinol Metab*. 2015;100(4):1376–1385.
282. Chioma L, Papucci G, Fintini D, Cappa M. Use of testosterone gel compared to intramuscular formulation for puberty induction in males with constitutional delay of growth and puberty: a preliminary study. *J Endocrinol Invest*. 2017;41(2):259–263.
283. Contreras MF, Raisingani M, Prasad K, Franklin B, Shah B. Transdermal testosterone gel for induction and continuation of puberty in adolescent boys with hepatic dysfunction. *J Pediatr Endocrinol Metab*. 2017;30(1):105–109.
284. Rogol AD, Swerdloff RS, Reiter EO, Ross JL, ZumBrunnen TL, Pratt GA, et al. A multicenter, open-label, observational study of testosterone gel (1%) in the treatment of adolescent boys with Klinefelter syndrome or anorchia. *J Adolesc Health*. 2014;54(1):20–25.
285. Decourt C, Robert V, Anger K, Galibert M, Madinier JB, Liu X, et al. A synthetic kisspeptin analog that triggers ovulation and advances puberty. *Sci Rep*. 2016;6: 26908.
286. Rastrelli G, Vignozzi L, Maggi M. Different medications for hypogonadotropic hypogonadism. *Endocr Dev*. 2016;30:60–78.
287. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab*. 2008;93(11):4210–4217.
288. Rosenbloom AL. Idiopathic short stature: conundrums of definition and treatment. *Int J Pediatr Endocrinol*. 2009;2009: 470378.
289. Hero M, Norjavaara E, Dunkel L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J Clin Endocrinol Metab*. 2005;90(12):6396–6402.
290. Wickman S, Sipila I, Ankarberg-Lindgren C, Norjavaara E, Dunkel L. A specific aromatase inhibitor and potential increase in adult height in boys with delayed puberty: a randomised controlled trial. *Lancet*. 2001;357(9270):1743–1748.
291. Hero M, Wickman S, Dunkel L. Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. *Clin Endocrinol*. 2006;64(5):510–513.
292. Maura N, Gonzalez de Pijem L, Hsiang HY, Desrosiers P, Rapaport R, Schwartz ID, et al. Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. *J Clin Endocrinol Metab*. 2008;93(3):823–831.
293. Shulman DI, Francis GL, Palmert MR, Eugster EA. Use of aromatase inhibitors in children and adolescents with disorders of growth and adolescent development. *Pediatrics*. 2008;121(4):e975–e983.
294. Ferris JA, Geffner ME. Are aromatase inhibitors in boys with predicted short stature and/or rapidly advancing bone age effective and safe? *J Pediatr Endocrinol Metab*. 2017;30(3):311–317.
295. Wit JM, Hero M, Nunez SB. Aromatase inhibitors in pediatrics. *Nat Rev Endocrinol*. 2011;8(3):135–147.
296. Warne DW, Decosterd G, Okada H, Yano Y, Koide N, Howles CM. A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle-stimulating hormone and human chorionic gonadotropin. *Fertil Steril*. 2009;92(2):594–604.

297. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2002;87(1):152–160.
298. Pitteloud N, Hayes FJ, Dwyer A, Boepple PA, Lee H, Crowley Jr WF. Predictors of outcome of long-term GnRH therapy in men with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2002;87(9):4128–4136.
299. Liu PY, Baker HW, Jayadev V, Zacharin M, Conway AJ, Handelsman DJ. Induction of spermatogenesis and fertility during gonadotropin treatment of gonadotropin-deficient infertile men: predictors of fertility outcome. *J Clin Endocrinol Metab.* 2009;94(3):801–808.
300. Dwyer, A.A., Phan-Hug, F., Hauschild, M., Elowe-Gruau, E., Pitteloud, N. Transition in endocrinology: hypogonadism in adolescence. *Eur J Endocrinol*, 173(1), R15–24.
301. Gong C, Liu Y, Qin M, Wu D, Wang X. Pulsatile GnRH is superior to hCG in therapeutic efficacy in adolescent boys with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2015;100(7):2793–3279.
302. Rohayem, J., Hauffa, B.P., Zacharin, M., Kliesch, S., Zitzmann, M., German Adolescent Hypogonadotropic Hypogonadism Study G. Testicular growth and spermatogenesis: new goals for pubertal hormone replacement in boys with hypogonadotropic hypogonadism? -a multicentre prospective study of hCG/rFSH treatment outcomes during adolescence. *Clin Endocrinol (Oxf)*. 2017;86(1):75–87.
303. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med.* 2012;366(19):1803–1813.
304. Kumanov P, Deepinder F, Robeva R, Tomova A, Li J, Agarwal A. Relationship of adolescent gynecomastia with varicocele and somatometric parameters: a cross-sectional study in 6200 healthy boys. *J Adolesc Health.* 2007;41(2):126–131.
305. Deepinder F, Braunstein GD. Drug-induced gynecomastia: an evidence-based review. *Expert Opin Drug Saf.* 2012;11(5):779–795.
306. Demura M, Martin RM, Shozu M, Sebastian S, Takayama K, Hsu WT, et al. Regional rearrangements in chromosome 15q21 cause formation of cryptic promoters for the CYP19 (aromatase) gene. *Hum Mol Genet.* 2007;16(21):2529–2541.
307. Gourgari E, Saloustros E, Stratakis CA. Large-cell calcifying Sertoli cell tumors of the testes in pediatrics. *Curr Opin Pediatr.* 2012;24(4):518–522.
308. Braunstein GD. Clinical practice. Gynecomastia. *N Engl J Med.* 2007;357(12):1229–1237.
309. Mauras N, Bishop K, Merinbaum D, Emeribe U, Agbo F, Lowe E. Pharmacokinetics and pharmacodynamics of anastrozole in pubertal boys with recent-onset gynecomastia. *J Clin Endocrinol Metab.* 2009;94(8):2975–2978.
310. Plourde PV, Reiter EO, Jou HC, Desrochers PE, Rubin SD, Bercu BB, et al. Safety and efficacy of anastrozole for the treatment of pubertal gynecomastia: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab.* 2004;89(9):4428–4433.
311. Huhtaniemi I, Howard S, Dunkel L, Anderson RA. The gonadal axis: a life perspective. In: Pfaff DW, Joels M, eds. *Hormones, Brain, and Behavior*. 3rd ed. Oxford: Academic Press; 2017.
312. Wit JM, Rekers-Mombarg LT. Final height gain by GH therapy in children with idiopathic short stature is dose dependent. *J Clin Endocrinol Metab.* 2002;87(2):604–611.
313. Wu FC, Brown DC, Butler GE, Stirling HF, Kelnar CJ. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab.* 1993;76(1):26–31.
314. Segal TY, Mehta A, Anazodo A, Hindmarsh PC, Dattani MT. Role of gonadotropin-releasing hormone and human chorionic gonadotropin stimulation tests in differentiating patients with hypogonadotropic hypogonadism from those with constitutional delay of growth and puberty. *J Clin Endocrinol Metab.* 2009;94(3):780–785.
315. Doty RL, Shaman P, Kimmelman CP, Dann MS. University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *Laryngoscope.* 1984;94(2 Pt 1):176–178.
316. Wickman S, Dunkel L. Inhibition of P450 aromatase enhances gonadotropin secretion in early and midpubertal boys: evidence for a pituitary site of action of endogenous E. *J Clin Endocrinol Metab.* 2001;86(10):4887–4894.

CHAPTER OUTLINE

INTRODUCTION	695
DEFINITIONS AND EPIDEMIOLOGY	695
BIOLOGICAL DETERMINANTS OF GENDER IDENTITY	695
EMERGENCE AND DEVELOPMENTAL TRAJECTORIES OF GENDER	697
Infancy	697
Early Childhood	697
Adolescence	697
MENTAL HEALTH COMORBIDITIES ASSOCIATED WITH GENDER DYSPHORIA	697
Internalizing Disorders and Gender Dysphoria	697
Suicidality and Nonlethal Self-Harm	698
Eating Disorders	698
Traits of Autism Spectrum Disorder	698
NATURAL HISTORY OF GENDER DYSPHORIA	698
Desistence Versus Persistence	698
Impact of Family Support and Treatment	698
ROLE OF MENTAL HEALTH IN MULTIDISCIPLINARY CARE	698
Social Transition	699
MEDICAL TREATMENT	699
Current Treatment Models	699
Outcomes and Potential Adverse Effects	700
CHALLENGES TO DELIVERY OF CARE	701
CONCLUSIONS	701
References	701

INTRODUCTION

Concurrent with increasing public awareness of individuals whose gender identity is not aligned with their physical sex characteristics, there has been an increasing number of gender nonconforming/transgender youth seeking medical services to enable the development of physical characteristics consistent with their experienced gender. In eligible individuals, current clinical practice guidelines endorse use of agents to block endogenous puberty at Tanner stage 2 development with subsequent use of gender-affirming sex hormones, and are based on longitudinal studies demonstrating that youth first identified as gender dysphoric in childhood and who continue to meet mental health criteria for gender dysphoria (GD)/gender incongruence (GI) at early puberty are likely to be transgender as adults. Limited outcomes data support current practice and long-term studies are necessary to optimize care. This chapter reviews definitions relevant to gender nonconforming/transgender youth, epidemiology, developmental trajectories of gender, evidence supporting a role for biology in gender identity development, mental health comorbidities associated with GD, current treatment models, barriers to care, and priorities for research.

DEFINITIONS AND EPIDEMIOLOGY

According to the *Merriam-Webster's Medical Dictionary*, sex and gender have distinct meanings. Sex refers to "either of two major forms of individuals that occur in many species and that are distinguished respectively as female or male, especially on the basis of their reproductive organs and structures." In contrast, gender refers to the "behavioral, cultural, or psychological traits typically associated with one sex." Gender itself is then subdivided into gender identity and gender role/behavior. Gender identity is a person's internal sense of being male or female, whereas gender role is the expression of masculinity or femininity.¹ There has been increasing recognition that gender identity exists on a spectrum and that some individuals identify as nonbinary.^{2,3} Sexual orientation is one's sexual attraction toward partners of the opposite sex/gender (heterosexual), same sex/gender (homosexual), or both (bisexual). Gender identity does not predict sexual orientation. A person of any gender may have any sexual orientation.

"Gender Dysphoria," listed in the *Diagnostic and Statistical Manual of Mental Disorders (DSM) V* refers to clinically significant distress of at least 6 months, duration, related to the incongruence between one's affirmed or experienced gender and one's "assigned (or natal) gender" (gender incongruence).⁴ This term replaces *gender identity disorder (GID)*, which was included in the earlier DSM IV. Replacing the term "disorder" with "dysphoria" underscores the concept that a transgender identity, in and of itself, is no longer considered pathological, and focuses clinical concern on the distress that an individual with GI may experience. A summary of terms used in this chapter is detailed in [Box 19.1](#).

A 2017 report from the Williams Institute of the University of California Los Angeles School of Law, informed by state level population-based surveys, indicated that 0.6% of US adults (25–64 years) and 0.7% of adolescents and young adults (13–24 years) identify as transgender.⁵ A population-based study of self-reported gender identity in 80,929 Minnesota high school students reported a prevalence of 2.7% gender nonconforming or transgender.⁶ Transgender prevalence estimates ranging from 0.5% to 1.3% of birth-assigned males and 0.4% to 1.2% of birth-assigned females have been reported in a recent international review, representing an estimate of 25 million transgender people worldwide.⁷ In recent years, there has been a striking inversion in the sex ratio of adolescents seeking services for GD, with a predominance of birth-assigned females.⁸

BIOLOGICAL DETERMINANTS OF GENDER IDENTITY

Studies from several biomedical disciplines—genetics, endocrinology, and neurology—support the concept that there are biological underpinnings to gender identity development. Results of these studies support the concept that gender identity is not simply a psychosocial construct but likely reflects a complex interplay of biological, environmental, and cultural factors.⁹

With respect to genetics and gender identity, a recent study reports heritability estimates for gender identity in the range of 30% to 60%.¹⁰ A study supporting a role for genetic factors in gender identity outcome in transgender individuals

BOX 19.1 Definitions of Terms Used in This Chapter

Biological sex, biological male or female: These terms refer to physical aspects of maleness and femaleness. As these may not be in line with each other (e.g., a person with XY chromosomes may have female-appearing genitalia), the terms biological sex and biological male or female are imprecise and should be avoided.

Cisgender: This means not transgender. An alternative way to describe individuals who are not transgender is “nontransgender people.”

Gender-affirming (hormone) treatment: See “gender reassignment”

Gender dysphoria: This is the distress and unease experienced if gender identity and designated gender are not completely congruent. In 2013 the American Psychiatric Association released the fifth edition of the DSM-5, which replaced “gender identity disorder” with “gender dysphoria” and changed the criteria for diagnosis.

Gender expression: This refers to external manifestations of gender, expressed through one’s name, pronouns, clothing, haircut, behavior, voice, or body characteristics. Typically, transgender people seek to make their gender expression align with their gender identity, rather than their designated gender.

Gender identity/experienced gender: This refers to one’s internal, deeply held sense of gender. For transgender people, their gender identity does not match their sex designated at birth. Most people have a gender identity of man or woman (or boy or girl). For some people, their gender identity does not fit neatly into one of those two choices. Unlike gender expression (see later), gender identity is not visible to others.

Gender identity disorder: This is the term used for GD/gender incongruence in previous versions of DSM (see “gender dysphoria”). The ICD-10 still uses the term for diagnosing child diagnoses, but the upcoming ICD-11 has proposed using “gender incongruence of childhood.”

Gender incongruence: This is an umbrella term used when the gender identity and/or gender expression differs from what is typically associated with the designated gender. Gender incongruence is also the proposed name of the gender identity–related diagnoses in ICD-11. Not all individuals with gender incongruence have gender dysphoria or seek treatment.

Gender variance: See “gender incongruence”

Gender reassignment: This refers to the treatment procedure for those who want to adapt their bodies to the experienced gender by means

of hormones and/or surgery. This is also called *gender-confirming* or *gender-affirming treatment*.

Gender-reassignment surgery (gender-confirming/gender-affirming surgery): These terms refer only to the surgical part of gender confirming/gender-affirming treatment.

Gender role: This refers to behaviors, attitudes, and personality traits that a society (in a given culture and historical period) designates as masculine or feminine and/or that society associates with or considers typical of the social role of men or women.

Sex designated at birth: This refers to sex assigned at birth, usually based on genital anatomy.

Sex: This refers to attributes that characterize biological maleness or femaleness. The best known attributes include the sex-determining genes, the sex chromosomes, the H-Y antigen, the gonads, sex hormones, internal and external genitalia, and secondary sex characteristics.

Sexual orientation: This term describes an individual’s enduring physical and emotional attraction to another person. Gender identity and sexual orientation are not the same. Irrespective of their gender identity, transgender people may be attracted to women (gynephilic), attracted to men (androphilic), bisexual, asexual, or queer.

Transgender: This is an umbrella term for people whose gender identity and/or gender expression differs from what is typically associated with their sex designated at birth. Not all transgender individuals seek treatment.

Transgender male (also: trans man, female-to-male, transgender male): This refers to individuals assigned female at birth but who identify and live as men.

Transgender woman (also: trans woman, male-to-female, transgender female): This refers to individuals assigned male at birth but who identify and live as women.

Transition: This refers to the process during which transgender persons change their physical, social, and/or legal characteristics consistent with the affirmed gender identity. Prepubertal children may choose to transition socially.

Transsexual: This is an older term that originated in the medical and psychologic communities to refer to individuals who have permanently transitioned through medical interventions or desired to do so.

(From Hembree, W.C. et al. (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrine Metab*, 102 (11), 1–35; by permission of the Endocrine Society.)

demonstrated a 39.1% concordance for GID (based on DSM-IV criteria) in 23 pairs of monozygotic twins, with no concordance for GID in 21 same-sex dizygotic female and male twin pairs or in seven opposite sex twin pairs.¹¹ Although a number of investigators have sought to identify polymorphisms in specific candidate genes that may be more prevalent in transgender versus nontransgender controls, such studies have been inconsistent and lacking strong statistical significance.^{12–15}

With respect to hormonal influences on gender identity, it should be noted that most transgender individuals do not have a disorder/difference of sex development (DSD) or any obvious abnormality in sex steroid production or response. However, studies in individuals with a variety of DSDs have informed our understanding of the role that hormones (prenatal and early postnatal androgens, in particular) may play in gender identity development. For example, in studies of 46 XX individuals reared female, with virilizing congenital adrenal hyperplasia (CAH) caused by mutations in the *CYP21A2* gene, there is a greater degree of a transgender identity outcome (female-to-male) than what would be expected in the general population.^{16–18} In a meta-analysis of 250 adults with this condition, raised female, although nearly 95% accepted a female gender identity, 5.2% reported either a male gender identity or

GD.¹⁶ By comparison, the prevalence of a transgender identity in adults in recent population estimates in the United States is 0.5% to 0.7%.⁵ A separate study of adult 46 XX individuals with classical 21-hydroxylase deficiency demonstrated a relationship between severity of disease and gender identity outcome. Of 42 patients with the salt-wasting form, three (7.1%) either had GD or a male gender identity; no GD was seen in less severely affected individuals.¹⁷ A study in 46 XX youth with 21-hydroxylase deficiency (salt-wasting or simple virilizing) found that 12.8% demonstrated cross-gender identification.¹⁸ In a recent cross-sectional study from Europe, of 221 individuals with 46 XX CAH, 28 were noted to have experienced a “gender change”; in 25, this was reported to have occurred prepubertally; in one postpubertally; and in two the timing of “gender change” was unknown.¹⁹ The 25 individuals in this study described as having a prepubertal “gender change,” were, in fact, individuals who underwent feminizing genital surgery in the newborn period (before one’s gender identity is known); furthermore, the one individual reported in this study to have a gender change postpubertally was, in fact, a 46 XX individual with a male gender identity who had undergone masculinizing genital surgery (personal communication with Dr. Baudewijntje P.C. Kreukels, VU University Medical Center, Amsterdam). The

report of this cross-sectional European study did not indicate the number of 46 XX CAH individuals reared female that actually developed either gender dysphoria or a male gender identity (personal communication with B.P.C. Kreukels). It is noteworthy that in 46 XX individuals with virilizing CAH from 21-hydroxylase deficiency, prenatal androgens are more likely to affect gender expression/behavior and sexual orientation than gender identity.^{20,21} A role for prenatal/early postnatal androgens in gender identity development is also supported by studies in a variety of other hormonal and nonhormonal DSDs.²²

With respect to brain and gender identity, numerous studies in transgender adults, carried out before treatment with gender-affirming sex hormones, indicate that some sexually dimorphic brain structures are more closely aligned with gender identity than with physical sex characteristics.^{23,24} A gray matter study in gender dysphoric youth has shown a similar trend.²⁵ In addition, functional studies (e.g., analysis of hypothalamic blood flow in response to smelling odorous compounds and brain-imaging studies carried out during mental rotation tasks) demonstrate that patterns typically observed to be sexually dimorphic were more closely aligned with gender identity than with physical sex characteristics, even before treatment with gender-affirming sex hormones, in both transgender adolescents and adults.^{26–28}

EMERGENCE AND DEVELOPMENTAL TRAJECTORIES OF GENDER

To identify when a child is exhibiting gender nonconforming behavior, it is necessary to understand what gender behaviors are typical at various developmental stages and how these behaviors may change over time.²⁹ It is also important to appreciate how some expressions of gender vary in different environments.^{30,31} Recent reports of higher estimated prevalence rates of GD among youth in Australasia, Western Europe, and North America^{32–36} may reflect a greater willingness of people to seek treatment, as a result increased access to multidisciplinary gender clinics, as well as societal changes in attitudes about gender diversity.

Infancy

Although sex differentiation begins during early fetal development, gender differences from birth throughout infancy are limited to gross movement and emotional expressivity. For example, boys produce fewer tongue movements and weaker suckling than girls during early life;^{37,38} however, infant boys spend more time awake and produce greater movement of their trunk and limbs.³⁹ Finally, infant girls smile more than boys and are less likely to exhibit angry facial expressions.^{39,40} Additional differences in behavior between boys and girls either do not yet exist, or are unable to be detected with current technology at this very young time.

Early Childhood

An important milestone that starts to occur between 18 months and the second year of life is the emergence of gender identity.^{29,41} This occurs around the time language skills develop so that young children increasingly use gender labels (e.g., girl, boy, woman, man) as their speech evolves.⁴² Boys begin to exhibit preferences for gender-typed toys, such as trucks by 2 years of age,^{43,44} and by the third year of life children prefer same-sex peers and this preference intensifies over time.^{45,46} Interestingly, young children who understand and use gender labels are more likely to prefer gender-typed toys,⁴⁷ in support of the self-socialization theory of gender development that posits children socialize themselves into gender categories.²⁹

Children referred for treatment of GD prefer cross-sex toys, activities,⁴⁸ and playmates⁴⁹ more than their gender conforming peers and siblings. These differences in early childhood are not surprising, as the majority of transgender teens and adults recall that the onset of their GD occurred before puberty.^{50,51} Unknown at this time, is whether children with GD use gender labels differently during early childhood or experience the emergence of gender identity differently from gender conforming peers, during the first 2 years of life. Also unknown at this time is whether or not preferences for same- or cross-sex toys, activities, and playmates are stable throughout childhood for either gender conforming or nonconforming children.

Adolescence

Fewer studies of gender development have been conducted in adolescents compared with younger children. The theory of gender intensification suggests that adolescents experience increased pressure to conform to societal expectations of masculinity and femininity, and this pressure acts to further strengthen their gender identity.⁵² Although some studies support this theory, others do not.⁵³ Some adolescents who experienced GD as younger children no longer do so as adolescents (desisters), whereas others continue to experience GD, as they mature (persisters).⁵⁴ Thus for some people, gender identity evolves during adolescence in ways not predicted from earlier childhood. As noted earlier, the stability of gender from early childhood through later life has not been well studied. However, some investigators have attempted to identify factors that predict GD “persisters” versus “desisters,” as detailed in the section on “Natural History of Gender Dysphoria,” later. One of the recommended areas of future research in youth with GD is to identify additional predictors of GD persistence.^{55,56}

MENTAL HEALTH COMORBIDITIES ASSOCIATED WITH GENDER DYSPHORIA

Gender nonconforming people have historically presented with psychological symptoms, such as anxiety, depression, and suicidality and self-harm at rates much higher than the general population.^{34,57} Until recently, most studies of psychiatric comorbidities associated with GD were conducted in transgender adults who had limited family and peer support, as well as poor access to gender-affirming treatment and counseling.⁵⁸ As research on mental health of gender nonconforming youth broadens to include those who are both supported during their social transition and who receive multidisciplinary care,³ comorbidities are fewer. Nonetheless, behavioral and emotional problems for gender nonconforming youth in the community who are, and are not, referred for treatment are increased.⁵⁹ As these youth continue to be followed, evidence will build to address long-term safety and efficacy of multidisciplinary treatment on their health and wellbeing.

Internalizing Disorders and Gender Dysphoria

Data from nationally representative samples of students,⁶⁰ and also clinic-based studies of youth seeking medical treatment for GD^{34,51,61,62} reveal marked increases in depression among transgender youth compared with their cisgender counterparts. Clinically significant anxiety is also common.⁵⁸ In contrast, children with GD who socially transition with the support of their family exhibit levels of depression that are no different, and anxiety levels that are only mildly elevated, from age-matched population norms.⁶³ Additional evidence that support of others is important to maintain good mental health for gender nonconforming youth are associations between poor peer relations (i.e., gets teased, not liked by others) and

emotional problems.⁶⁴ Thus internalizing disorders, such as depression and anxiety are not necessarily a comorbidity of GD per se. Rather, psychological distress is likely caused by social ostracism and maltreatment by others.

Suicidality and Nonlethal Self-Harm

Transgender youth are more likely to think about suicide, attempt suicide, and inflict nonlethal self-harm (i.e., cutting, burning, or hitting) than cisgender youth.^{58,62} Self-harm in youth with GD is most common among assigned females and those impacted by psychologic symptoms, such as anxiety and depression.⁶⁵ A report from Ontario, Canada, found that transgender adolescents and young adults were more likely to have greater self-esteem and life satisfaction, as well as decreased depression, suicidal ideation, and suicide attempts if their parents were supportive of their gender identity, in comparison to those individuals whose parents were “somewhat to not at all supportive.”⁶⁶ Further investigations of the impact of social support and of gender-affirming multidisciplinary care warrant greater resources and effort, as studies of transgender youth continue to expand in pediatric medicine.

Eating Disorders

In the largest survey study of transgender youth to date, a higher rate of eating disorders was observed compared with cisgender women.⁶⁷ Furthermore, assigned females are at particular risk.⁶⁸ A smaller study reported high rates of overweight and obesity among transgender youth.⁵¹ For these youth, having too little or too much body fat may be a way to hide undesired physical characteristics. Thus it is recommended to screen for eating disorders in gender nonconforming children and adolescents.⁶⁸

Traits of Autism Spectrum Disorder

There is growing evidence from parent and teacher ratings, as well as review of medical records, that traits of autism spectrum disorder (ASD) and GD cooccur in some youth and adults.^{36,69–72} Although ASD is more prevalent in boys than in girls, in gender conforming individuals,⁷³ the association between ASD and GD is similar for both natal males and females.^{70,72} Although there are no clinical guidelines for the delivery of care to children and adolescents with cooccurring GD and ASD traits, initial consensus guidelines for assessment and care are available.⁷⁴

NATURAL HISTORY OF GENDER DYSPHORIA

Information about the natural history of GD is limited. This is in part because medical treatment has been inaccessible to many because of expense and/or stigmatization surrounding this condition. In addition, much of what is known about GD focuses on adults. Thus basic understanding about the natural history of GD in youth is only now being elucidated. Here we review what is currently understood about remission versus persistence of GD in children and adolescents, and also the impact of family support and medical treatment on mental health status of young people whose gender identity is incongruent with their natal sex.

Desistence Versus Persistence

Historically, the majority of children who presented for treatment of GD experienced remission (desisters) by late childhood or early adolescence.⁵⁴ Studies of desisters, and also those who continue to experience GD into later adolescence

and adulthood (persisters), are beginning to reveal factors that distinguish between these groups. Specifically, natal females, those with more intense GD in childhood and adolescence, and those who experience greater dissatisfaction with their primary and secondary sex characteristics are more likely to be persisters. Persisters are also more likely to be sexually attracted to members of their natal sex.⁵³ In addition, when asked if they are a boy or a girl, children whose GD persists are more likely to report that they believe themselves to be the sex opposite their natal sex, whereas desisters are more likely to report that they wished they were the other sex.⁵⁴ The possibility of remission of GD in later childhood or early adolescence, coupled with the ability to use the onset of puberty, as a diagnostic tool for persistence of GD, motivate recommendations for delaying pubertal suppression (see “Medical Treatment,” later) until after transyouth enter the first stages of puberty (Tanner stages 2–3).^{3,75}

Impact of Family Support and Treatment

Among prepubertal children with GD who have the support of their families and who have socially transitioned, depression is no different from population averages and anxiety is only mildly elevated.⁶³ Later in development, puberty suppression is associated with improved psychosocial functioning in adolescents whose GD persists.⁷⁶ Among older adolescents and young adults with GD who received puberty suppression, cross-sex hormone treatment, and in some cases gender reassignment surgery—psychological function was comparable with the general population.⁶⁴ In all of these studies, mental health support was part of multidisciplinary care delivered by experienced healthcare teams. Thus many of the mental health comorbidities of GD discussed earlier are ameliorated when family support is coupled with access to multidisciplinary care for children and adolescents of various developmental stages.

ROLE OF MENTAL HEALTH IN MULTIDISCIPLINARY CARE

The Standards of Care of the World Professional Association for Transgender Health (WPATH) (WPATH, 7th Version) and the Endocrine Society Clinical Practice Guideline for Gender-Dysphoric/Gender-Incongruent Persons^{2,3} promote several approaches for the psychological support of youth with GD and their families. It is recommended that youth receive mental healthcare before, during, and after their social and medical transition. Roles for mental healthcare providers include¹: the ability to assess GD/GI in children and adolescents and appropriately use the DSM and International Classification of Diseases for diagnosing these conditions²; provide counseling and supportive psychotherapy to youth and their families³; diagnose and treat other psychiatric conditions apart from GD⁴; refer for pubertal suppression, cross-sex hormone therapy; and gender reassignment surgery when appropriate, including assessment of comprehension of the risks and benefits of these treatments⁵; refer patients and families to peer support; and⁶ educate and advocate on behalf of patients and their families.

Both the WPATH and the Endocrine Society outline competency requirements for mental health professionals who provide services to children and adolescents with GD. These include¹ training in child/adolescent gender development (including gender nonconforming identities and roles) and child/adolescent psychopathology, with a minimum of a Master’s degree in clinical psychology from an accredited program and relevant licensing,² and supervised training and competency in psychotherapy or counseling, including treatment of GD, as well as continued education in these areas.^{2,3}

Social Transition

Some children benefit from a social (nonmedical) transition to help determine if their GD will remit or persist. Reversible changes that allow a child to live according to the gender they identify with at home and school (such as hair length, clothing, and name change) are associated with improved mental health among this group.⁶³ Children who undergo social transition are less likely to experience remission of their GD,⁵³ and it is unclear if this is because only those with stronger GD initiate social transition, or if social transition itself impacts the evolution of GD in youth. Because current understanding of the effects of childhood social transition on GD is limited, mental health professionals may work with families to determine if this option is best for their child. For example, social transition may be considered exploratory (as opposed to finite) and may be first attempted while families vacation or in the privacy of the home.²

MEDICAL TREATMENT

Current Treatment Models

GD that either emerges or worsens with the onset of physical puberty is highly predictive of a transgender identity during adulthood.⁷⁷ This observation is central to the rationale for medical intervention in eligible transgender adolescents. Medical care of transgender youth has been primarily informed by Clinical Practice Guidelines from the Endocrine Society³ and cosponsoring organizations and by Standards of Care from WPATH.² These documents endorse the use of gonadotropin-releasing hormone (GnRH) agonists at Tanner Stage 2 of pubertal development (testicular volume ≥ 4 mL for assigned males at birth or initial stages of breast budding for assigned females at birth) in adolescents who meet the criteria for GD (optimally determined by a qualified mental health gender specialist) that has either emerged or worsened with the onset of puberty. Additional criteria for initiation of pubertal suppression with GnRH agonists include the following: the adolescent has requested treatment and has provided informed assent and the parents or legal guardians have provided informed consent, and any coexisting medical or psychosocial concerns that could interfere with treatment have been addressed.³ GnRH agonists should not be used in prepubertal gender dysphoric children to block the initiation of puberty.³

Considered fully reversible, GnRH agonists, by pausing puberty, provide additional time for gender identity exploration, without the pressure of continued pubertal progression, and prevent irreversible development of secondary sex characteristics associated with the puberty that is not aligned with the person's affirmed gender identity.³ Such undesired physical changes include breast development, female body habitus, and potentially short stature in assigned females at birth, and Adam's apple, lowered voice, male bone configuration, and potentially tall stature in assigned males at birth.²² A protocol for baseline and follow-up monitoring of physical examination, and laboratory testing during pubertal suppression with GnRH agonists is outlined in Box 19.2. GnRH agonists, while the preferred option for pubertal suppression, are costly and often inaccessible. Alternatives for pubertal suppression include depot and oral progestins.³

Adolescents who have undergone pubertal suppression at early puberty and continue to meet the criteria for GD may request phenotypic transition with sex steroids. Age-specific recommendations for initiation of gender-affirming sex steroids in gender dysphoric adolescents are not delineated in the WPATH Standards of Care seventh version.² The most recent version of the Endocrine Society Clinical Practice

BOX 19.2 Baseline and Follow-Up Protocol During Suppression of Puberty

Every 3–6 mo
Anthropometry: height, weight, sitting height, blood pressure, Tanner stages
Every 6–12 mo
Laboratory: LH, FSH, E2/T, 25OH vitamin D
Every 1–2 y
Bone density using DEXA
Bone age on x-ray of the left hand (if clinically indicated)

DEXA, Dual-energy x-ray absorptiometry; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone. (From Hembree, W.C. et al. (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrine Metab*, 102 (11), 1–35; by permission of the Endocrine Society.)

Guidelines recommends initiating treatment “using a gradually increasing dose schedule after a multidisciplinary team of medical and mental health professionals has confirmed the presence of GD/GI, and sufficient mental health capacity to give informed consent, which most individuals have by age 16 years.”³ In addition, the most recent version of the Endocrine Society Clinical Practice Guidelines acknowledges that there may be compelling reasons to initiate sex hormone treatment before 16 years of age in some gender dysphoric adolescents. In gender dysphoric adolescents whose puberty is blocked at Tanner stage 2, delaying gender-affirming sex hormone treatment until 16 years of age could be detrimental to bone health; furthermore, keeping someone suspended in a prepubertal state until the age of 16 years could have detrimental mental health effects.³ A protocol for induction of puberty in gender dysphoric adolescents, and recommendations for baseline and follow-up physical examination and laboratory monitoring during pubertal induction are provided in Boxes 19.3 and 19.4, respectively.

During pubertal induction with gradually increasing doses of estrogen or testosterone, initial sex steroid levels will not be sufficient to suppress endogenous sex steroid secretion. In transgender females, even when adult levels of estrogen are reached, it is recommended that GnRH agonist treatment (or another antiandrogen) be continued until gonadectomy.³ Given that some transgender adults may not choose to have gonadectomy, long-term studies are needed to assess potential risks of prolonged GnRH agonist treatment. GnRH agonist treatment can typically be stopped in transgender males, once adult levels of testosterone have been achieved.³

It is not uncommon for some transgender adolescents to initially present for care when they have either virtually completed puberty or are already postpubertal. In such cases, transgender females are treated with estrogen, as well as an agent that blocks testosterone secretion and/or action, using protocols typical for transgender adults. With respect to estrogen treatment, 17 β -estradiol (transdermal, oral, or parental) is preferred to conjugated (e.g., premarin) or synthetic estrogens (e.g., ethinyl estradiol) because conjugated and synthetic estrogen levels cannot be monitored in the serum and ethinyl estradiol is associated with increased risk for venous thromboembolic disease and death from cardiovascular causes in studies of adults.³ Postpubertal transgender male adolescents can be treated with testosterone alone, following protocols typical for transgender adults. Although testosterone typically induces amenorrhea within a few months, a progestin or other agent may be used if uterine bleeding persists.⁷⁸

BOX 19.3 Protocol Induction of Puberty

Induction of female puberty with oral 17 β -estradiol, increasing the dose every 6 mo:

- 5 mcg/kg/day
- 10 mcg/kg/day
- 15 mcg/kg/day
- 20 mcg/kg/day
- Adult dose = 2–6 mg/day

In postpubertal transgender female adolescents, the dose of 17 β -estradiol can be increased more rapidly:

- 1 mg/day for 6 mo
- 2 mg/day

Induction of female puberty with transdermal 17 β -estradiol, increasing the dose every 6 mo (new patch is placed every 3.5 day):

- 6.25–12.5 mcg/24 hour (cut 25-mcg patch into quarters, then halves)
- 25 mcg/24 hour
- 37.5 mcg/24 hour
- Adult dose 50–200 mcg/24 hour

Adjust maintenance dose to mimic physiological estradiol levels.

Induction of male puberty with testosterone esters increasing the dose every 6 mo (IM or SC):

- 25 mg/m²/2 wk (or alternatively, half this dose weekly, or double the dose every 4 wk)
- 50 mg/m²/2 wk
- 75 mg/m²/2 wk
- 100 mg/m²/2 wk
- Adult dose = 100–200 mg every 2 wk

In postpubertal transgender male adolescents the dose of testosterone esters can be increased more rapidly:

- 75 mg/2 wk for 6 mo
- 125 mg/2 wk

Adjust maintenance dose to mimic physiologic testosterone levels.

IM, Intramuscularly; SC, subcutaneously.

(From Hembree, W.C. et al. (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrine Metab*, 102 (11), 1–35; by permission of the Endocrine Society.)

BOX 19.4 Baseline and Follow-up Protocol During Induction of Puberty

Every 3–6 mo

- Anthropometry: height, weight, sitting height, blood pressure, Tanner stages

Every 6–12 mo

- In transgender males: hemoglobin/hematocrit, lipids, testosterone, 25OH vitamin D
- In transgender females: prolactin, estradiol, 25OH vitamin D

Every 1–2 y

- BMD using DEXA
- Bone age on x-ray of the left hand (if clinically indicated)

BMD should be monitored into adulthood (until the age of 25–30 y or until peak bone mass has been reached).

BMD, Bone mineral density; DEXA, dual-energy X-ray absorptiometry.

(From Hembree, W.C. et al. (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrine Metab*, 102 (11), 1–35; by permission of the Endocrine Society.)

With respect to surgery, some transgender adolescents seek such procedures to bring their bodies into closer alignment with their gender identity. It is recommended that gender-affirming genital surgery involving gonadectomy and/or hysterectomy not take place until the individual has reached at least 18 years of age, or the age of legal majority in their country.³ For transgender males seeking breast reduction surgery, the Endocrine Society Guidelines acknowledge that some adolescents may consider this procedure before 18 years of age, but note there is insufficient evidence to recommend a specific age requirement.³ These guidelines suggest that clinicians should consider the physical and mental health status of the individual patient, when determining the timing of this procedure.

Outcomes and Potential Adverse Effects

Limited outcomes data are available based on current treatment models. As noted earlier, one published study has thus far evaluated mental health in transgender adolescents/young adults before and after GnRH agonist treatment, following gender-affirming sex steroid treatment, and 1 year after “gender reassignment surgery.”⁶⁴ At the completion of the observation period, GD was resolved, general psychological functioning had improved, and a sense of “wellbeing” was observed to be equal or greater to that found in age-matched controls. In addition, none of the 55 study participants regretted treatment.⁶⁴

Potential adverse effects of pubertal suppression with GnRH agonists in transgender youth, as recently reviewed, include impaired bone mineral density (BMD) and compromised fertility. In addition, there are unclear effects on brain development, body mass index (BMI), and body composition.⁷⁹

With respect to skeletal health, a 6-year longitudinal study (that spanned the period of pubertal suppression, gender-affirming sex hormone treatment, and gonadectomy), observed a significant decrease in lumbar spine areal BMD z-scores (relative to natal sex) in transgender females, with a similar decrease, following pubertal suppression in transgender males.⁸⁰ Potential study limitations, as acknowledged by the authors, included a relatively small number of study participants, relatively low doses of sex hormones, and lack of information regarding other factors that can influence BMD, including vitamin D status, dietary calcium intake, and weight-bearing exercise.⁸⁰ During pubertal suppression with GnRH agonists in early pubertal transgender adolescents, it is recommended to monitor vitamin D status and supplement if necessary, and to encourage adequate dietary calcium intake and weight-bearing exercise.²² In a separate study, bone turnover markers and bone mineral apparent density (BMAD) z-scores decreased, following GnRH agonist treatment in younger transgender adolescents, whereas an increase in BMAD was observed after 2 years of gender-affirming sex hormone treatment, in both younger and older transgender adolescents.⁸¹ A 22-year follow-up study of a gender dysphoric adolescent, treated initially with GnRH agonist and subsequently with gender-affirming sex hormones, found that BMD was in the normal range for both sexes when evaluated at 35 years of age.⁸²

A discussion about implications for fertility must precede any treatment of gender dysphoric adolescents with either GnRH agonists or gender-affirming sex hormones. Transgender adolescents may wish to preserve fertility, which will likely be compromised if puberty is suppressed at an early stage, and the individual subsequently transitions with gender-affirming sex hormones. In vitro maturation of human germ cells has not yet been achieved, although some families elect to freeze a section of prepubertal gonadal tissue for potential future use.⁸³ Cryopreservation of mature sperm or eggs is an option for late pubertal/ fully pubertal adolescents. However, recent reports indicate that even when provided with counseling regarding

potential impact of sex hormone treatment on fertility and options for fertility preservation, only a small percentage of such adolescents opted to pursue fertility preservation.^{84,85} Questionnaires to assess fertility and fertility preservation attitudes in transgender youth and their parents have been recently developed.^{86,87}

With respect to brain function, few studies have thus far evaluated potential adverse effects of GnRH agonists in transgender adolescents. When comparing small groups of GnRH agonist-treated versus untreated transgender adolescents (both male-to female and female-to male), there was no significant compromise of executive functioning, a developmental milestone typically achieved during puberty.⁸⁸ A 28-month longitudinal study in one transgender adolescent, undergoing treatment with GnRH agonist, showed lack of expected variation in white matter fractional anisotropy, a measure of brain maturation thought to normally occur during puberty, as well as a 9-point drop in operational memory testing after 22 months of pubertal suppression.⁸⁹ Given this relative lack of data, further longitudinal studies are needed to assess the impact of GnRH agonist treatment on brain development and function in transgender adolescents.

Studies assessing the impact of GnRH agonist treatment on BMI and body composition have also been carried out. Although variable results have been observed with respect to BMI,^{80,90} an increase in fat percentage and a decrease in lean body mass percentage, after 1 year of treatment with GnRH agonist, have been reported in both transgender male and female adolescents.⁹⁰ In a separate study, significant weight gain was reported in one of 27 transgender adolescents treated with GnRH agonist, although this individual's BMI was noted to be greater than the 85th percentile before treatment.⁹¹

A small number of short-term studies have thus far evaluated potential adverse effects of gender-affirming sex hormones in transgender adolescents. No change in blood pressure, BMI standard deviation score, lean body mass percentage, or fat percentage were observed in a study from the Netherlands of 28 transgender females treated for 1 to 3 years, primarily with gradually increasing doses of 17 β -estradiol.⁹² In addition, no abnormalities were observed with liver enzymes or creatinine, and there was no change in hematocrit or hemoglobin A1c. Hyperprolactinemia was observed in one individual who had received high-dose ethinyl estradiol treatment to limit statural growth.⁹²

Two studies from the United States have assessed potential adverse effects of gender-affirming sex hormones in transgender adolescents and young adults. Following treatment with 17 β -estradiol in 44 transgender females, no abnormalities were seen in blood pressure, BMI, hemoglobin/hematocrit, lipids, renal and liver function studies, or in prolactin.⁹³ Following treatment with testosterone in 72 transgender males, there was an increase in BMI and in hemoglobin/hematocrit (supraphysiologic hematocrit levels were seen in 4% of the individuals) and a decrease in high-density lipoprotein cholesterol levels; no abnormalities were seen in blood pressure, renal and liver function studies, or in hemoglobin A1c.⁹³ A separate prospective study, after 21 to 31 months of treatment with gender-affirming hormones in 25 transfeminine and 34 transmasculine individuals, showed no clinically significant adverse effects in a variety of metabolic parameters.⁹⁴

CHALLENGES TO DELIVERY OF CARE

Multidisciplinary care for transgender youth represents a relatively new focus of clinical service and research. Although there have been significant advances, beginning with pioneering work from the Netherlands, there are only limited safety and efficacy studies, with virtually no published data on the use of GnRH agonists to suppress puberty in gender dysphoric

youth younger than 12 years of age, or cross-sex hormones in transgender adolescents younger than 16 years of age. Randomized controlled trials are often considered the gold standard; however, this approach to study hormonal interventions in gender dysphoric youth has not been considered feasible or ethical.^{95,96} Although an increasing number of clinical programs have emerged around the world in recent years, there are many geographic areas where no such services exist, requiring patients and their families to travel long distances. In addition, there are significant limitations to access for medical treatments, given that all hormonal interventions for gender dysphoric youth are considered "off-label" in the United States, are expensive (GnRH agonists, in particular), and are often denied by insurance companies. Furthermore, lack of formalized training of providers and prejudice and misunderstanding on the part of family, community, and both medical and mental health providers limit access to optimal care.⁹⁷

There are additional challenges to providing optimal care for transgender youth. Whereas almost all published research has been conducted in the context of a binary gender model, there are increasing numbers of youth seeking care that identify as gender nonbinary.⁹⁸ Another limitation to the delivery of evidence-based care is that most studies of children, adolescents, and young adults with GD include only those with early-onset GD. A phenomenon known as *rapid-onset gender dysphoria* (ROGD), in which GD is first expressed by older youth during or after the completion of puberty, is poorly understood at this time.⁹⁹ Methodological concerns have been raised regarding the ROGD report (calling into question the existence of ROGD, itself), including the fact that only parents (recruited from websites) and none of the gender dysphoric youth participated in the study, and that parents were not recruited from websites supportive of transgender youth.¹⁰⁰ More research is needed to better understand those youth who have been described to have ROGD to optimally provide safe and effective care for these individuals.

CONCLUSIONS

Significant advances in our understanding of transgender/gender nonconforming youth have been achieved, although significant gaps in knowledge remain. Compelling studies have emerged supporting the concept that gender identity is not simply a psychosocial construct, but reflects a complex interplay of biological, environmental, and cultural factors. The removal of the term "gender identity disorder," replacing it with "gender dysphoria" in the DSM-V, underscores that a transgender identity, in and of itself, is no longer considered pathological, and that clinical concern should focus on GD that may be present, along with concomitant mental health challenges. The first long-term study, based on current models of care, indicates that mental health comorbidities in gender dysphoric youth either significantly diminish or resolve, when such individuals are provided with gender-affirming care, optimally delivered in a multidisciplinary clinical setting.⁶⁴ Further prospective, long-term outcome studies are needed to optimize care for transgender/gender nonconforming youth.

REFERENCES

1. Money J, Hampson JG, Hampson JL. An examination of some basic sexual concepts: the evidence of human hermaphroditism. *Bull Johns Hopkins Hosp.* 1955;97(4):301–319.
2. Coleman E, Bockting W, Botzer M, et al. Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgender.* 2012;13(4):165–232.
3. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender dysphoric/gender-incongruent persons: an

- Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102(11):3869–3903.
4. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Association Publishing; 2013.
 5. Herman JL, Flores AR, Brown TNT, Wilson BDM, Conron KJ. *Age of individuals who identify as transgender in the United States*. The Williams Institute, UCLA School of Law; 2017.
 6. Rider GN, McMorris BJ, Gower AL, et al. Health and care utilization of transgender and gender nonconforming youth: a population-based study. *Pediatrics.* 2018;141. e20171683.
 7. Winter S, Diamond M, Green J, et al. Transgender people: health at the margins of society. *Lancet.* 2016;388(10042):390–400.
 8. Aitken M, Steensma TD, Blanchard R, et al. Evidence for an altered sex ratio in clinic-referred adolescents with gender dysphoria. *J Sex Med.* 2015;12:756–763.
 9. Rosenthal SM. Transgender youth: current concepts. *Ann Pediatr Endocrinol Metab.* 2016;21:185–192.
 10. Polderman, TJC., Kreukels, BPC, Irwig, MS, et al; International Gender Diversity Genomics Consortium. The biological contributions to gender identity and gender diversity: bringing data to the table. *Behav Genet.* 2018;48(2):95–108.
 11. Heylens G, De Cuypere G, Zucker KJ, et al. Gender identity disorder in twins: a review of the case report literature. *J Sex Med.* 2012;9(3):751–757.
 12. Henningson S, Westberg L, Nilsson S, et al. Sex steroid-related genes and male-to-female transsexualism. *Psychoneuroendocrinology.* 2005;30(7):657–664.
 13. Hare L, Bernard P, Sánchez FJ, et al. Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. *Biol Psychiatry.* 2009;65(1):93–96.
 14. Ujike H, Otani K, Nakatsuka, et al. Association study of gender identity disorder and sex hormone-related genes. *Progr Neuropsychopharmacol Biol Psychiatry.* 2014;38(7):1241–1244.
 15. Bentz EK, Hefler LA, Kaufmann U, Huber JC, Kolbus A, Tempfer CB. A polymorphism of the CYP17 gene related to sex steroid metabolism is associated with female-to-male but not male-to-female transsexualism. *Fertil Steril.* 2008;90(1):56–59.
 16. Dessens AB, Slijper FM, Drop SL. Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. *Arch Sex Behav.* 2005;34(4):389–397.
 17. Meyer-Bahlburg HF, Dolezal C, Baker SW, Ehrhardt AA, New MI. Gender development in women with congenital adrenal hyperplasia as a function of disorder severity. *Arch Sex Behav.* 2006;35(6):667–684.
 18. Pasternski V, Zucker KJ, Hindmarsh PC, et al. Increased cross-gender identification independent of gender role behavior in girls with congenital adrenal hyperplasia: Results from a standardized assessment of 4-to 11-year-old children. *Arch Sex Behav.* 2015;44(5):1363–1375.
 19. Kreukels BPC, Koler B, Nordenstrom A, et al. Gender dysphoria and gender change in disorders of sex development/ intersex conditions: results from the dsd-LIFE study. *J Sex Med.* 2018;15:777–785.
 20. Meyer-Bahlburg HF, Dolezal C, Baker SW, New MI. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. *Arch Sex Behav.* 2008;37:85–99.
 21. Frisén L, Nordenström A, Falhammar H, et al. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab.* 2009;94:3432–3439.
 22. Rosenthal SM. Approach to the patient: transgender youth: endocrine considerations. *J Clin Endocrinol Metab.* 2014;99:4379–4389.
 23. Luders E, Sánchez FJ, Gaser C, et al. Regional gray matter variation in male-to-female transsexualism. *Neuroimage.* 2009;46(4):904–907.
 24. Rametti G, Carrillo B, Gómez-Gil E, et al. White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study. *J Psychiatric Res.* 2011;45(2):199–204.
 25. Hoekzema E, Schagen SE, Kreukels BP, et al. Regional volumes and spatial volumetric distribution of gray matter in the gender dysphoric brain. *Psychoneuroendocrinology.* 2015;55:59–71.
 26. Berglund H, Lindström P, Dhejne-Helmy C, Savic I. Male-to-female transsexuals show sex-atypical hypothalamus activation when smelling odorous steroids. *Cereb Cortex.* 2007;18(8):1900–1908.
 27. Burke SM, Cohen-Kettenis PT, Veltman DJ, Klink DT, Bakker J. Hypothalamic response to the chemo-signal androstadienone in gender dysphoric children and adolescents. *Front Endocrinol.* 2014;5:1–10.
 28. Burke SM, Kreukels BP, Cohen-Kettenis PT, Veltman DJ, Klink DT, Bakker J. Male-typical visuospatial functioning in gynephilic girls with gender dysphoria - organizational and activation effects of testosterone. *J Psychiatry Neurosci.* 2016;41(6):395–404.
 29. Martin CL, Ruble DN. Patterns of gender development. *Annu Rev Psychol.* 2010;61:353–381.
 30. Fagot BI, Leinbach MD. The young child's gender schema: environmental input, internal organization. *Child Dev.* 1989;60:663–672.
 31. Fagot BI, Leinbach MD. Gender knowledge in egalitarian and traditional families. *Sex Roles.* 1995;32:513–526.
 32. Chen M, Fuqua J, Eugster EA. Characteristics of referrals for gender dysphoria over a 13-year period. *J Adolesc Health.* 2016;58:369–371.
 33. Delahunt JW, Denison HJ, Sim DA, Bullock JJ, Krebs JD. Increasing rates of people identifying as transgender presenting to Endocrine Services in the Wellington region. *NZ Med J.* 2018;131:33–42.
 34. Spack NP, Edwards-Leeper L, Feldman HA, et al. Children and adolescents with gender identity disorder referred to a pediatric medical center. *Pediatrics.* 2012;129(3):418–425.
 35. Weijes CM, Nota NM, de Blok CJM, et al. The Amsterdam cohort of gender dysphoria study (1972–2015): trends in prevalence, treatment and regrets. *J Sex Med.* 2018;15(4):582–590.
 36. Zucker KJ. Epidemiology of gender dysphoria and transgender identity. *Sex Health.* 2017;14(5):404–411.
 37. Korner AF. Sex differences in newborns with special reference to differences in the organization of oral behavior. *J Child Psychol Psychiatry.* 1973;14:19–29.
 38. Lundqvist C, Hafstrom M. Non-nutritive sucking in full-term and preterm infants studied at term conceptual age. *Acta Paediatr.* 1999;88:1287–1289.
 39. Phillips S, King S, DuBois L. Spontaneous activities of female versus male newborns. *Child Dev.* 1978;49(3):590–597.
 40. Weinberg MK, Tronick EZ, Cohn JF, Olson KL. Gender differences in emotional expressivity and self-regulation during early infancy. *Dev Psych.* 1999;35:175–188.
 41. Martin CL, Ruble DN. Patterns of gender development. *Annu Rev Psychol.* 2010;61:353–381.
 42. Bussey K, Bandura A. Social cognitive theory of gender development and differentiation. *Psychol Rev.* 1999;106:676–713.
 43. Berenbaum SA, Martin CL, Hanish LD, Briggs PT, Fabes RA. Sex differences in children's play. In: Becker JB, Berkley KJ, Geary N, Hampson E, Herman JP, Young EA, eds. *Sex Differences in the Brain: From Genes to Behavior*. Oxford, UK: Oxford Univ. Press; 2008:275–290.
 44. Ruble DN, Martin CL, Berenbaum SA. Gender development. In: Eisenberg N, ed. *Handbook of Child Development*. New York: Wiley; 2006:858–932.
 45. La Freniere P, Strayer FF, Gauthier R. The emergence of same-sex affiliative preferences among preschool peers: a developmental/ethological perspective. *Child Dev.* 1984;55:1958–1965.
 46. Maccoby EE. *The Two Sexes: Growing up Apart, Coming Together*. Cambridge, MA: Belknap; 1998.
 47. Zosuls KM, Ruble DN, Tamis-LeMonda CS, Shrout PE, Bornstein MH, Greulich FK. The acquisition of gender labels in infancy: implications for sex-typed play. *Dev Psychol.* 2009;45:688–701.
 48. Zucker KJ, Nabbijohn ANN, Santarossa A, et al. Intense/obsessional interests in children with gender dysphoria: a cross-validation study using the Teacher's Report Form. *Child Adolesc Psychiatry Ment Health.* 2017;11:51.
 49. Fridell SR, Owen-Andersen A, Johnson LL, Bradley SJ, Zucker KJ. The playmate and play style preferences structured interview: a comparison of children with gender identity disorder and controls. *Arch Sex Behav.* 2006;35(6):729–737.

50. Cohen-Kettenis PT, Pfafflin F. The DSM diagnostic criteria for gender identity disorder in adolescents and adults. *Arch Sex Behav*. 2010;39(2):499–513.
51. Olson J, Schrager SM, Belzer M, Simons LK, Clark LF. Baseline physiologic and psychosocial characteristics of transgender youth seeking care for gender dysphoria. *J Adolesc Health*. 2015;57:374–380.
52. Hill JP, Lynch ME. The intensification of gender-related role expectations during early adolescence. In: Brooks-Gunn J, Petersen A, eds. *Girls at Puberty: Biological and Psychosocial Perspectives*. New York: Plenum; 1983:201–228.
53. Steensma TD, McGuire JK, Kreukels BPC, Beekman AJ, Cohen-Kettenis PT. Factors associated with desistance and persistence of childhood gender dysphoria: a quantitative follow-up study. *J Am Acad Child & Adolesc Psychiatry*. 2013;52(6):582–590.
54. Steensma TD, Biemond R, de Boer F, Cohen-Kettenis PT. Desisting and persisting gender dysphoria after childhood: a qualitative follow-up study. *Clin Child Psychol Psychiatry*. 2011;16(4):499–516.
55. Hidalgo MA, Ehrensaft D, Tishelman AC, et al. The gender affirmative model: what we know and what we aim to learn. *Hum Dev*. 2013;56:285–290.
56. Olson-Kennedy J, Cohen-Kettenis PT, Kreukels BP, et al. Research priorities for gender nonconforming/transgender youth: gender identity development and biopsychosocial outcomes. *Curr Opin Endocrinol Diabetes Obes*. 2016;23(2):172–179.
57. Becerra-Culqui TA, Liu Y, Nash R, et al. Mental health of transgender and gender nonconforming youth compared with their peers. *Pediatrics*. 2018;141(5). e20173845.
58. Connolly MD, Zervos MJ, Barone II CJ, Johnson CC, Joseph CLM. The mental health of transgender youth: advances in understanding. *J Adolesc Health*. 2017;59:489–495.
59. Van der Miesen AIR, Nabbijohn AN, Santarossa A, VanderLaan DP. Behavioral and emotional problems in gender-nonconforming children: a Canadian community-based sample. *J Am Acad Child Adolesc Psychiatry*. 2018;57:491–499.
60. Clark TC, Lucassen MF, Bullen P, et al. The health and well-being of transgender high school students: results from the New Zealand adolescent health survey (Youth '12). *J Adolesc Health*. 2014;55(1):93–99.
61. de Vries ALC, Cohen-Kettenis PT. Clinical management of gender dysphoria in children and adolescents: The Dutch approach. *J Homosex*. 2012;59(3):301–320.
62. Reisner SL, Vettes R, Leclerc M, et al. Mental health of transgender youth in care at an adolescent urban community health center: a matched retrospective cohort study. *J Adolesc Health*. 2015;56:274–279.
63. Olson KR, Durwood L, DeMeules M, McLaughlin KA. Mental health of transgender children who are supported in their identities. *Pediatrics*. 2016;137(3). e20153223.
64. de Vries ALC, McGuire JK, Steensma TD, Wagenaar ECF, Doreleijers AH, Cohen-Kettenis PT. Young adult psychological outcome after puberty suppression and gender reassignment. *Pediatrics*. 2014;134:696–704.
65. Davey A, Arcelus J, Meyer C, Bouman WP. Self-injury among trans individuals and matched controls: prevalence and associated factors. *Health Soc Care Community*. 2016;24(4):485–494.
66. Travers R, Bauer G, Pyne J, et al. Impacts of strong parental support for trans youth: a report prepared for Children's Aid Society of Toronto and Delisle Youth Services. *Trans Pulse*. 2012;1–5.
67. Diemer EW, Grant JD, Munn-Chernoff MA, Patterson DA, Duncan AE. Gender identity, sexual orientation, and eating-related pathology in a national sample of college students. *J Adolesc Health*. 2015;57(2):144–149.
68. Feder S, Isserlin L, Seale E, Hammond N, Norris ML. Exploring the association between eating disorders and gender dysphoria in youth. *Eat Disord*. 2017;25(4):310–317.
69. Heylens G, Aspeslagh L, Dierickx J, et al. The co-occurrence of gender dysphoria and autism spectrum disorder in adults: an analysis of cross-sectional and clinical chart data. *J Autism Dev Disord*. 2018;48(6):2217–2223.
70. Janssen A, Huang H, Duncan C. Gender variance among youth with autism spectrum disorders: a retrospective chart review. *Transgend Health*. 2016;1(1):63–68.
71. Shumer DE, Reisner SL, Edwards-Leeper L, Tishelman A. Evaluation of Asperger Syndrome in youth presenting to a gender dysphoric clinic. *LGBT Health*. 2016;3(5):387–390.
72. van der Miesen AIR, de Vries ALC, Steensma TD, Hartman CA. Autistic symptoms in children and adolescents with gender dysphoria. *J Autism Dev Disord*. 2018;48(5):1537–1548.
73. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. *J Am Acad Adolesc Psychiatry*. 2017;56(6):466–474.
74. Strang JF, Meagher H, Kenworthy L, et al. Initial clinical guidelines for co-occurring autism spectrum disorder and gender dysphoria or incongruence in adolescents. *J Clin Child Adolescent Psychol*. 2018;47:105–115.
75. Cohen-Kettenis PT, van Goozen S. Pubertal delay as an aid in diagnosis and treatment of a transsexual adolescent. *Eur Child Adolesc Psychiatry*. 1998;7:246–248.
76. Costa R, Dunsford M, Skagerberg E, et al. Psychological support, puberty suppression, and psychosocial functioning in adolescents with gender dysphoria. *J Sex Med*. 2015;12:2206–2214.
77. Cohen-Kettenis PT, Delemarre-van de Waal HA, Gooren LJ. The treatment of adolescent transsexuals: changing insights. *J Sex Med*. 2008;5(8):1892–1897.
78. Carswell JM, Roberts SA. Induction and maintenance of amenorrhea in transmasculine and nonbinary adolescents. *Transgend Health*. 2017;2(1):195–201.
79. Perl L, Lee JY, Rosenthal, SM. Medical side effects of GnRH agonists. In: Finlayson, C., ed. *Pubertal Suppression in Transgender Youth*. Elsevier, In press.
80. Klink D, Caris M, Heijboer A, van Trotsenburg M, Rotteveel J. Bone mass in young adulthood following gonadotropin-releasing hormone analog treatment and cross-sex hormone treatment in adolescents with gender dysphoria. *J Clin Endocrinol Metab*. 2015;100(2):E270–E275.
81. Vlot MC, Klink DT, den Heijer M, Blankenstein MA, Rotteveel J, Heijboer AC. Effect of pubertal suppression and cross-sex hormone therapy on bone turnover markers and bone mineral apparent density (BMAD) in transgender adolescents. *Bone*. 2017;95:11–19.
82. Cohen-Kettenis PT, Schagen SE, Steensma TD, de Vries AL, Delemarre-van de Waal HA. Puberty suppression in a gender-dysphoric adolescent: a 22-year follow-up. *Arch Sex Behav*. 2011;40(4):843–847.
83. Johnson EK, Finlayson C, Rowell EE, et al. Fertility preservation for pediatric patients: Current state and future possibilities. *J Urol*. 2017;198:186–194.
84. Nahata L, Tishelman AC, Caltabellotta BA, et al. Low fertility preservation utilization among transgender youth. *J Adolesc Health*. 2017;61:40–44.
85. Chen D, Simons L, Johnson EK, et al. Fertility preservation for transgender adolescents. *J Adolesc Health*. 2017;61:120–123.
86. Chen D, Matson M, Macapagal K, et al. Attitudes toward fertility and reproductive health among transgender and gender nonconforming adolescents. *J Adolesc Health*. 2018;63:62–68.
87. Strang JF, Jarin J, Call D, et al. Transgender youth fertility attitudes questionnaire: Measure development in nonautistic and autistic transgender youth and their parents. *J Adolesc Health*. 2018;62:128–135.
88. Staphorsius AS, Kreukels BP, Cohen-Kettenis PT, et al. Puberty suppression and executive functioning: an fMRI-study in adolescents with gender dysphoria. *Psychoneuroendocrinology*. 2015;56:190–199.
89. Schneider MA, Spritzer PM, Soll BMB, et al. Brain maturation, cognition and voice pattern in a gender dysphoria case under pubertal suppression. *Front Hum Neurosci*. 2017;11:528.
90. Schagen SE, Cohen-Kettenis PT, Delemarre-van de Waal HA, Hannema SE. Efficacy and safety of gonadotropin-releasing hormone agonist treatment to suppress puberty in gender dysphoric adolescents. *J Sex Med*. 2016;13(7):1125–1132.
91. Khatchadourian K, Amed S, Metzger DL. Clinical management of youth with gender dysphoria in Vancouver. *J Pediatr*. 2014;164(4):906–911.
92. Hannema SE, Schagen SE, Cohen-Kettenis PT, Delemarre-van de Waal HA. Efficacy and safety of pubertal induction using 17 β -Estradiol in transgirls. *J Clin Endocrinol Metab*. 2017;102(7):2356–2363.

93. Jarin J, Pine-Twaddell E, Trotman G, et al. Cross-sex hormones and metabolic parameters in adolescents with gender dysphoria. *Pediatrics*. 2017;139(5). e20163173.
94. Olson-Kennedy J, Okonta V, Clark LF, et al. Physiologic response to gender-affirming hormones among transgender youth. *J Adolesc Health*. 2018;62:397–401.
95. Drescher J, Byne W. Gender dysphoric/gender variant (GD/GV) children and adolescents: summarizing what we know and what we have yet to learn. *J Homosex*. 2012;59:501–510.
96. Vance Jr SR, Ehrensaft D, Rosenthal SM. Psychological and medical care of transgender youth. *Pediatrics*. 2014;134:1184–1192.
97. Vance Jr SR, Halpern-Felsher BL, Rosenthal SM. Health care providers' comfort with and barriers to care of transgender youth. *J Adolesc Health*. 2015;56:251–253.
98. Veale JF, Watson RJ, Peter T, Saewyc EM. Mental health disparities among Canadian transgender youth. *J Adolesc Health*. 2017;60:44–49.
99. Littman L. Rapid-onset gender dysphoria in adolescents and young adults: a study of parental reports. *PLOS One*. 2018;13(8). e0202330.
100. Wadman M. 'Rapid onset' of transgender identity ignites storm. *Science*. 2018;361:958–959.

Allen W. Root, Michael A. Levine

CHAPTER OUTLINE

HYPOCALCEMIA	705
Hypocalcemia in the Neonate and Infant	705
Hypocalcemia in the Child and Adolescent	722
HYPERCALCEMIA	728
Hypercalcemia in the Neonate and Infant	728
Hypercalcemia in the Child and Adolescent	735
DISORDERS OF MAGNESIUM METABOLISM	744
Hypomagnesemia	745
Hypermagnesemia	747
DISORDERS OF BONE	747
Disorders of Bone Formation and Mineralization in the Neonate and Infant	748
Disorders of Bone Mineralization and Formation in the Child and Adolescent	750
Chronic Kidney Disease-Mineral and Bone Disorder	768
Disorders of Bone Mineralization	769
Fibrous Dysplasia	785
High Bone Mass	786
Heterotopic Bone Formation/Ectopic Calcification	793
OSTEOCHONDRODYSPLASIAS	795
CONCLUDING REMARKS	801

Disorders of calcium, magnesium, and phosphate metabolism and of bone formation, accrual, and maintenance during the first 2 decades of life result from suboptimal ingestion, absorption, or retention of constituent nutrients, abnormal vitamin D metabolism or bioactivity, disorders of parathyroid hormone (PTH) synthesis, secretion, or action, and intrinsic aberrations in cartilage and bone cells. The origins of these illnesses may be intrinsic because of pathological variations in the genes controlling these processes or to acquired insults (Table 20.1). Serum concentrations of calcium vary by age.¹ Although serum total and ionized calcium (Ca^{2+}) concentrations generally are intrinsically related, dissociation between these analytes may be observed in patients with hyperproteinemia or hypoproteinemia and at extremes of plasma pH—either alkalosis or acidosis.² Hypocalcemia is present when the serum concentrations of Ca^{2+} are below, and hypercalcemia is identified by serum values of Ca^{2+} above the normal range for age, respectively.¹ For an integrated overview of calcium, mineral, and skeletal homeostasis, the reader is referred to Chapter 9.

HYPOCALCEMIA

In children older than 1 year of age, depending on the analytic laboratory hypocalcemia is defined by a decrease in Ca^{2+} values below the lower limits of normal for age of 4.64 to 4.80 mg/dL = 1.16 to 1.20 mmol/L; the lower normal range for age of the total calcium concentration is 8.5 to 8.9 mg/dL = 2.20 to 2.3 mmol/L.^{1,2} If the serum concentration of albumin declines by 1 g/dL, the total calcium value will fall by 0.8 mg/dL (0.2 mmol/L), whereas the level of Ca^{2+} does not vary. Symptoms of hypocalcemia reflect heightened neuromuscular irritability, such as paresthesias, tetany, carpopedal spasm, laryngospasm, muscular cramps and/or myotonic spasms, and focal or generalized convulsions; physical signs of hypocalcemia include presence of a Chvostek sign (tapping on facial nerve elicits twitching of facial muscles ipsilaterally) and/or a Trousseau sign (carpopedal spasm after maintaining an inflated blood pressure cuff slightly above systolic pressure for 3 minutes).³ During hypocalcemia the electrocardiographic QT interval is prolonged.

Hypocalcemia in the Neonate and Infant

In utero, fetal concentrations of total and ionized calcium are higher than they are postnatally; total calcium levels exceed maternal values by approximately 2.0 mg/dL = 0.5 mmol/L, because placental calcium transport is stimulated by parathyroid hormone-related protein (PTHrP); in term umbilical cord blood, mean total calcium values are 10 to 11 mg/dL and Ca^{2+} levels approximate 6.4 mg/dL = 1.6 mmol/L, respectively.^{4,5} In the fetus, serum concentrations of phosphate, PTHrP, and calcitonin are higher than in the pregnant woman, while fetal levels of 1,25-dihydroxyvitamin D₃ (calcitriol) and PTH are low and those of 25-hydroxyvitamin D₃ (calcidiol) approximate maternal values.⁵ In the neonate, total calcium and Ca^{2+} concentrations decline in the first 24 hours after birth to values approximating 8 to 9 mg/dL (2.0–2.75 mmol/L) and 4.4 to 5.4 mg/dL (1.1–1.35 mmol/L), respectively; calcium levels then plateau and subsequently increase to the midnormal range by the third day of life. Neonatal concentrations of PTHrP and calcitonin decline after delivery, whereas values of PTH and calcitriol increase over the first 2 postnatal days. Subsequently, in healthy infants, Ca^{2+} concentrations vary by postnatal age: 1 month—5.2 to 6.1 mg/dL = 1.28 to 1.52 mmol/L; 3 months—5.2 to 6.0 mg/dL = 1.30 to 1.49 mmol/L; 12 months—5.0 to 5.6 mg/dL = 1.24 to 1.39 mmol/L.²

Clinical manifestations of hypocalcemia occurring in the neonate (defined as values of total calcium <7.5–8.0 mg/dL and/or Ca^{2+} <4.4 mg/dL [1.1 mmol/L] in newborns with birth weights >1500 g and <7.0 mg/dL and/or Ca^{2+} <3.6 mg/dL [0.9 mmol/L] in newborns with birth weights <1500 g) are principally those of neuromuscular hyperexcitability:

TABLE 20.1 Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism

Gene	Chromosome	OMIM	Disease	OMIM
ACVR1	2q24.1	102576	Fibrodysplasia ossificans progressiva	135100
AIRE	21q22.3	607358	Autoimmune polyendocrine syndrome, type I	240300
ALPL	1p36.12	171760	Hypophosphatasia, infantile	241500
			Hypophosphatasia, childhood	241510
			Hypophosphatasia, adult	146300
AP2S1	19q13.31	602242	Hereditary hypocalciuric hypercalcemia 3	600740
BAZ1B	7q11.23	605681	Williams-Beuren syndrome	194050
BMP1	8p21.3	112264	Osteogenesis imperfecta type XIII	614856
BSND	1p32.2	606412	Bartter syndrome type 4a	602522
CA2	8q22	611492	Osteopetrosis - renal tubular acidosis	259730
CASR	3q13.3-q21	601199	Hereditary hypocalciuric hypercalcemia 1	145980
			Neonatal severe hyperparathyroidism	239200
			Hypercalcemic hypercalciuria	601199
			Hypoparathyroidism, familial isolated	146200
			Acquired hypocalciuric hypercalcemia	145980
CDC73	1q31.2	607393	Familial isolated hypoparathyroidism type 1	145000
			Hyperparathyroidism - jaw tumor syndrome	145001
CDKN1B	12p13.1	600778	Multiple endocrine neoplasia, type 4	610755
CHD7	8q12.2	608092	CHARGE syndrome (Coloboma, Heart defect, Choanal atresia Retardation, Genital and Ear anomalies)	214800
CDKN1B	12P13.1	600778	Multiple endocrine neoplasia type 4	610755
CLCN5	Xp11.23	300008	X-linked recessive hypophosphatemic rickets	300554
			Dent disease 1	300009
			Nephrolithiasis, X-linked recessive	310468
CLCN7	16p13.3	602727	Osteopetrosis, autosomal recessive Type IV	611490
			Osteopetrosis, autosomal dominant type II	166600
CLDN10	13q32.1	617579	HELIX = Hypohidrosis, Electrolyte imbalance, Lacrimal Dysfunction, Ichthyosis, Xerostomia	617671
CLDN16	3q27	603959	Type 3 hypomagnesemia	248250
CLDN19	1p34.2	610036	Type 5 hypomagnesemia, hypercalciuria, visual impairment	248190
CLCNKB	1p36.13	602023	Bartter syndrome type 3	607364
CNNM2	10q24.32	607803	Type 6 hypomagnesemia with normomagnesuria	613882
COL1A1	17q21.31-q22	120150	Osteogenesis imperfecta type I	166200
			Osteogenesis imperfecta type IIA	166210
			Osteogenesis imperfecta type III	259420
			Osteogenesis imperfecta type IV	166220
COL1A2	7q22.1	120160	Osteogenesis imperfecta type IIA	166210
			Osteogenesis imperfecta type III	259420
			Osteogenesis imperfecta type IV	166220
CREB3L1	11p11.2	616215	Osteogenesis imperfecta type XV1	616229
CRTAP	3p22	605497	Osteogenesis imperfecta type IIB	610854
			Osteogenesis imperfecta type VII	610682
CTSK	1q21.3	601105	Pycnodysostosis	265800
CYP2R1	11p15.2	608713	Vitamin D hydroxylation-deficient rickets, type 1B (25-Hydroxylase deficiency)	600081
CYP3A4	7q22.1	124010	Vitamin D-dependent rickets type 3	
CYP24A1	12q13.2	126065	Infantile hypercalcemia type 1	143880
CYP27B1	12q14.1	609506	Vitamin D hydroxylation-deficient rickets, type IA (25 α -Hydroxyvitamin D-1 α - hydroxylase deficiency)	264700
DMP1	4q22.1	600980	Hypophosphatemic rickets, autosomal recessive type 1	241520
ELN	7q13.23	120160	Williams-Beuren syndrome	194050
ENPP1	6q23.2	173335	Hypophosphatemic rickets, autosomal recessive type 2	613312
FAM111A	11q12.1	615292	Kenny-Caffey syndrome 2	127000
			Gracile bone dysplasia	602361

TABLE 20.1 Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism—cont'd

Gene	Chromosome	OMIM	Disease	OMIM
FAM20C	7p22.3	611061	Raine syndrome	259775
FERMT3	11q13.1	607901	Osteopetrosis, autosomal recessive	
FGF23	12p13.3	605380	Hypophosphatemic rickets, autosomal dominant	193100
			Familial tumoral calcinosis	211900
			Hyperostosis hyperphosphatemia syndrome	610233
FGFR3	4p16.3	134934	Achondroplasia	100800
FKBP10	17q21.2	607063	Osteogenesis imperfecta type XI	610968
			Bruck syndrome 1	259450
FOXP3	Xp11.23	300292	Immunodysregulation, polyendocrinopathy, enteropathy (IPEX)	304790
FXYD2	11q23.3	601814	Autosomal dominant hypomagnesemia type 2 with hypocalciuria	154020
GALNT3	2q24-q31	601756	Familial tumoral calcinosis	211900
			Hyperostosis hyperphosphatemia syndrome	610233
GATA3	10p13-14	131320	Hypoparathyroidism, sensorineural deafness, renal disease (hypoparathyroidism-deafness-renal dysplasia/Barakat syndrome)	146255
GCM2	6p24.2	603716	Hypoparathyroidism, familial isolated	146200
GNA11	19p13.3	139313	Hereditary hypocalciuric hypercalcemia 2	145981
GNAS	20q13.32	139320	Pseudohypoparathyroidism, type 1A	103580
			Pseudohypoparathyroidism, type 1B	603233
			Pseudohypoparathyroidism, type 1C	612462
			Pseudopseudohypoparathyroidism	612463
			Osseous heteroplasia, progressive	166350
			Fibrous dysplasia/McCune-Albright	174800
GNPTAB	12q23.2	607840	Mucopolidosis type II	252500
GTF21	7q11.23	601679	Williams-Beuren syndrome	194050
HADHB	2p23.3	143450	MELAS—Mitochondrial encephalomyopathy, lactic acidosis, stroke, hypoparathyroidism	540000
HNF1B	17q12	189907	Hypomagnesemia with maturity onset diabetes of youth ⁵ and renal cysts	137920
HNRNPC	14q11.2	164020	Vitamin D-dependent rickets type 2B	600785
HRPT2	1q24-q31	607393	Familial hyperparathyroidism 2 - jaw tumor syndrome	145001
IKBKG	Xq28	300248	Osteopetrosis, X-linked	300301
IFITM5	11p15.5	6147577	Osteogenesis imperfecta type V	610967
KCNA1	12p13.32	176260	Hypomagnesemia with myokymia	160120
KCNJ1	11q24	600359	Hypomagnesemia/Antenatal Bartter syndrome type 2	600839
KCNJ10	1q23.2	612780	Hypomagnesemia/sesame syndrome	612780
KL	13q13.1	604824	Familial tumoral calcinosis	211900
			Hypophosphatemia & hyperparathyroidism	612089
LEPRE1	1p34	610339	Osteogenesis imperfecta type VIII	610915
LRP4	604270	11p11.2	Sclerosteosis 2	614305
LRP5	11.13.4	603506	Osteoporosis-pseudoglioma syndrome	259770
			Idiopathic juvenile osteoporosis	259750
			High bone mass variation	601884
			Autosomal dominant osteopetrosis type I	607634
			Van Buchem disease, type 2	607636
MBTPS2	Xp22.12	300294	Osteogenesis imperfecta type XIX	301014
MEN1	11q13	613733	Multiple endocrine neoplasia type I	131100
NEBL	10p12.31	605491	DiGeorge syndrome type 2	605491
			Velocardiofacial syndrome complex 2	
NPR2	9p21-p12	108961	Acromesomelic dysplasia (Maroteaux)	602875
OSTM1	6q21	607649	Autosomal recessive osteopetrosis type V	259700
PCBD1	10q22.1	126090	Renal hypomagnesemia, maturity onset diabetes of the young, type 5	

Continued

TABLE 20.1 Genetic Origins of Disorders of Mineral, Cartilage, and Bone Metabolism—cont'd

Gene	Chromosome	OMIM	Disease	OMIM
PDE4D	5q11.2-q12.1	600129	Acrodysostosis type 2	614613
PHEX	Xp22.2-p22.1	300550	Hypophosphatemic rickets, X-linked dominant	307800
PLEKHM1	17q21.3	611466	Autosomal recessive osteopetrosis type VI	611497
PPIB	15q21-q22	123841	Osteogenesis imperfecta type IX	259440
PRKAR1A	17q24.3	188830	Acrodysostosis type 1	101800
PTH	11p15.3	168450	Hypoparathyroidism, familial isolated	146200
PTH1R	3p21.31	168468	Blomstrand osteochondrodysplasia	215045
			Murk-Jansen metaphyseal chondrodysplasia	156400
			Enchondromatosis (Ollier disease)	166000
RET	10q11.2	164761	Multiple endocrine neoplasia type IIA	171400
			Multiple endocrine neoplasia type IIB	162300
			Familial medullary carcinoma of thyroid	155240
SAMD9	7q21.2	610456	Tumoral calcinosis, normophosphatemic	610455
SERPINF1	17p13.2	172860	Osteogenesis imperfecta gene type VI	613982
SERPINH1	11q13.5	600943	Osteogenesis imperfecta gene type X	613848
SLC2A2	3q26.2	138160	Fanconi-Bickel syndrome	227810
SLC34A1	5q35.3	182309	Autosomal dominant hypophosphatemia with urolithiasis 1	612286
			Autosomal recessive Fanconi syndrome with hypophosphatemic rickets	613388
			Infantile hypercalcemia, type 2	616963
SLC34A3	9q34.3	609826	Hypophosphatemic rickets with hypercalciuria	241530
SLC9A3R1	17q25.1	604990	Autosomal dominant hypophosphatemia with urolithiasis/osteoporosis 2	612287
SLC12A1	15q21.1	600839	Antenatal Bartter syndrome type 1	601678
SLC12A3	16q13	600968	Hypomagnesemia/Gitelman syndrome	263800
SLC34A1	5q35	182309	Infantile hypercalcemia 2	616963
SLC34A3	9q34	609826	Hypophosphatemic rickets with hypercalciuria, hereditary	241530
SLC7A7	14q11.2	603593	Lysinuric protein intolerance	222700
SNX10	7p15.2	614780	Autosomal recessive osteoporosis type VIII	615085
SOST	17q12-q21	605740	Scleroosteosis	269500
			Hyperostosis corticalis generalisata (Van Buchem disease type 1)	239100
SOX3	Xq26.3	313430	Hypoparathyroidism, X-linked	307700
SP7	12q13.13	606633	Osteogenesis imperfecta XII	613849
SPARC	5q33.1	182120	Osteogenesis imperfecta XVII	6616597
STK3	11q23.3	614766	Spondyloepimetaphyseal dysplasia, Krakow	618162
STX16	20q13.32	603666	Pseudohypoparathyroidism, type 1B	603233
TBX1	22q11.12	602054	DiGeorge syndrome	188400
TBCE	1q42.3	604934	Sanjad-Sakati (HRD) syndrome	241410
			Kenney-Caffey syndrome, type 1	244460
TCIRG1	11q13.2	604592	Autosomal recessive osteopetrosis type I	259700
TENT5A	6q14.1	611357	Osteogenesis imperfecta XVIII	617592
TGFB1	19q13.1	190180	Progressive diaphyseal dysplasia	131300
TMEM38B	611236	9q31.1	Osteogenesis imperfecta gene type XIV	615066
TNFRSF11A	18q22.1	603499	Autosomal recessive osteopetrosis type VII	612301
			Hereditary (familial) expansile polyostotic osteolytic dysplasia	174810
TNFRSF11B	8q24	602643	Paget disease, juvenile	239000
TNFSF11	13q14.11	602642	Autosomal recessive osteopetrosis type III	259730
TRPM6	9q21.13	607009	Type 1 hypomagnesemia with hypocalcemia	602014
TRPV6	7q34	606680	Transient neonatal hyperparathyroidism	618188
VDR	12q13.11	601769	Vitamin D-resistant rickets, type IIA	277440
WNT1	12q13.12	164820	Osteogenesis imperfecta type XV	615220

See Tables 20.13 and 20.14 for genes associated with osteochondrodysplasias.

irritability, hyperacusis, jitteriness, tremulousness, facial spasms, tetany, laryngospasm, and focal or generalized seizures.^{2,4} Nonspecific symptoms, such as apnea, tachycardia, cyanosis, emesis, and feeding problems may also occur. Causes of neonatal hypocalcemia may be considered in relation to the age of onset (before or after 72 hours of life = early/late) (Tables 20.2A, 20.2B).

Early Neonatal Hypocalcemia

In the absence of hypoproteinemia, hypocalcemia occurring within the first 72 hours after birth is considered “early

neonatal hypocalcemia.” It occurs most commonly in prematurely delivered or small-for-gestational-age, low birth weight (LBW), or asphyxiated neonates, or in those born to women with gestational or permanent forms of diabetes mellitus (rarely unsuspected maternal hyperparathyroidism) and is the consequence of subnormal PTH secretion in response to declining serum calcium values and delayed renal tubular phosphaturic response to PTH characteristic of the neonate, unusually prolonged secretion of calcitonin, and/or hypomagnesemia.² Total calcium and Ca^{2+} concentrations decline more rapidly from high intrauterine values to lower nadir levels in

TABLE 20.2A Causes of Hypocalcemia

I Neonatal

A Maternal disorders

- 1 Diabetes mellitus
- 2 Toxemia of pregnancy
- 3 Vitamin D deficiency
- 4 High intake of alkali or magnesium sulfate
- 5 Use of anticonvulsant medications
- 6 Hyperparathyroidism

B Neonatal disorders

- 1 Low birth weight: prematurity, intrauterine growth restriction
- 2 Peripartum asphyxia, sepsis, critical illness
- 3 Hyperbilirubinemia, phototherapy, exchange transfusion
- 4 Hypomagnesemia, hypermagnesemia
- 5 Acute/chronic renal failure
- 6 Nutrients/medications—high phosphate intake, fatty acids, phytates, bicarbonate infusion, citrated blood, anticonvulsants, aminoglycosides
- 7 Hypoparathyroidism
- 8 Vitamin D deficiency or resistance
- 9 Osteopetrosis type II

II Hypoparathyroidism

A Congenital

- 1 Transient neonatal
- 2 Congenital hypoparathyroidism - see also Table 20.2B
 - a Familial isolated hypoparathyroidism
 - (1) Autosomal recessive hypoparathyroidism (*GCMB*, *PTH*)
 - (2) Autosomal dominant hypoparathyroidism (*CASR*, *GNA11*, *PTH*)
 - (3) X-linked hypoparathyroidism (*SOX3*)
 - b DiGeorge syndrome (*TBX1*)
 - c Sanjad-Sakati syndrome (short stature, developmental delay, dysmorphism—HRD)
 - Kenny-Caffey syndrome 1 (short stature, osteosclerosis, immunodeficiency) (*TBCE*)
 - d Kenny-Caffey syndrome 2 (developmental delay, dysmorphism) (*FAM111A*)
 - e Barakat syndrome (sensorineural deafness, renal dysplasia—HDR) (*GATA3*) Lymphedema-hypoparathyroidism-nephropathy, nerve deafness
 - f Mitochondrial deletions Kearns-Sayre: ophthalmoplegia, pigmented retina, cardiomyopathy; MELAS: mitochondrial encephalopathy, lactic acidosis, stroke-like episodes, MTPD: mitochondrial trifunctional protein deficiency (*HADHB*)
- 3 Insensitivity to parathyroid hormone
 - a Blomstrand chondrodysplasia (*PTH1R1*)
 - b Pseudohypoparathyroidism - type Ia (*GNAS*)
 - Pseudohypoparathyroidism - type IB (*GNASAS1*) IB (*STX16*)
 - 1b (*GNAS*)
 - Pseudohypoparathyroidism - type Ic (*GNAS*)
 - Pseudohypoparathyroidism - type II (?)
 - Pseudopseudohypoparathyroidism (*GNAS*)
 - c Acrolyostosis with hormone resistance (*PRKAR1A*, *PDE4D*)
 - d Hypomagnesemia (see Tables 20.7A, 20.7B)

B Acquired

- 1 Autoimmune polyglandular syndrome - type I (*AIRE*)
- 2 Activating antibodies to the calcium sensing receptor
- 3 Postsurgical, radiation destruction
- 4 Infiltrative (excessive iron [hemochromatosis, thalassemia] or copper [Wilson disease] deposition; granulomatous inflammation, neoplastic invasion; amyloidosis, sarcoidosis)
- 5 Maternal hyperparathyroidism
- 6 Hypomagnesemia/hypermagnesemia

III Vitamin D Deficiency (See Disorders of Mineralization)

IV Other Causes of Hypocalcemia

A Calcium deficiency

- 1 Nutritional deprivation
- 2 Hypercalciuria

B Disorders of magnesium homeostasis - see Tables 20.7A, 20.7B

- 1 Congenital hypomagnesemia
- 2 Acquired
 - a Acute renal failure
 - b Chronic inflammatory bowel disease, intestinal resection
 - c Diuretics

C Hyperphosphatemia

- 1 Renal failure
- 2 Phosphate administration (intravenous, oral, rectal)
- 3 Tumor cell lysis
- 4 Muscle injuries (crush, rhabdomyolysis)

D Miscellaneous

- 1 Hypoproteinemia
- 2 Hyperventilation
- 3 Drugs - furosemide, bisphosphonates calcitonin, anticonvulsants, ketoconazole, antineoplastic agents (plicamycin, asparaginase, cisplatin, cytosine arabinoside, doxorubicin), citrated blood products
- 4 Hungry bone syndrome
- 5 Acute and critical illness - sepsis, acute pancreatitis, toxic shock a
 - a Organic acidemia - propionic, methylmalonic, isovaleric

(From Carpenter T. Etiology of hypocalcemia in infants and children. *UpToDate*. 2018;1–17; Shaw N. A practical approach to hypocalcaemia in children. In: Allgrove J, Shaw N. (eds). *Calcium and Bone Disorders in Children and Adolescents*. Basel, Karger, *Endocr Dev*. 2018;16:73–92; Thakker R.V. Hypocalcemia: pathogenesis, differential diagnosis, and management. In: Favus, M.J. (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:213–215.)

TABLE 20.2B Genetic Variants Associated With Hypocalcemia/Hypoparathyroidism

Gene Protein Chromosome OMIM	Disorder OMIM	Clinical/Biochemical Manifestations	Gene Function/Transmission
Autosomal dominant hypoparathyroidism <i>CASR</i> Calcium sensing receptor 3q13.3-q21.1 601199	Autosomal dominant hypocalcemia type 1, (isolated hypoparathyroidism) 601198	Hypocalcemia-mediated increased neuromuscular irritability: paresthesias, tetany, seizures; may also result in hypokalemia & secondary hyperaldosteronism	Encodes calcium sensing receptor expressed on plasma membrane of parathyroid gland & renal tubules; gain-of-function variants increase sensitivity & response of CaSR to low serum concentrations of calcium, AD (may be associated with Barter syndrome type 5 caused by coimpairment of renal tubular reabsorption of sodium chloride, AD 601198)
<i>GNA11</i> Guanine nucleotide-binding protein, alpha-11 19p13.3 139313	Autosomal dominant hypocalcemia type 2 (isolated hypoparathyroidism) 615361	Hypocalcemia-mediated increased neuromuscular irritability: paresthesias, tetany, seizures	Encodes G-protein alpha subunit (G α 11) that initiates intracellular signal transduction after binding of Ca ²⁺ to CaSR, AD
Familial isolated hypoparathyroidism <i>PTH</i> Parathyroid hormone 11p15.3 168450	Familial isolated hypoparathyroidism 146200	Hypocalcemia-mediated increased neuromuscular irritability: paresthesias, tetany, seizures	Encodes parathyroid hormone, AD/AR
<i>GCM2</i> Glial cells missing, drosophila, homolog of, 2 6p24.2 603716	Familial isolated hypoparathyroidism 146200	Hypocalcemia-mediated increased neuromuscular irritability: paresthesias, tetany, seizures	Encodes transcription factor essential for differentiation of the parathyroid glands, AD/AR
<i>FHL1</i> Four-and-a-half Lim domains 1 300163 Xq26.3	Isolated hypoparathyroidism 146200	Hypocalcemic seizures	Encodes gene essential for differentiation of the parathyroid glands, X-linked
Complex hypoparathyroidism <i>TBX1</i> T-box 1 22q11.21 602054	DiGeorge syndrome (DGS) type 1 (deletion chromosome 22q11 syndrome) 188400	Hypoparathyroidism, thymic hypoplasia, congenital heart anomalies, cleft palate, impaired renal function, dysmorphic facial features	T-box transcription factor embryonically expressed in the tissues adversely impacted in DGS; AD
<i>NEBL</i> Nebulette 10p12.31 605491	DiGeorge syndrome type (complex) 2, 601362	Thymic aplasia, congenital heart anomalies, cleft palate, impaired renal function, dysmorphic facial features	Protein expressed in cardiac & striated muscle, associated with actin, myofibrils, & cellular adhesion complexes, AD
<i>CHD7</i> Chromodomain helicase DNA-binding protein 7 8q12.2 608092	CHARGE syndrome 214800	CHARGE = Coloboma, Heart anomaly, Choanal atresia, Retardation, Genital, & Ear anomalies	Regulator of neural crest gene expression & ribosomal RNA formation, AD
<i>GATA3</i> GATA-binding protein 3 10p15 131320	HDR (Barakat syndrome) 146255	Hypoparathyroidism, sensorineural deafness, renal dysplasia	Transcription factor/ enhancer element required for development of parathyroid glands, auditory system, kidneys & for expression of genes encoding T-cell receptor subunits, AD
<i>TBCE</i> Tubulin-specific chaperone E 1q42.3 604934	HRD (Sanjad-Sakati syndrome—SSS), 244460; Kenny-Caffey syndrome type 1 (KCS1) 241410	HRD: Hypoparathyroidism, retardation, dysmorphism; KCS1: aforementioned + osteosclerosis & recurrent infections	Chaperone protein necessary for correct folding of tubulin subunits & stability of cellular cytoskeleton, AR
<i>FAM111A</i> Family with sequence similarity 111, Member A 11q12.1 615292	Kenny-Caffey syndrome type 2 (KCS2), 127000; Gracile bone dysplasia (GBD), 602381	KCS2: Hypoparathyroidism, retardation, dysmorphism; GBD: Hypocalcemia, thin but dense & fragile bones, may be lethal perinatally	Functional effect(s) in man unknown, (host range restriction factor in viruses), AD

TABLE 20.2B Genetic Variants Associated With Hypocalcemia/Hypoparathyroidism—cont'd

Gene Protein Chromosome OMIM	Disorder OMIM	Clinical/Biochemical Manifestations	Gene Function/Transmission
Mitochondriopathic hypoparathyroidism Deletion mitochondrial chromosome	Kearns-Sayre syndrome (KSS) 530000	Hypoparathyroidism, ophthalmoplegia, retinitis pigmentosa, sensorineural deafness, cerebellar ataxia, abnormal cardiac conductivity, myopathy, growth retardation, renal tubular dysfunction, hypoadrenocorticism, hypogonadism, diabetes mellitus	Mitochondrial genes encoding energy-generating electron transport proteins, Maternal, AD
Deletion mitochondrial chromosome	Mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes (MELAS) 540000	Hypoparathyroidism, myopathy, ophthalmoplegia, neuropathy, cardiomyopathy, impaired cognition	Mitochondrial genes encoding energy-generating electron transport proteins, Maternal, AD
<i>HADHB</i> Hydroxylacyl-CoA dehydrogenase/3-Ketoacyl-CoA thiolase/Enoyl-CoA hydratase, beta subunit 2p23.3 143450	Mitochondrial trifunctional protein deficiency syndrome, 609015	Because of inability to metabolize an energy source, clinical presentation may vary from acute & lethal in the perinatal period to hepatic Reye-like syndrome in older infants to skeletal myopathy in adolescents	Nuclear gene encoding beta subunit of mitochondrial trifunctional protein catalyzing beta oxidation of long chain fatty acids, AR
Autoimmune hypoparathyroidism <i>AIRE</i> Autoimmune regulator 21q21.3 607358	Autoimmune polyendocrinopathy syndrome, type I 240300	Hypoparathyroidism, hypoadrenocorticism, mucocutaneous candidiasis, alopecia, pernicious anemia, hypogonadism	Expressed in thymus, essential for recognition of self-antigens, AD/dominant negative/AR
Pseudohypoparathyroidism <i>GNAS</i> GNAS complex locus 20q13.32 139320	Pseudohypoparathyroidism type 1A, 103580	Hypocalcemia, hyperphosphatemia, subnormal renal tubular cyclic AMP response to exogenous PTH, Albright hereditary osteodystrophy phenotype (AHO)	Inactivating mutations or deletions of maternal <i>GNAS</i> or biallelic paternal expression of <i>GNAS</i> (isochromosomes) lead to proximal renal tubular & skeletal resistance to PTH, AD
<i>GNAS</i> GNAS complex locus 20q13.32 139320	Pseudohypoparathyroidism type 1B, 603233	Hypocalcemia, hyperphosphatemia, subnormal renal tubular cyclic AMP response to exogenous PTH, normal erythrocyte cyclic AMP activity, normal phenotype	Maternally transmitted epigenetic methylation defect of maternal <i>GNAS</i> leads to its silencing & proximal renal tubular resistance to PTH, AD
<i>GNASAS1</i> GNAS complex locus, antisense transcript 1 20q13.32 610540	Pseudohypoparathyroidism type 1B 603233	Hypocalcemia, hyperphosphatemia, absent renal tubular cAMP response to exogenous PTH, normal phenotype	Maternally transmitted deletion leads to paternal expression of <i>GNASAS1</i> resulting in silencing of maternal <i>GNAS</i> because of an epigenetic methylation defect of & proximal renal tubular resistance to PTH, AD
<i>STX16</i> Syntaxin 16 20q13.32 603666	Pseudohypoparathyroidism type 1B 603233	Hypocalcemia, hyperphosphatemia, absent renal cyclic AMP response to exogenous PTH, normal phenotype	Maternally transmitted epigenetic methylation defect of maternal <i>GNAS</i> leads to its silencing & proximal renal tubular resistance to PTH, AD
<i>GNAS</i> GNAS complex locus 20q13.32 139320	Pseudohypoparathyroidism type 1C	Hypocalcemia, hyperphosphatemia, absent renal cyclic AMP response to exogenous PTH, AHO phenotype, normal erythrocyte cyclic AMP activity	Inactivating mutations or deletions in exon 13 of maternal <i>GNAS</i> lead to proximal renal tubular & skeletal resistance to PTH, AD
?	Pseudohypoparathyroidism type 2, 203330	Hypocalcemia, hyperphosphatemia, partial resistance to PTH, normal phenotype	Despite normal increase in urinary cyclic AMP after PTH administration, patients are resistant to phosphaturic effect of PTH
<i>GNAS</i> GNAS complex locus 20q13.32 139320	Pseudopseudohypoparathyroidism, 612463	Phenotype of AHO but normocalcemia	Skeletal resistance to PTH because of loss-of-function variants of paternal <i>GNAS</i>

Continued

TABLE 20.2B Genetic Variants Associated With Hypocalcemia/Hypoparathyroidism—cont'd

Gene Protein Chromosome OMIM	Disorder OMIM	Clinical/Biochemical Manifestations	Gene Function/Transmission
<i>PRKAR1A</i> Protein kinase, cAMP dependent, regulatory, type 1 alpha 17q24.2 188830	Acrodysostosis 1 101800	Skeletal dysplasia: short stature, brachydactyly, facial dysostosis, nasal hypoplasia; with/without hormone resistance	Encodes a regulatory subunit of cyclic AMP-dependent protein kinase A requisite for intracellular signal transduction
<i>PDE4D</i> Phosphodiesterase 4D, cAMP-specific 5q11.2-q21.1 600129	Acrodysostosis 2 614613	<i>Vide supra</i> , developmentally challenged	Encodes enzyme that degrades cyclic AMP, thereby inhibiting intracellular signal transduction

AD, Autosomal dominant; AR, autosomal recessive; cAMP, cyclic adenosine monophosphate; PTH, parathyroid hormone; RNA, ribonucleic acid. (From Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers*. 2017;3:17055; Thiele S, Mantovani G, Barlier A, et al. From pseudohypoparathyroidism to inactivating PTH/PTHrP signalling disorder (IPPSD), a novel classification proposed by the EuroPHP network. *Eur J Endocrinol*. 2016;175:P1–P17; Vahe C, Benomar K, Espiard S, et al. Diseases associated with the calcium-sensing receptor. *Orphanet J Rare Dis*. 2017;12:19.)

preterm than in term neonates. In LBW neonates, hypocalcemia may be further attributed to the rapid accretion of skeletal calcium in the presence of relative resistance to the calcium absorptive and reabsorptive effects of calcitriol on the intestinal tract and bone, respectively. Offspring of severely vitamin D-deficient mothers may become hypocalcemic shortly after birth. Hypocalcemia develops in approximately one-third of asphyxiated newborns who are products of complicated and compromised deliveries, including those associated with maternal diabetes mellitus, toxemia of pregnancy, vitamin D deficiency, use of anticonvulsant drugs, and (unsuspected) maternal hyperparathyroidism.² In these infants, increased phosphate load caused by cellular injury, reduced calcium intake, and hypercalcitonemia are important pathogenetic factors in the development of hypocalcemia. Neonates born prematurely and those with intrauterine growth restriction, asphyxia, infections, respiratory distress syndrome, or other critical illness are often hypocalcemic.

Some 50% of infants of mothers with diabetes mellitus develop early neonatal hypocalcemia; the incidence may be reduced by strict maternal glycemic control.² Its causes are multifactorial and include reduced placental transfer of calcium because of substantial maternal urinary excretion of calcium and magnesium, decreased neonatal secretion of PTH, hypercalcitonemia, hypomagnesemia (occurring in 40% of offspring of diabetic women), and limited intake and impaired absorption of ingested calcium. Maternal hypercalcemia caused by unsuspected hyperparathyroidism leads to increased transfer of calcium to the fetus and still further increase in in-utero serum calcium concentrations that suppress fetal PTH synthesis and release and stimulate calcitonin secretion—aberrations in homeostatic mechanisms that persist postpartum and may result in hypocalcemic tetany/seizures in her offspring. Suppression of PTH secretion may persist for several months and be undetected until symptomatic hypocalcemia develops after weaning of the infant from breast milk to higher phosphate containing cow milk formula. Maternal ingestion of large quantities of calcium carbonate in antacids has also led to neonatal hypocalcemia.

Hypocalcemia may occur in neonates with hyperbilirubinemia undergoing exchange transfusion and in those exposed to phototherapy. Neonates with acute rotavirus infection and severe diarrhea may present with hypocalcemic seizures. Aminoglycoside antibiotics (e.g., gentamycin) increase urinary excretion of calcium and magnesium, thereby facilitating the

development of neonatal hypocalcemia. Compounds that complex with and sequester calcium, such as citrate (present in transfused blood), phosphates (that alter the calcium x phosphate product), and fatty acids (given as caloric supplements) lower Ca²⁺ levels. Bicarbonate administered to correct acidosis increases calcium binding to albumin and thus lowers Ca²⁺ values. Hypomagnesemia impairs release of PTH from the parathyroid glands. Hypocalcemia may also occur in hyperventilated infants with severe respiratory alkalosis, as well as in those with other causes of metabolic alkalosis. Phytates in soy milk bind calcium and phosphate and interfere with their absorption. Neonates and infants with malignant osteopetrosis type II and impaired osteoclastogenesis⁶ may present with either early or late neonatal hypocalcemia.^{6–8}

Late Neonatal Hypocalcemia

Late transient hypocalcemia (first developing when the neonate is >72 hours of postnatal age) may be caused by increased intake of phosphate, hypomagnesemia, hypoparathyroidism, or vitamin D deficiency (see Tables 20.2A, 20.2B). Neonatal hypocalcemia may develop after 3 days of age in offspring born in the late winter-early spring of the year to multiparous women with inadequate intake of vitamin D or exposure to sunlight. High phosphate content of evaporated milk or modified cow milk formulas may lead to formation of poorly soluble calcium salts limiting the intestinal absorption of calcium while raising serum phosphate values. Premature introduction of fiber-containing cereals into the infant's diet also decreases calcium absorption. Affected infants may have an associated defect in renal phosphate excretion or coexisting vitamin D deficiency. Hyperphosphatemia and hypocalcemia may initially suggest hypoparathyroidism, but serum PTH concentrations are usually normal or modestly elevated in infants with excessive phosphate loading in response to reciprocal reduction in serum calcium; markedly elevated or persistently high PTH values raise the question of whether pseudohypoparathyroidism (PHP, rarely acrodysostosis—a skeletal dysplasia with hormone resistance related to inactivating variants of *PRKAR1A* or *PDE4D*, vide infra) may be present. Newborns and infants with chronic renal insufficiency because of renal hypoplasia or obstructive nephropathies often are hypocalcemic and hyperphosphatemic with elevated serum PTH levels as well, but they are also azotemic. Hypomagnesemia leads to impaired secretion of PTH and decreased

peripheral responsiveness to PTH and may be transient or related to congenital abnormalities of intestinal absorption or renal tubular reabsorption of magnesium.² Hypermagnesemia may occasionally be associated with neonatal hypocalcemia.

Hypocalcemia and hypophosphatemia caused by fetal/neonatal deficiency of vitamin D occurs in offspring of mothers with substantial deficiency of vitamin D (either for cultural or socioeconomic reasons), impaired hepatic 25-hydroxylation of cholecalciferol or renal 25-hydroxyvitamin D-1 α hydroxylase activity or loss-of-function mutations of the vitamin D receptor (VDR). Hypovitaminosis D may develop in an older breastfed infant of a vegetarian mother who shields herself from sunlight and ingests a diet low in vitamin D. Marginal deficiency of vitamin D in neonates and infants is much more common than has been recognized heretofore.^{9,10} "Late-late" neonatal hypocalcemia occurs in premature infants with low bone mass at 3 to 4 months of age in whom the intake of calcium, phosphate, and vitamin D has been marginal; it is perhaps caused by avid deposition of available calcium into bone.² Hypocalcemia caused by vitamin D deficiency may develop rather acutely and in the absence of clinical or radiographic signs of rickets in the older infant and young child ingesting an elimination diet low in vitamin D because of severe allergies and/or maintained indoors with limited exposure to sunlight.

Hypocalcemia initially manifesting after 72 hours of age often heralds the presence of significant compromise of calcium homeostatic mechanisms, such as those associated with hypoparathyroidism because of malformation of the parathyroid glands (e.g., the DiGeorge syndrome or variant of a gene critical for embryogenesis of these structures) or functional error in PTH secretion (e.g., an abnormality in the activity of the calcium sensing receptor [CaSR]).

Hypoparathyroidism

Hypoparathyroidism presenting in infancy is often transient and related to delayed developmental maturation of parathyroid gland function; it may resolve within the first several weeks of life (see Tables 20.2A, 20.2B and Fig. 20.1). When persistent, hypoparathyroidism may be caused by an error in the embryogenesis of the parathyroid glands or in the synthesis or secretion of PTH or to peripheral unresponsiveness to PTH (functional hypoparathyroidism, i.e., PHP, occurs in patients who are resistant to PTH [vide infra]). Familial isolated congenital hypoparathyroidism may be transmitted as an autosomal dominant, autosomal recessive, or X-linked recessive trait caused by loss-of-function mutations in genes required for differentiation of the parathyroid glands leading to congenital aplasia or hypoplasia of these structures occurring as a unitary disorder. Thus, familial isolated hypoparathyroidism (OMIM 146200) has been related to inactivating mutations in *PTH*, glial cells missing (*GCM2*), and four-and-a-half Lim domains 1 (*FHL1*) and autosomal dominant hypocalcemia with suppressed secretion of PTH because of gain-of-function variants of *CASR* and guanine nucleotide binding protein alpha 11 (*GNA11*).^{3,11} Inactivating mutations in the exons encoding the signal peptide of *PTH* interfere with the processing of preproPTH to the bioactive 84 aa functional PTH molecule resulting in hypoparathyroidism that may be transmitted as an autosomal dominant or recessive characteristic.¹² Depending on the specificity of the immunoassay for PTH, serum levels of PTH may be low, normal, or even high in these patients. *GCM2* is a gene with five exons that encodes a 506 aa deoxyribonucleic acid (DNA)-binding transcription factor whose expression is restricted to the parathyroid glands. Intra-genic deletions or homozygous missense inactivating mutations in exons 2, 3, and 5 of *GCM2* result in hypoparathyroidism in humans.¹²⁻¹⁵ Mutations in *GCM2* exons 2 and 3 (encoding

DNA binding and transactivation domain 1) lead to impaired protein synthesis and stability and autosomal recessive transmission of congenital hypoparathyroidism whereas those in exon 5 (encoding transactivation domain 2) lead to mutations with a dominant negative effect and autosomal dominant transmission of this disorder.¹⁵ (Expression of *GCM2* takes place soon after specification of parathyroid cells and is dependent on normal transcriptional function of *GATA3*, the gene mutated in patients with the Barakat syndrome of hypoparathyroidism-deafness-renal dysplasia [HDR] [vide infra].¹⁶ Activating mutations of *GCM2* are associated with hyperparathyroidism [vide infra].) X-linked hypoparathyroidism is associated with agenesis of the parathyroid glands; the disorder is caused by loss-of-function variants of *FHL1* (encoding Four-and-a-half Lim domains 1, OMIM 300163), a gene whose product is essential for differentiation of the parathyroid glands.¹¹ *FHL1* is a 280-amino-acid (aa) protein with a LIM domain (double zinc finger motif) that is also expressed in testes, cardiac, and skeletal muscle.

Hypocalcemia associated with impaired secretion of PTH caused by monoallelic activating mutations in *CASR* have been identified in neonates, infants, children, and adults.^{17,18} Hypercalciuric hypocalcemia is an autosomal dominantly transmitted form of hypoparathyroidism that is caused by gain-of-function mutations in *CASR* (autosomal dominant hypocalcemia type 1) or *GNA11* (autosomal dominant hypocalcemia type 2) that result in enhanced "sensitivity" of the CaSR to the PTH-suppressive effects of Ca²⁺ in the chief cells of the parathyroid glands and in renal cells in the thick ascending limb of the loop of Henle (TALH). The CaSR monitors plasma concentrations of Ca²⁺, whereas *GNA11* is the alpha subunit—guanine nucleotide binding protein of the trisubunit guanosine protein binding receptor (GPCR) transmitting the signal of the CaSR to intracellular signal transduction pathways. A lowered "set-point" of the CaSR or enhancement of the postreceptor signal transduction system itself enables PTH secretion to be suppressed and renal tubular reabsorption of calcium to be depressed (thus increasing urine calcium excretion) by extremely low concentrations of Ca²⁺ resulting in marked hypercalciuria. Activating mutations (e.g., p.Lys47Asn, p.Leu616Val, p.Phe788Leu) may be scattered throughout *CASR* but occur predominantly in the second peptide loop of its extracellular domain.¹⁸ Some activating monoallelic variants (e.g., p.Cys141Trp, p.Leu125Pro, p.Ala843Glu) of *CASR* may also inhibit function of the renal outer medullary potassium channel (encoded by *KCNJ1*, OMIM 600359), leading to a Bartter-like syndrome with hypokalemic metabolic alkalosis, hyperreninemia, hyperaldosteronism, and hypomagnesemia, as well as hypercalciuric hypocalcemia (designated Bartter syndrome type 5, OMIM 601198). These paired metabolic defects are partially responsive to treatment with hydrochlorothiazide and low doses of calcitriol. However, children with hypercalciuric hypocalcemia caused by gain-of-function mutations in *CASR* are very sensitive to even low doses of calcitriol that can lead to even more marked hypercalciuria and to nephrocalcinosis. Thus management of these patients has been difficult. Administration of recombinant human PTH¹⁻³⁴ to a 14-month-old hypocalcemic male infant with a de novo nonsense mutation in *CASR* (p.Leu727Gln) for 17 months partially restored calcium homeostasis with increased but still subnormal serum levels of calcium, while urinary excretion of calcium decreased into the normal range.¹⁷ During treatment, the child was clinically asymptomatic, did not develop nephrocalcinosis, and tolerated the drug well. However, administration of PTH to such a patient may increase urine calcium excretion and the risk of nephrocalcinosis. Use of calcilytic agents (type II calcimimetics), such as cinacalcet that antagonize the effects of calcium upon the CaSR in the parathyroid glands and renal tubules may prove therapeutically useful in these patients. Neonates with

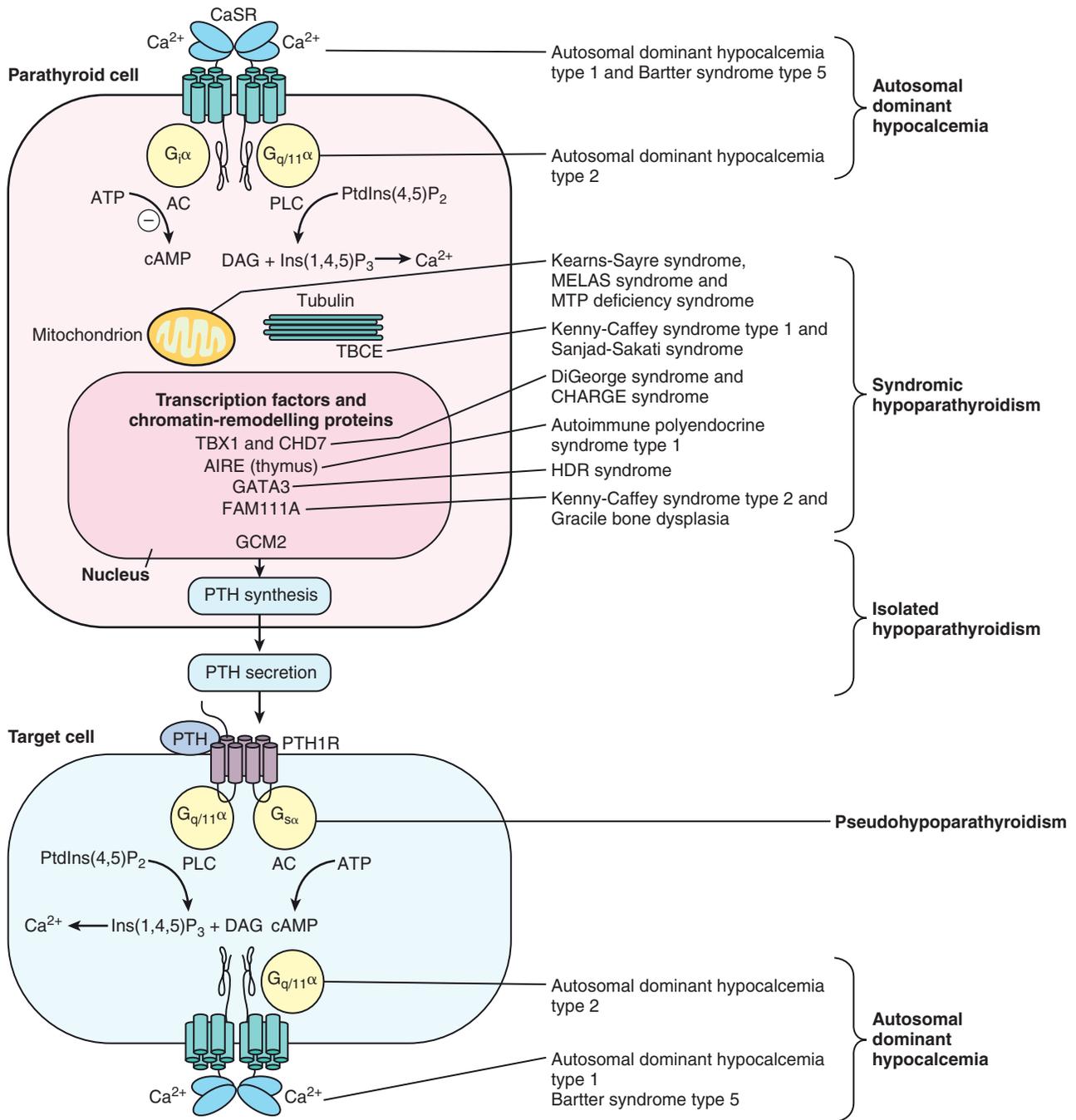


Fig. 20.1 Regulation of parathyroid hormone (PTH) secretion and genetic disorders resulting in hypoparathyroidism. In the parathyroid gland the calcium sensing receptor (CaSR) monitors the circulating Ca^{2+} concentration and mediates the secretion of PTH that acts through the PTH receptor on the plasma membrane of the target bone or renal cell. Genetic errors in this pathway lead to hypoparathyroidism. AC, Adenylyl cyclase; AIRE, autoimmune regulator; ATP, adenosine triphosphate; Ca^{2+} , ionized calcium, CaSR, calcium sensing receptor, cAMP, cyclic adenosine monophosphate; CHD, chromodomain; DAG, diacylglycerol; FAM111A, family with sequence similarity 111A; GATA3, GATA binding protein 3; GCM2, glial cell missing transcription factor 2; Ins(1,4,5)P₃, inositol trisphosphate; PLC, phospholipase C; PtdIns, phosphatidylinositol; PTH, parathyroid hormone; PTH1R, PTHrP receptor; TBCE, tubulin-specific chaperone E; TBX1, T-box transcription factor 1. (From Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers*. 2017;3:17055, with permission.)

variants of *GNA11* may not have the same degree of hypercalciuria as do those with mutant *CASR*.

The most commonly encountered forms of hypoparathyroidism are those associated with constellations of congenital abnormalities (see Tables 20.2A, 20.2B and see Fig. 20.1). The most frequent complex form of hypoparathyroidism is that associated with the DiGeorge syndrome type 1 (OMIM 188400), a disorder that occurs with a frequency of 1:4000 births and is present in approximately 70% of children with

isolated hypoparathyroidism.^{19,20} In many infants with the DiGeorge syndrome, hypoparathyroidism partially remits over time only to reappear in periods of stress, such as infections or trauma. The DiGeorge syndrome is a neurocristopathy—the result of disturbed migration of cervical neural crest cells and consequent maldevelopment of tissues of neural crest origin derived from the third and fourth pharyngeal pouches and first to fifth branchial arches. DiGeorge syndrome type 1 is associated with microdeletions of chromosome region 22q11.2

(del22q11.2—the DiGeorge critical region), a segment on which more than 35 genes are sited and thus is a contiguous gene syndrome (a disorder caused by deletion of several adjacent genes that when individually mutated may result in a distinctive clinical feature but when collectively lost leads to a group of apparently unrelated clinical findings). The chromosome 22q11.2 microdeletion is contained within regions of low copy number repeats, and it is this characteristic that results in unequal segmental exchange between the paired 22nd chromosomes during meiosis.²⁰ Subjects with the DiGeorge syndrome usually have the triad of: (1) hypocalcemia because of hypoplasia of the parathyroid glands often manifest in the neonatal period but which may not be detected until an older age, (2) defective T lymphocyte function and impaired cell-mediated immunity because of partial or complete absence of thymic differentiation leading to increased frequency of viral and fungal infections and propensity of autoimmune disorders, and (3) conotruncal defects of the heart or aortic arch (Tetralogy of Fallot, ventricular septal defect, interrupted or right aortic arch, truncus arteriosus, vascular ring).²⁰ To a substantial extent, DiGeorge syndrome type 1 is related to loss or deleterious variants of T-box 1 (*TBX1*, OMIM 602054) within the 22q11.2 chromosomal segment. Experimental disruption of *Tbx1* impairs development of the pharyngeal arch arterial vasculature, whereas introduction of null mutations in *Tbx1* results in anomalies of the cardiac outflow track and hypoplasia of the thymus and parathyroid glands. The transcription factor encoded by *TBX1* is part of a network of gene products (including those encoded by *ISL1*, *SHH*, *FOXA2*, *FOXC2*) that controls development of the parathyroid glands and thymus by regulating expression of *GATA3*, *GCM2*, and *PAX9*.²⁰ Mutations of *TBX1* specifically account for five major manifestations of the DiGeorge syndrome: parathyroid hypoplasia and hypocalcemia, thymic aplasia, cardiac anomalies, unusual facial features (low set ears, micrognathia, slanted eyes, short palpebral fissures and philtrum, small mouth), and cleft palate with velopharyngeal insufficiency. *TBX1* haploinsufficiency can also cause isolated hypoparathyroidism. Also within this two megabase microdeletion at chromosome 22q11.2 is *HIRA* (histone cell cycle regulation, OMIM 600237), a transcription regulatory factor that is expressed in developing heart and upper body neural crest elements and is necessary for normal cardiac development. Another critical gene sited at chromosome 22q11.2 is *UFD1L* (ubiquitin fusion degradation 1-like, OMIM 601754), whose product is important for the posttranslational processing of proteins and/or their degradation by interaction with the ubiquitin fusion protein. Experimentally, the DiGeorge syndrome has been linked to genes encoding endothelin-1, vascular endothelial growth factor, and fibroblast growth factor-8 (*Fgf8*, a target gene for *TBX1*). In the mouse hypomorphic for *Fgf8*, there are cardiovascular, craniofacial, parathyroid, and thymic defects—an experimental phenocopy of the human del22q11.2 syndrome.²¹ (Maternal diabetes mellitus, alcoholism, or ingestion of retinoic acid may occasionally be associated with the DiGeorge syndrome in the offspring.)

Prenatally, the presence of the DiGeorge syndrome may be considered when fetal ultrasonography reveals an interrupted aortic arch or truncus arteriosus and may be confirmed by appropriate studies (microarray, fluorescent in situ hybridization [FISH]) on samples of chorionic villi or amniotic fluid. Other clinical features of the DiGeorge syndrome include: growth retardation, renal dysplasia, gastrointestinal malformations (esophageal atresia, anal atresia), cervical spine instability, impaired vision, and ocular malformations, malformation of the cerebral cortex (perisylvian polymicrogyria), and developmental delay.²⁰ DiGeorge syndrome type 1 (OMIM 188400) may occur sporadically or be transmitted as an autosomal dominant trait. DiGeorge syndrome type 2/Velocardiofacial syndrome complex 2 (OMIM 601362) has been

associated with interstitial deletion of chromosome 10p13 but attributed, in part, to loss of *NEBL* (Nebulette, OMIM 605491), sited at chromosome 10p12.31. *NEBL* encodes a protein expressed in cardiac and striated muscle that associates with actin, myofibrils, and cellular adhesion complexes; DiGeorge syndrome type 2 is transmitted as an autosomal dominant characteristic.³ The DiGeorge syndrome has also been associated with microdeletions of chromosomes 18q21.33 and 4q21.2-q25—indicative of the cascade of genes likely involved in the generation of this phenotype. In addition to DiGeorge syndrome type 1, deletion of chromosome 22q11.2 has been associated with the velocardiofacial and other syndromes. Collectively, these syndromes display similar facial features (ocular hypertelorism, lateral displacement of inner canthi, short palpebral fissures, swollen eyelids, dysmorphic “segmented” nose, small mouth, low set ears with abnormally folded pinnae, short philtrum, micrognathia, malar hypoplasia, velopharyngeal insufficiency with/without cleft palate), olfactory dysfunction, short stature, nonverbal learning disabilities, and various psychological maladies.^{19,22} Takao velocardiofacial syndrome (included in OMIM 188440) consists primarily of the typical cardiac defects described earlier that may also be associated with hypocalcemia; Shprintzen velocardiofacial syndrome (OMIM 192430) is characterized by craniofacial and palatal defects and cardiac anomalies; Cayler cardiofacial syndrome (OMIM 125520) is associated with partial unilateral facial paresis because of hypoplasia of the depressor angulioris muscle and anomalies of the heart and aorta. These syndromes have been grouped as the CATCH-22 syndromes of cardiac defects, abnormal face, thymic hypoplasia, cleft palate, hypocalcemia.

Hypocalcemia has been observed in some subjects with microduplication of chromosome 22q11.2, a copy number variant; clinical characteristics of individuals with this genetic anomaly vary from those who are entirely normal to patients with multiple congenital anomalies, severe developmental delay, autism, and schizophrenia.^{23,24} The duplication chromosome 22q11.2 syndrome appears to be transmitted as an autosomal dominant characteristic whose expression is modified by other factors. The pathophysiology of hypocalcemia in affected subjects is uncertain. In one family in which the proband had the DiGeorge syndrome associated with del22q11.2, the normal father had the same anomaly on one of his 22nd chromosomes and dup22q11.2 on his other 22nd chromosome; paternal quantitative expression of the genes located on chromosome 22q11.2 was normal indicating that the adverse effects of the 22q11.2 deletion were compensated by the 22q11.2 duplication.²⁵

There are several other syndromes with multisystem involvement and hypoparathyroidism. Hypoplasia of the parathyroid glands and consequent hypoparathyroidism may be observed in patients with the CHARGE syndrome (coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies, OMIM 214800) as a result of deletion of chromosome 8q12 or specifically to a heterozygous inactivating variant of *CHD7* (chromodomain helicase DNA-binding protein 7, OMIM 608092).²⁶ *CHD7* encodes a transcription regulating factor essential for differentiation of the neural crest that may coassociate with *TBX1*. *CHD7* is an adenosine triphosphate (ATP)-dependent chromatin remodeler that regulates movement of nucleosomes. The incidence of the CHARGE syndrome is approximately 1/10,000 births. The CHARGE and DiGeorge syndromes share several anomalies including hypoparathyroidism, cardiac and renal anomalies, cleft palate, ear abnormalities, and developmental delay. Indeed, hypoparathyroidism is more common (72%) in neonates with CHARGE than in those with the DiGeorge syndrome (26%).

The Barakat or HDR syndrome of hypoparathyroidism, sensorineural deafness, and renal disease (dysplasia, steroid-resistant

nephrosis with progressive renal failure—OMIM 146255) has been attributed to monoallelic deleterious variants of *GATA3* encoding GATA-binding protein-3 (OMIM 131320), a zinc-finger transcription factor/enhancer element that is required for development of the parathyroid glands, auditory system, and kidneys and for expression of genes encoding the four T-cell receptor subunits.¹² Heterozygous inactivating mutations of *GATA3* primarily involving loss of its carboxyl terminal DNA-binding segment are transmitted as an autosomal dominant disorder. Insertions, missense, and nonsense mutations in *GATA3* have also been identified in patients and families with HDR.²⁷ *GATA3* is a zinc-finger transcription factor that regulates expression of *GCM2* and thus is critical for the embryonic development of the parathyroid glands, as well as for the kidneys, otic vesicles, and thymus. The parathyroid glands of these children are hypoplastic or absent. Hypocalcemia may be present in the newborn period or unrecognized until later childhood. Malformations of the uterus and vagina (didelphic uterus, septate vagina) may be present in females with this disorder.

The Sanjad-Sakati syndrome of congenital hypoparathyroidism, mental retardation, and facial dysmorphism (depressed nasal bridge, long philtrum, thin upper lip, long filtrum) (HRD, OMIM 241410) is caused by biallelic loss-of-function mutations (often a 12 base pair deletion in the third coding exon) in *TBCE* (tubulin-specific chaperone E, OMIM 604934).²⁸ *TBCE* is a chaperone protein that is essential for formation/folding and stability of microtubules—cytosolic structures composed of heterodimeric α - and β -tubulin subunits that form the cytoskeleton, mitotic apparatus, cilia, and other cellular components; this chaperonin assists in the correct folding of α - and β -tubulin subunits and the formation of α - β -tubulin heterodimers. The α - and β -tubulin subunits and *TBCE* are necessary for normal embryogenesis of the parathyroid glands. Mutations in *TBCE* result in lowered microtubule formation and consequently in decrease in subcellular components, such as the cytoskeleton, Golgi apparatus, and endosomal compartments required for normal intracellular movement of proteins, as well as formation of the mitotic apparatus and cilia. The majority of infants with HRD sustain intrauterine growth retardation and manifest hypocalcemic seizures with low serum concentrations of PTH and normal phosphaturic responses to exogenous PTH in the first several weeks and months after birth. Children with HRD are short, developmentally delayed, and seizure prone; they have medullary stenosis of the long bones and other skeletal anomalies; they are microcephalic with faces characterized by deeply recessed eyes or microphthalmia, depressed nasal bridge, beaked nose, long philtrum, thin upper vermilion border, micrognathia, and large floppy earlobes. The cardiovascular system of these patients is intact, but as infants, they are susceptible to life-threatening pneumococcal infections.²⁹ Additional features in HRD subjects as assessed by neuroimaging include adenohipophyseal hypoplasia and attenuation of the pituitary stalk, infundibulum, and corpus callosum.

When a patient with the Sanjad-Sakati syndrome—HRD triad also manifests cortical thickness and medullary stenosis of the long bones, osteosclerosis of the skull, and susceptibility to recurrent infections and has biallelic variants of *TBCE*, the complex is termed the *Kenny-Caffey syndrome type 1* (KCS1, OMIM 244460). Neonates with KCS1 are often severely hypocalcemic early in the postnatal period. As children they are short, with microcephaly and craniofacial anomalies because of absence of diploic space in the skull, osteosclerosis, and thickening of the cortices of the long bones with narrowing of the medullary compartment, normal or mildly delayed development, and increased susceptibility to recurrent bacterial infections. Interestingly, the identical mutation in *TBCE*—a homozygous 12-bp deletion in exon 2—may result in either the HRD or KCS1 phenotype in a specific family.³⁰ The clinical,

laboratory, and radiographic findings in patients with Kenny-Caffey syndrome 2 (KCS2, OMIM 127000) are similar to those in subjects with KCS1, except that patients with KCS2 have normal intelligence. KCS2 is the result of monoallelic loss-of-function mutations in *FAM111A* (family with sequence similarity 111, Member A, OMIM 615292). Allelic to KCS2 is gracile bone dysplasia (OMIM 602361), in which the bones are thin, slender, and brittle, the diaphyses are dense, the basal cranial sutures close prematurely, there is microphthalmia, and hypocalcemia because hypoparathyroidism is common. Hypomagnesemia is often present as well. Heterozygous variants in *FAM111A* have been identified in these patients. *FAM111A* encodes a factor whose expression is lowest in stage G0 of the cell cycle but whose primary function is unknown; it has been suggested that *FAM111A* may interact with *TBCE* to regulate gene expression or function.³¹

Mitochondria are cytoplasmic organelles that are primarily donated to the embryo within the cytoplasm of the fertilized ovum and are therefore of maternal origin. Mitochondria are the primary intracellular site of respiration and energy utilization. The mitochondrial genome consists of a single circular chromosome with 37 embedded genes. Thirteen mitochondrial genes encode proteins required for electron transport and energy generation; 22 mitochondrial genes encode transfer ribonucleic acids (RNAs) required for protein synthesis. In addition, there are mitochondrial proteins whose genes reside in the cell's nucleus but that are expressed within mitochondria. Thus mitochondrial dysfunction leading to hypoparathyroidism may be caused by deletion and/or duplication of mitochondrial DNA encoding genes intrinsic to the mitochondrion itself or to variants of nuclear DNA encoding a protein expressed within the mitochondrion.³ Clinical manifestations of mitochondrial DNA mutations almost always involve muscle dysfunction, as well as other tissues depending upon the postconceptual age and cellular site(s) in which the mitochondrial DNA variation occurs. The Kearns-Sayre syndrome (OMIM 530000) is associated with agenesis or dysgenesis of the parathyroid glands leading to hypoparathyroidism. Primary manifestations of this syndrome include progressive external ophthalmoplegia, pigmentary retinopathy, sensorineural deafness, cerebellar ataxia, abnormal cardiac conductivity, myopathy, growth retardation, and renal tubular dysfunction, as well as hypoadrenocorticism, hypogonadism, and diabetes mellitus.³² Both deletions and duplications of mitochondrial DNA may be found in patients with this syndrome. The syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS, OMIM 540000) is also associated with hypoparathyroidism, as well as myopathy, ophthalmoplegia, neuropathy, cardiomyopathy, impaired cognition, and diabetes mellitus. Biallelic mutations in *HADHB* (Hydroxylacyl-CoA dehydrogenase/3-Ketoacyl-CoA thiolase/Enoyl-CoA hydratase, beta subunit, beta subunit, OMIM 143450) encoding mitochondrial trifunctional protein impair intramitochondrial beta oxidation of fatty acids resulting in the inability to use an energy source. Depending upon the extent of the enzymatic deficiency, clinical presentations may vary from acute and lethal in the perinatal period to a hepatic Reye-like syndrome in older infants to a skeletal myopathy in adolescents. Occasionally, hypoparathyroidism may occur in these patients.³³

PHP is a conglomerate of clinical disorders most often characterized by end-organ insensitivity to the biological effects of PTH (and PTHrP) resulting in hypocalcemia despite substantial secretion of endogenous PTH. The PTH receptor (*PTH1R*, OMIM 168468) is a heptahelical transmembrane G-protein-coupled structure that after binding to extracellular PTH or PTHrP initiates intracellular signal transduction by changing its configuration, thereby enabling the linked submembrane stimulatory guanine nucleotide-binding protein (Gs-protein)

to exchange guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the alpha subunit (*GNAS*, OMIM 139320) of the heterotrimer of alpha, beta, and gamma subunits that together comprise the G_s-protein.³⁴ After substitution of GTP for GDP on the α subunit, G_{s α} dissociates from its linked $\beta\gamma$ subunits. G_{s α} then stimulates intracellular adenylyl cyclase (*ADCY3*, OMIM 600291) enzymatic activity, thereby converting ATP to cyclic adenosine monophosphate (AMP) and releasing it from the intracellular surface of the plasma membrane of the PTH-responsive cell. Cyclic AMP in turn binds to the regulatory alpha subunit of protein kinase A or PKA (*PRKAR1A*, OMIM 188830), thereby initiating intracellular signaling.³⁴ The catalytic subunits of PKA then phosphorylate a number of intracellular proteins including the cyclic AMP-responsive binding protein (*CREB1*, OMIM 123810) that in turn initiate transcription of cyclic AMP target genes. CREB1 is inactivated by one of several phosphodiesterases encoded by *PDE4D* (OMIM 600129) and *PDE3A* (OMIM 123805). After G_{s α} has propagated signal transmission through activation of adenylyl cyclase, the intrinsic GTPase activity of G_{s α} hydrolyzes the attached GTP to GDP, thereby halting further signal transduction.

Neonates with biallelic loss-of-function mutations in *PTH1R* are functionally hypoparathyroid despite elevated serum concentrations of PTH and thus represent a form of "PHP."^{35,36} Because of subresponsiveness to PTHrP in utero, fetal bone formation is abnormal resulting in Blomstrand chondrodysplasia—an osteochondrodystrophy characterized by short extremities and advanced skeletal and dental maturation—abnormalities detectable in utero by fetal ultrasonography. Histologically, the proliferative zone of the cartilage growth plate is narrowed with relatively few resting and proliferating chondrocytes, whereas the hypertrophic zone is composed of irregular columns of chondrocytes. Transmitted as an autosomal recessive trait, its clinical characteristics include polyhydramnios, hydrops fetalis, short-limbed dwarfism, facial anomalies, aberrant tooth development, aplasia of the nipples and breasts, hypoplastic lungs, preductal aortic coarctation, and neonatal hypocalcemia and hyperphosphatemia despite elevated serum concentrations of PTH. Although Blomstrand osteochondrodysplasia (OMIM 215045) is usually lethal, skeletal malformations may be more (type I) or less severe (type II). Mutations in *PTH1R* that result in complete absence of normal protein (e.g., Arg104Ter) are designated type I while mutations that permit some *PTH1R* synthesis (Pro132Leu) result in type II Blomstrand osteochondrodysplasia.³⁷ Eiken chondrodysplasia (OMIM 600002) is also caused by biallelic loss-of-function mutations in *PTH1R* but is clinically and radiographically distinct from Blomstrand osteochondrodysplasia as affected subjects have mild growth retardation, markedly delayed epiphyseal ossification, multiple epiphyseal dysplasia, and persistent islands of cartilage in the pelvis.³⁸ Variants of *PTH1R* that occur in patients with the Eiken syndrome are located in the carboxyl terminal portion of the gene/protein (e.g., pArg485Ter). Heterozygous inactivating mutations of *PTH1R* may result in an autosomal dominant nonsyndromic failure of tooth eruption (OMIM 125350).³⁹

Classically, PHP is the term applied to the clinical state that is associated with abnormalities of the signaling pathway that transmits the message conveyed by interaction of PTH/PTHrP with *PTH1R*. Neonates and infants with PHP are often hypocalcemic and hyperphosphatemic with elevated serum levels of PTH, whereas older patients with PHP exhibit the characteristic phenotype and skeletal malformations of Albright hereditary osteodystrophy ([AHO]; growth retardation; brachydactyly of the third, fourth, and fifth metacarpal bones; round face; impaired dentinogenesis; and subcutaneous ossifications) with or without biochemical abnormalities.^{40,41} The AHO phenotype

of growth retardation and brachydactyly is the result of accelerated closure of cartilaginous growth plates in long bones—the consequence of G_{s α} haploinsufficiency in chondrocytes and early phase osteoblasts that accelerates osteoblast maturation. Palpable heterotopic subcutaneous ossifications, at times associated with a bluish discoloration of the overlying skin, develop in 70% of patients with AHO associated with PHP1a and pseudopseudohypoparathyroidism (PPHP); their number and size may increase over time and become acutely or chronically painful. Other unusual consequences of PHP1a include spinal stenosis, carpal tunnel syndrome, sensorineural and conductive hearing loss, olfactory impairment, sleep apnea, and asthma. Consistent with resistance to endogenous PTH, administration of exogenous PTH does not reduce the number of sodium-phosphate cotransporters (NaPi2a, NaPi2c) and thus does not increase renal excretion of phosphate or cyclic AMP.

GNAS is an imprinted gene; although *GNAS* is expressed from both maternal and paternal *GNAS* genes in many tissues, *GNAS* is expressed only from the maternal gene in the proximal renal tubule, thyroid, gonads, and adenohypophysis (vide infra).⁴² Thus in the proximal renal tubule *GNAS* expression is "imprinted"; that is, there is differential gene expression depending on the parent of origin of the allele. *GNAS* is composed of 13 exons; it has multiple transcripts that arise through splicing of four unique first exons onto shared exons 2 to 13 (Fig. 20.2).⁴² Transcripts of *GNAS* include: (1) the G_{s α} transcript—a protein that stimulates adenylyl cyclase and generates cyclic AMP—G_{s α} is expressed by both maternal and paternal alleles in most tissues (including skin, white adipose tissue, chondrocytes, bone); however, only the maternal allele of *GNAS* is expressed in the proximal renal tubule, thyroid, gonads, and anterior pituitary (because of silencing of paternal *GNAS* caused by an epigenetic imprint)⁴¹; (2) XL α s yields a G_{s α} isoform that is specifically expressed in neuroendocrine and nerve tissues and is identical to G_{s α} except that it has a very long amino terminal sequence of amino acids; it is expressed only by the paternal allele; (3) the neuroendocrine secretory protein-55 (NESP55) transcript is a chromogranin-like protein that is expressed in neuroendocrine tissues but only by the maternal *GNAS* allele; and (4) the alternative first exon A/B (exon 1A) transcript is expressed ubiquitously but only at low levels and by the paternal *GNAS* allele and is not translated. The promoters of XL α s, NESP55, and exon 1A transcripts lie within the 5' differentially methylated region (DMR) of *GNAS*. Methylation of the promoter usually silences expression of that *GNAS* transcript.

Loss-of-function mutations in *GNAS* or epigenetic (methylation) aberrations that result in failure of expression of a parental G_{s α} transcript of *GNAS* lead to PHP types IA, IB, and IC and PPHP. PHP type 1A (PHP1a, OMIM 103580) is associated with resistance to protein hormones that signal through GPCRs and is the consequence of loss-of-function mutations in the maternal allele that encodes *GNAS*. Inactivating variants of maternal *GNAS* (functionally resulting in biallelic inactivity of *GNAS* and near total loss of G_{s α} activity in the proximal renal tubule) lead to resistance to the biological effects of PTH in the proximal renal tubule and decreased reabsorption of filtered calcium resulting in hypocalcemia, exaggerated proximal renal tubular reabsorption of filtered phosphate leading to hyperphosphatemia, and elevated serum concentrations of PTH. In response to exogenous PTH, neither the urinary excretion of cyclic AMP nor that of phosphate increases. Erythrocyte G_{s α} activity is subnormal in patients with PHP1a.³⁴ (Resistance to thyroid-stimulating hormone [TSH] also occurs in patients with PHP1a. Thus PHP1a may be suspected in a neonate with hypocalcemia in whom hyperthyrotropinemia has been detected in the neonatal screening study for congenital hypothyroidism.⁴³) More than 200 heterozygous loss-of-function mutations in maternal

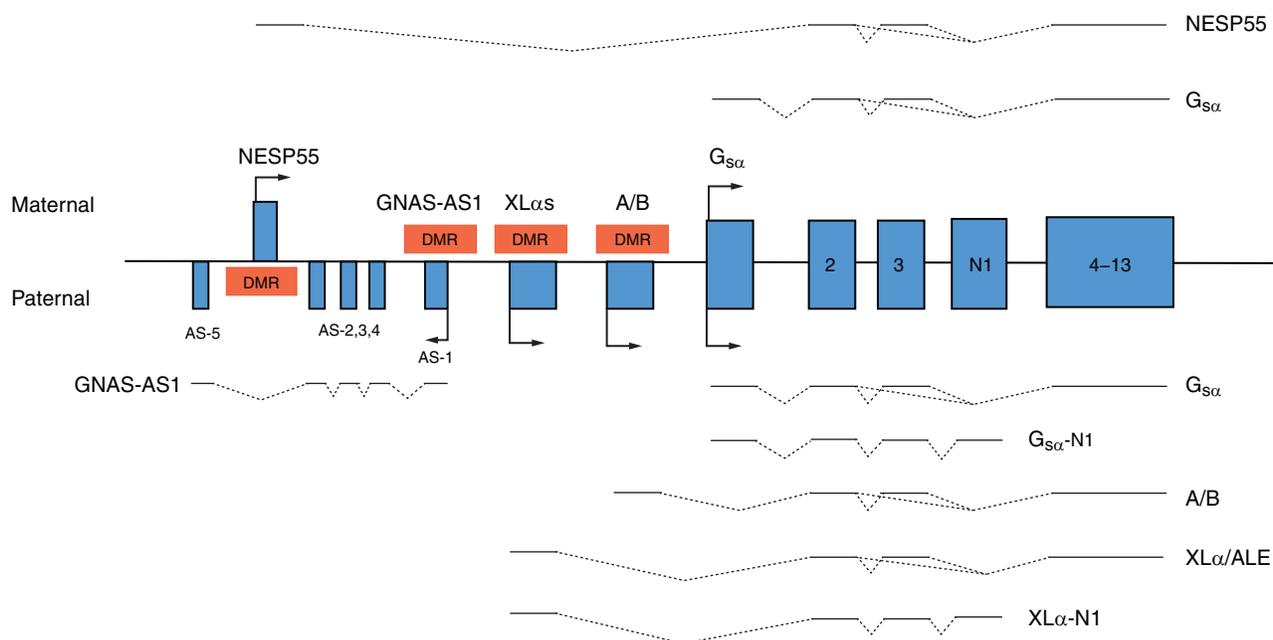


Fig. 20.2 Schematic view of the *GNAS* gene complex. Three major transcripts that encode proteins are derived from *GNAS* (neuroendocrine secretory protein-55 [NESP55], XL α s, and G $_{S\alpha}$) by linking of their first exon to the common exons 2–13. The transcript GNAS-AS1 is transcribed in the reverse/antisense direction. Through epigenetic imprinting regulated by the differentially methylated regions (DMR), the transcripts of XL α s and GNAS-AS1 are expressed only by the paternal allele, whereas NESP55 is expressed only by the maternal *GNAS* gene. G $_{S\alpha}$ is expressed from both parental *GNAS* alleles except in the proximal renal tubule, thyroid, gonads and adenohypophysis where only the maternal G $_{S\alpha}$ allele is expressed. The maternal *GNAS* transcripts are shown above the horizontal line, while the paternal transcripts are represented below the line. Bold lines represent exons; dashed lines indicate introns. (From Pignola RJ, Ramaswamy G, Fong JT, et al. Progressive osseous heteroplasia: diagnosis, treatment, and prognosis. *Appl Clin Genet*. 2015;8:37–48, with permission.)

GNAS have been described. A four base pair deletion in exon 7 (codons 188–189) in *GNAS* that leads to a frameshift and premature stop codon has been found in a number of families with PHP1a and appears to be a “mutational hotspot” as it impairs DNA polymerization and replication. Other mutations alter intracellular movement of *GNAS* protein (p.Leu99Pro, p.Ser250Arg), increase the rate of release of GDP (p.Arg258Trp, p.Ala366Ser), or impair coupling of G-protein to PTH1R (p.Arg385His). Inasmuch as *GNAS* is expressed by both maternal and paternal alleles in the distal renal tubule where filtered calcium is also reabsorbed, nephrocalcinosis does not usually develop in patients with PHP1a.³

Although patients with PHP type IB (PHP1b, OMIM 603233) generally have a normal phenotype (except for mild brachydactyly or obesity), they are hypocalcemic, hyperphosphatemic, and resistant to the biological effects of exogenous PTH and to TSH. PHP type IB occurs only in the offspring of obligate female carriers in whom loss of maternal *GNAS* expression in the kidney results in selective proximal renal tubular resistance to PTH; inasmuch as skeletal expression of both maternal and paternal *GNAS* is intact, bone formation is normal. PHP type IB is the result of defects in methylation of cytosine nucleotides within maternal *GNAS* resulting in its inactivation/conversion to a paternal *GNAS* epigenotype and the same functional consequences as those observed in patients with PHP type IA inasmuch as maternal *GNAS* is “silenced” in imprinted tissues, particularly the kidney. In some instances, this maternal epigenetic error has been transmitted by a maternal carrier to her offspring. In some patients, PHP type IB is caused by deletions within a DMR of *GNAS* or to loss of a 5' cis-acting imprinting control center that is essential for methylation of the *GNAS* DMR that regulates expression of maternal *GNAS* in the proximal renal tubule.^{40,44} PHP type IB may also be caused by microdeletions of maternal *GNAS* exons AS3–4 or

loss of methylation in maternal exons A/B of the differentially methylated region resulting in silencing of maternal *GNAS* or to variants of *GNAS*-AS1 or *STX16*.^{3,34} *GNAS*-AS1 (*GNAS* complex locus, antisense transcript 1, OMIM 610540) is embedded within the *GNAS* coding region, whereas *STX16* (Syntaxin16, OMIM 603666) is positioned immediately centromeric (5') to *GNAS* on the long (q) arm of chromosome 20; mutations (loss of exons 3–6) of maternal *STX16* are associated with loss of methylation of *GNAS* exons A/B. Nevertheless, the genetic basis for most patients with sporadic PHP1b remains unknown; some of these patients appear to have global epigenetic methylation defects. Paternal uniparental isodisomy of the long arm of chromosome 20 (site of *GNAS*) may be the cause of PHP1b in some patients. These patients have two normal paternal 20th chromosomes but no functional *GNAS* in specific tissues (i.e., proximal renal tubules). Imprinting defects involving *GNAS* have also been identified in patients with AHO IB and microdeletions of chromosome 2q37. Inasmuch as bone is responsive to PTH in patients with AHO IA/IB, an occasional subject may develop osteitis fibrosa cystica over time. Nevertheless, because PTH also has anabolic effects on endocortical bone, bone mineralization in some patients with PHP1a/1b may be increased.

Patients with PHP type IC have the AHO phenotype and are hypocalcemic and hyperphosphatemic, but erythrocyte Gas activity is normal.³⁴ PHP type I is related to variants of the maternal *GNAS* allele in exon 13 (see Fig. 20.2) near its carboxyl terminal, often leaving intact the adenylate cyclase activity region. Patients with PHP type II (OMIM 203330) have hypocalcemia, hyperphosphatemia, and elevated serum levels of PTH but a normal phenotype. In response to exogenous PTH, patients with PHP type II increase the urinary excretion of cyclic AMP but do not increase urine phosphate excretion, indicating a defect in intracellular signaling distal to generation

of adenylyl cyclase. Erythrocyte $G_{s\alpha}$ activity is normal in patients with PHP type II. The pathogenesis of PHP type II has not as yet been identified, but in some subjects it has been suggested that it may be related to vitamin D deficiency and PTH resistance secondary thereto; others may be ingesting anti-convulsant medications that accelerate degradation of vitamin D and its bioactive metabolites. Subjects with PPHP (OMIM 612463) caused by inactivating mutations of paternal *GNAS* have the AHO phenotype because of haploinsufficiency of *GNAS* expression in bone but are not obese and are intellectually intact; their kidneys, where maternal *GNAS* is expressed, are normally responsive to endogenous and exogenous PTH, and hence these patients are normocalcemic and normophosphatemic.³ Heterozygous mutations in *GNAS* on either parental allele have been associated with intrauterine growth retardation, with severity greatest when the variant is in the paternal allele, suggesting that a paternally derived *GNAS* transcript is required for normal fetal growth. The postnatal growth retardation of PHP and PPHP is possibly the extended consequence of cartilage growth plate resistance to PTH in utero and postnatally.³⁴ Patients with PHP type IA are resistant to many hormones that signal through *GNAS* including: TSH that may present as congenital hypothyroidism with hyperthyrotropinemia, growth hormone releasing hormone (GHRH) associated with growth hormone (GH) deficiency, luteinizing (LH) and follicle-stimulating (FSH) hormones presenting as delayed puberty, and melanocortin (MSH) associated with obesity of early onset.

Acrodysostosis is a chondrodysplasia with many features of PHP1a (short stature, obesity, brachydactyly, abnormal face with nasal and maxillary hypoplasia, hypertelorism, markedly advanced skeletal maturation) that is caused by gain-of-function variants of *PRKARIA* (OMIM 188830) or *PDE4D* (OMIM 600129) encoding components of the signal transduction systems activated by $G_{s\alpha}$ (vide supra).^{45,46} *PRKARIA* encodes the cyclic AMP-dependent regulatory alpha subunit of PKA, the protein kinase that is downstream of $G_{s\alpha}$ and cyclic AMP and whose activation leads to the cascade of intracellular signal transduction that regulates cell division, differentiation, metabolism, function, and apoptosis. Paradoxically, prolonged activation of the regulatory alpha subunit of PKA results in decline in the functional activity of the catalytic subunit of PKA. In a patient with acrodysostosis type 1 (OMIM 101800), a de novo germline gain-of-function mutation (pArg368Stop) in *PRKARIA* is present that leads to functional resistance to PTH and TSH.^{45,47} The mutant *PRKARIA* is a shortened protein whose binding avidity to the catalytic subunit of PKA is increased, because it lacks one of two cyclic AMP binding domains; hence it can only be slowly released from the catalytic subunit of PKA by cyclic AMP, thereby maintaining the catalytic subunit of PKA in the inactive state. In some patients with acrodysostosis type 1, resistance to PTH, GHRH, TSH, and the gonadotropins is present. *PDE4D* encodes phosphodiesterase 4D, an enzyme that hydrolyzes and inactivates cyclic AMP; hormone resistance is not present in patients with acrodysostosis type 2 (OMIM 614613) related to variants of *PDE4D*, although these patients are developmentally challenged.⁴⁵ In patients with inactivating mutations in *PDE4D*, the rate of degradation of cyclic AMP is increased.

(An alternative nomenclature for PHP has been presented by the EuroPHP network and termed the *inactivating PTH/PTHrP signaling disorder* [iPPSD] classification.³⁴ In this schema, the clinical disorders are linked to the genetic mutation in a component of the signal transduction pathways transmitting the messages of PTH and PTHrP that initiate cellular responses. For example, iPPSD1 is associated with biallelic inactivating mutations of *PTHRI* leading to the usually fatal Blomstrand chondrodysplasia [OMIM 215045] of short limbs, sclerotic

bones, advanced bone maturation, and facial dysmorphism associated with polyhydramnios and hydrops fetalis; Eiken syndrome [OMIM 600002] is an osteodysplasia with markedly delayed epiphyseal maturation that is also associated with biallelic mutations in *PTHRI*; Murk-Jansen chondrodysplasia [OMIM 156400] is the result of monoallelic activating mutations of *PTHRI* and is characterized by small stature, short and bowed limbs, clinodactyly, abnormal face, hypercalcemia, and hypophosphatemia despite normal to low serum concentrations of PTH. Inactivating mutations of *GNAS* leading to PHP1a are designated iPPSD2. Methylation defects involving *GNAS* giving rise to PHP1b are designated iPPSD3. Heterozygous mutations in *PRKARIA* [OMIM 188830] leading to deficient PKA activity result in acrodysostosis type 1 [OMIM 101800] characterized by growth retardation, variable facial dysmorphism, brachydactyly, and advanced skeletal maturation often associated with end-organ resistance to PTH and other G-protein-associated hormones are designated iPPSD4. Monoallelic variants of *PDE4D* [OMIM 600129] resulting in abnormalities of cyclic AMP-specific phosphodiesterase hydrolytic activity leading to acrodysostosis type 2 [OMIM 614613] whose phenotype resembles that of type 1 in association with developmental delay is termed iPPSD5. Brachydactyly with hypertension [OMIM 112410] has been related to variants of *PDE3A* [OMIM 123805, chr. 12p12.2] and assigned to iPPSD6. The designation iPPSD7 is used for disorders of unknown genetic pathogenesis.)

Progressive osseous heteroplasia (POH, OMIM 166350) is one of a group of disorders associated with intra- or subdermal ossification/calcification, in addition to the AHO phenotype of PHP, that is related to inactivating variants of paternal *GNAS* (vide infra).⁴² POH is a disorder caused by loss-of-function mutations involving the paternal *GNAS* allele that is characterized clinically by dermal ossifications that begin in infancy and progress to diffuse heterotopic bone formation in skeletal muscle and deep fascia.^{40,42,48} POH is to be distinguished from other syndromes associated with ectopic calcification and ossification, including fibrodysplasia ossificans progressiva (OMIM 135100; *ACVR1*—OMIM 135100) and hyperphosphatemic tumoral calcinosis (OMIM 211900; *GALNT3*—OMIM 211900). Although not as yet clinically described, an inactivating mutation in *LRP6* (encoding lipoprotein receptor-related protein 6—OMIM 603507) might also be associated with resistance to the biological effect of PTH. In addition to its primary role in receptor-mediated endocytosis of lipoproteins, *LRP6* is essential for the movement of $G_{s\alpha}$ to the plasma membrane and for its coupling to *PTH1R*.⁴⁹

Evaluation and Management

Evaluation of the neonate with hypocalcemia begins with review of the maternal preconceptional history, the conduct of gestation, and the peripartum, postnatal, and family histories followed by a comprehensive physical examination (Fig. 20.3). Historical data include those related to maternal parity and complications of pregnancy, such as maternal diabetes mellitus, toxemia of pregnancy or ingestion of agents that may cause maternal hypercalcemia (excessive alkali), intrauterine growth restriction, abnormalities of delivery, neonatal sepsis, or other early postpartum illnesses. The family history is examined for members with abnormalities of mineral metabolism, such as renal calculi, rickets, or hypocalcemia (e.g., seizure disorders). The social history provides information about the socioeconomic status of the mother and her cultural milieu that may have impacted maternal diet and exposure to sunlight during gestation. Physical examination of the neonate (abnormal face, cardiac murmur consistent with congenital heart disease) may suggest a complex form of hypocalcemia. Determination of a

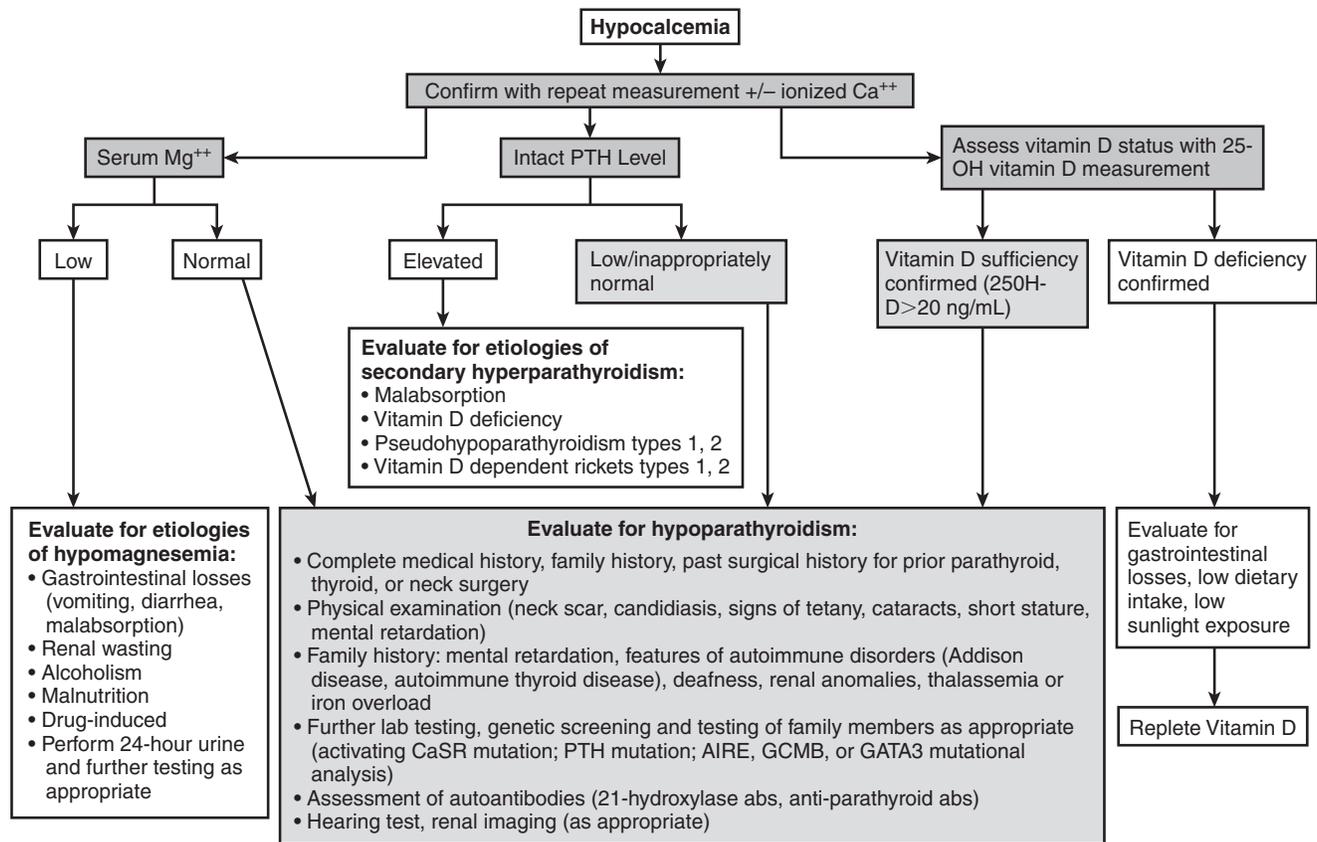


Fig. 20.3 Evaluation of hypocalcemia. (From Bilezikian JP, Khan A, Potts JT Jr, et al. Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Mineral Res.* 2011;26:2317–2337, with permission.)

complete blood count, serum concentrations of total calcium, Ca^{2+} , magnesium, phosphate, creatinine, intact PTH, calcidiol, and calcitriol, and urinary calcium and creatinine concentrations in a spot urine should precede initial therapy of the hypocalcemic newborn whenever possible. Decreased serum concentrations of PTH are common in neonates with early-onset hypocalcemia, but persistently low PTH levels suggest impaired PTH secretion, at times because of inactivating variants of *PTH* or to gain-of-function mutations of *CASR* or *GNA11*. High PTH concentrations are present in patients with vitamin D deficiency or insensitivity, PTH resistance caused by loss-of-function mutations in *PTH1R*, *GNAS* (PHP), or *PRKARIA* (acrodysostosis type 1) or impaired renal function. Low levels of calcidiol signify decreased maternal (and hence fetal) vitamin D stores or rarely a defect in *CYP27B1* encoding vitamin D-25 hydroxylase, whereas calcitriol concentrations are inappropriately low in subjects with severely compromised renal function, hypoparathyroidism, or those with deficiency of 25OHD-1 α -hydroxylase. Elevated calcitriol values suggest vitamin D resistance perhaps caused by an abnormality in VDR, a disorder that may be associated with alopecia. Skeletal radiographs may disclose osteopenia, whereas chest x-ray may not identify a thymic shadow (but is an unreliable sign in a severely ill or stressed neonate). Serum levels of calcium, Ca^{2+} , phosphate, and intact PTH should be measured in the mothers of neonates with unexplained hypocalcemia as some may have unsuspected hyperparathyroidism.

In neonates with hypocalcemia not otherwise explained, evaluation for possible DiGeorge syndrome should be undertaken—particularly when physical examination reveals an

abnormal face, and a congenital anomaly of the outflow tract of the heart is present. The white blood and T (CD4) lymphocyte counts are low and the thymic shadow often absent in these subjects. The diagnosis of the DiGeorge syndrome is confirmed by the presence of a microdeletion of chromosome 22q11.2 as demonstrated by single nucleotide polymorphism based chromosomal microarray or FISH. Single nucleotide polymorphism microarray enables detection of both copy number variation (deletions or duplications) and copy-neutral structural variants, such as regions of homozygosity and uniparental disomy. Occasionally, sequence analysis of *TBX1* may be needed to establish this diagnosis, if the other studies are normal. Because DiGeorge syndrome may be heritable, genetic evaluation of the parents and siblings of an affected infant is indicated. It should be noted that the majority of neonates and infants with DiGeorge syndrome are recognized primarily because of cardiac anomalies and that subjects without these lesions may not be identified until mid or late childhood or adolescence.⁵⁰ Neonates with PHP1a may present with elevated serum levels of TSH in the neonatal metabolic screening survey but often do not have the characteristic skeletal phenotype (brachymetacarpals) of AHO; if no cause of congenital hypothyroidism is identified, the diagnosis of PHP1a should be suspected and measurement of serum calcium levels and, if appropriate, genotyping and methylation studies of *GNAS* are indicated.⁴⁰

Early neonatal hypocalcemia is often asymptomatic, but nevertheless treatment is indicated when the total serum calcium concentration is below 8 mg/dL (2 mmol/L) (Ca^{2+} <4.4 mg/dL [1.1 mmol/L]) in term infants or prematurely born

neonates with birth weights over 1500 g, or in a very LBW newborn with a total calcium value less than 7 mg/dL (1.75 mmol/L) ($\text{Ca}^{2+} < 4.0 \text{ mg/dL}$ [1.0 mmol/L]).⁴ Asymptomatic neonates are most easily managed by increasing the oral intake of calcium and establishing an overall ratio of calcium:phosphate intake of 4:1 (including that in feedings with a low-phosphate formula, such as Similac PM 60/40—calcium:phosphate ratio 1.6:1) with calcium gluconate or calcium carbonate administered in divided doses every 4 to 6 hours (Table 20.3). Eucalcemia is almost always restored in these subjects within 3 weeks after birth and often earlier. In the hypocalcemic infant with tetany or frank seizures, 10% calcium gluconate (elemental calcium 9.3 mg/mL) at a dose of 1 to 3 mg/kg and rate of less than 1 mL/min and a total dose not to exceed 20 mg of elemental calcium/kg may be administered by intravenous infusion over 15 minutes; often seizures will cease after 1 to 3 mL of 10% calcium gluconate have been administered.⁴ Cardiac rate and rhythm must be carefully

monitored to prevent bradycardia and asystole. Further intravenous bolus doses of calcium (~10 mg/kg at 6-hour intervals) should be used sparingly as they result in wide excursions in serum calcium values. After initial treatment of neonatal hypocalcemia, 500 mg of calcium gluconate/kg/24 hours may be administered by continuous intravenous infusion, taking care to prevent extravasation or infiltration as extracellular calcium will precipitate in soft tissues.⁵¹ These infants may receive supplemental oral calcium if necessary (vide supra). Depending on the cause of the hypocalcemia, supplemental vitamin D or calcitriol may also be needed. In a neonate with hypocalcemia caused by hypoparathyroidism, administration of synthetic PTH¹⁻³⁴ (teriparatide) or recombinant human PTH¹⁻⁸⁴ may occasionally be required to restore eucalcemia.⁵² Serum and urine calcium and creatinine levels should be determined frequently and treatment modified to maintain eucalcemia and the urine calcium/creatinine ratio under 0.2 in an effort to avoid iatrogenic hypercalcemia, hypercalciuria, nephrocalcinosis, and

TABLE 20.3 Preparations of Calcitriol, Calcium, Magnesium, and Phosphate

Content	Elemental Mineral	
Vitamin D		
Vitamin D		
Calciferol	8000 IU/mL	
	50,000 IU/capsule	
Calcidiol	20 or 50 mcg/tablet	
Calcitriol		
Rocaltrol	1 mcg/mL (oral solution)	
	0.25 mcg or 0.5 mcg/capsule	
Dihydroxycholesterol		
Hytachero	0.2 mg/5 mL	
	0.125, 0.2, 0.4 mg/tablet	
Calcium		
Calcium acetate		
Phoslyra	667 mg/5 mL	667 mg/capsule
Calcium gluconate (iv)	93 mg/10 mL	93 mg/g
	500, 650 mg/tablet	
Calcium gluconate (solution)	64 mg/g	115 mg/5 mL
Calcium carbonate	400 mg/g	500, 600, 750, 1250 mg/tablet
Titralac	420 mg tablet	168 mg/tablet
	780 mg tablet	300 mg/tablet
Os-Cal	650 mg tablet	260 mg/tablet (Vitamin D)
	1250 mg tablet	500 mg/tablet (Vitamin D)
Tums	500 mg tablet	200 mg/tablet
	750 mg tablet	300 mg/tablet
	1000 mg tablet	400 mg/tablet
	1250 mg tablet	280 mg/tablet
Caltrate	1500 mg tablet	600 mg/tablet (Vitamin D)
Calcium carbonate (suspension)	1250 mg/5 mL	500 mg/5 mL
Calcium chloride	100 mg/mL	
Calcium citrate	210 mg/g	200 mg/tablet
Citracal	950 mg tablet	200 mg/tablet
Calcium lactate	130 mg/g	84 mg/tablet
Magnesium		
Magnesium sulfate	49 mg/mL (50% intramuscular solution)	
	0.32, 0.64, 4 mEq/mL (intravenous solution)	
Magnesium oxide	603 mg/g	241 mg/tablet
Magnesium gluconate	54 mg/g	27 mg/tablet
Magnesium chloride	120 mg/g	64 mg/tablet
Phosphorus		
Sodium phosphate (Phospha-Soda)		127 mg/mL
Sodium/potassium phosphate (Phos-NaK)		250 mg/packet (powder)
Potassium phosphate (Neutraphos-K)		250 mg/packet (powder)
Potassium phosphate (K-Phos Original)		114 mg/tablet
Sodium/potassium phosphate (K-Phos #2)		250 mg/coated tablet
Sodium/potassium phosphate (K-Phos Neutral)		250 mg/tablet

(From Alon US. Hypophosphatemic vitamin D-resistant rickets. In: Favus MJ (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:342–345; Shoback D. Hypoparathyroidism. *N Engl J Med*. 2008;359:391–403.)

renal insufficiency. In neonates that require parenteral alimentation, 50 mg of elemental calcium/kg/24 hours should be incorporated into the infused solution; elemental phosphate must also be administered as permitted and indicated but separately from calcium.

After restoration of eucalcemia in the infant with DiGeorge syndrome, other components of this disorder must be addressed. Cardiac anomalies often require surgical correction as do palatal clefts. In affected infants who are immunocompromised and experience recurrent infections because of thymic aplasia, appropriate antiinfectious therapy is mandatory. Transplantation of fetal or cultured postnatal thymic tissue, bone marrow, or peripheral blood mononuclear cells has restored immune function in infants with this disorder. Supplemental calcitriol (20–60 ng/kg/d) and calcium are necessary for restoration and maintenance of eucalcemia in infants with hypoparathyroidism. Poor growth caused by feeding difficulties and learning disabilities caused by developmental delay must be managed on an individual basis and illustrate the need for a multidisciplinary approach to the care of these patients.⁵⁰ Hypocalcemia caused by hypomagnesemia is managed acutely by the intravenous infusion or intramuscular injection of 50% magnesium sulfate at a dose of 0.1 to 0.2 mL/kg while monitoring cardiac status.

Hypocalcemia in the Child and Adolescent

Etiology

Causes of hypocalcemia in the child and adolescent include many that also result in neonatal hypocalcemia and are listed in Tables 20.2A, 20.2B and Fig. 20.1. Hypocalcemia is defined by the norms of the analytical laboratory; total serum calcium and phosphate concentrations vary by age.^{1,2} (A general guideline for age-related total calcium concentrations is: 1–5 years: 9.4–10.8; 6–12 years: 9.4–10.2; >20 years: 8.8–10.2 mg/dL.) Total calcium levels are low in the hypoalbuminemic patient—a correction for hypoalbuminemia may be calculated by adding 0.8 mg/dL to the recorded total calcium concentration for every decrease in albumin concentration of 1 g/dL. Thus it is appropriate to measure both total and Ca^{2+} values when evaluating the hypocalcemic child. It is particularly important to monitor Ca^{2+} values in the hypocalcemic critically ill child.⁵³ In the very ill child/adolescent, a low total calcium value may at times be related to hypoproteinemia or to altered acid-base balance and variable binding of calcium to serum proteins, but it may also be caused by impaired secretion of PTH related to hypomagnesemia or to decreased synthesis of vitamin D and consequent subnormal intestinal absorption of calcium. Mechanistically, hypocalcemia develops as a consequence of either too little inflow of calcium from the gastrointestinal tract, bone, or kidney into the extracellular and vascular spaces or excessive loss of calcium from these spaces into urine, stool, or bone. Thus hypocalcemia may be caused by decreased intake or absorption or excessive loss of calcium, decreased production of bioactive PTH because of congenital abnormalities of parathyroid gland development or PTH synthesis or of the CaSR, destruction of parathyroid glands by autoantibodies, metal overload (copper, iron), surgical or radiation insults, granulomatous infiltration, or to impaired cellular responsiveness to PTH. Restricted exposure to sunlight or reduced intake, absorption, metabolism, or activity of vitamin D leads to hypocalcemia. Hypomagnesemia impairs the secretion (but not the synthesis of PTH) and blunts tissue responsiveness to PTH. Hypocalcemia occurs after exposure to a number of drugs and medications. Thus hypocalcemic tetany may develop after the administration of phosphate containing enemas by rectum or laxatives by mouth.⁵⁴ At times, the hypocalcemic child or adolescent may be asymptomatic and identified by chemical

screening for an unrelated problem or may present with intermittent muscular cramping either at rest or during exercise (when the increase in systemic pH caused by hyperventilation lowers still further the concentration of Ca^{2+}); paresthesias of fingers, toes, or circumoral regions; tetany (carpopedal spasm, laryngospasm, bronchospasm); or seizures (grand mal, focal, petit mal, adynamic or syncopal). Prolonged and severe hypocalcemia may lead to congestive heart failure.⁵⁵ Physical examination often reveals a positive Chvostek (twitching of the cheek upon tapping of the ipsilateral seventh cranial nerve) and/or Trousseau sign (carpopedal spasm) and hyperreflexia. However, a positive Chvostek sign is commonly present in normal adolescents.

Hypoparathyroidism may occur as a solitary disorder, as part of a multidimensional autoimmune polyendocrinopathy, or as one manifestation of a group of complex congenital anomalies (as described in the section on neonatal hypocalcemia).^{3,55} Hypoparathyroidism may be the consequence of total thyroidectomy performed to excise a malignant thyroid lesion because of inadvertent removal of the four parathyroid glands or insults to their arterial blood supply.⁵⁶ There are sporadic and familial forms of hypoparathyroidism; when familial, hypoparathyroidism may be transmitted as an autosomal dominant, autosomal recessive, or X-linked recessive trait (see Tables 20.2A, 20.2B and see Fig. 20.1). Abnormalities in the development of the parathyroid glands, transcription of *PTH*, and in processing of the translated product have been associated with inherited forms of hypoparathyroidism. Familial isolated/autosomal dominant dysmaturational hypoparathyroidism may be caused by a monoallelic T \Rightarrow C transition in codon 18 (p.Cys18Arg) of the 25 aa signal peptide of preproPTH that impairs efficient transport of protein from the ribosome and interaction of preproPTH with the signal recognition particle, movement of the precursor peptide into and exit from the rough endoplasmic reticulum, its cleavage by a signal peptidase, and its incorporation into a secretory granule. Familial isolated/autosomal recessive dysmaturational hypoparathyroidism has also been associated with a homozygous G \Rightarrow C transversion in nucleotide 1 of intron 2 of *PTH* within the signal sequence that prevented normal cleavage of preproPTH and decreased secretion of PTH. Occasionally, isolated hypoparathyroidism may be found in patients with deletion of chromosome 22q11.2 without other signs or symptoms of the DiGeorge or related syndromes.

Autosomal dominant hypocalcemia type 1 (OMIM 601198) is caused by heterozygous gain-of-function mutations in the extracellular, transmembrane, and intracellular domains of *CASR* that transcribe a CaSR that is not intrinsically constitutively active but is exceptionally sensitive to and easily activated by very low serum Ca^{2+} concentrations may be identified in infancy or in older subjects (Fig. 20.4). In this disorder, even at hypocalcemic levels, Ca^{2+} binds avidly to the CaSR and activates phospholipase C- β 1 increasing cytosolic levels of inositol phosphate and Ca^{2+} and stimulating the mitogen-activated protein kinase (MAPK) signal transduction pathway in parathyroid chief cells, thus suppressing PTH synthesis and secretion, and in the kidney, there decreasing renal tubular reabsorption of calcium and magnesium, leading to urinary wasting of these cations (hypercalciuric hypocalcemia) and decreased urinary concentrating ability. In these subjects, serum levels of phosphate are increased and magnesium values decreased; PTH concentrations are low or inappropriately normal. Autosomal dominant hypocalcemia type 2 is caused by heterozygous variants of *GNA11* (OMIM 139313) that encodes a G-protein alpha subunit ($\text{G}\alpha$ 11) that initiates intracellular signal transduction after binding of Ca^{2+} to the CaSR. Its clinical and biochemical manifestations are similar to those caused by variants of *CASR* with seizures, laryngospasm, hypocalcemia, hyperphosphatemia, and relatively low serum levels of PTH. Because

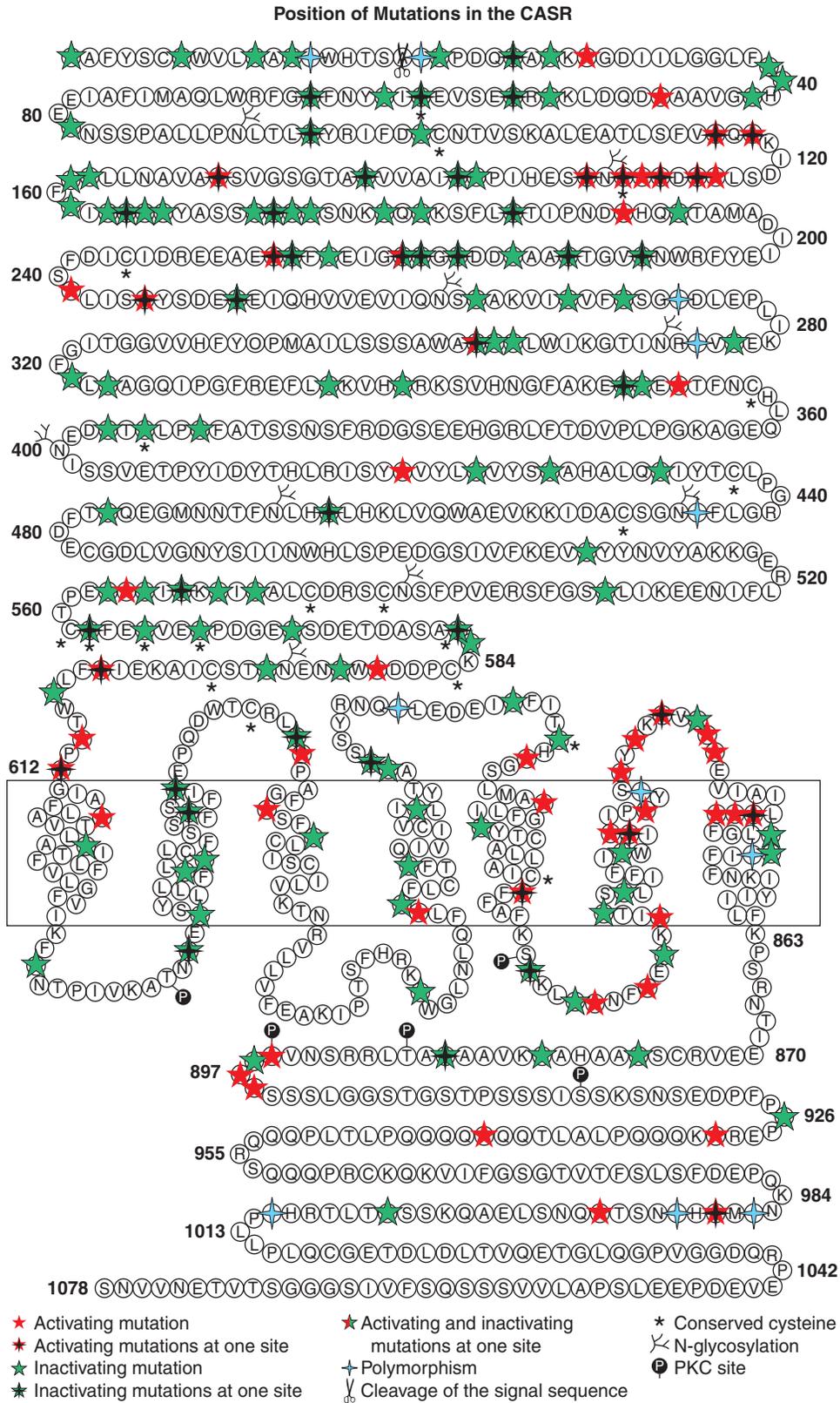


Fig. 20.4 Schematic view of the calcium sensing receptor (CaSR) depicting inactivating (hereditary/familial hypocalciuric hypercalcemia) and activating (autosomal dominant hypoparathyroidism) mutations. Note that mutations at adjacent codons may result in either a gain- or loss-of-function of the mutant CASR product (e.g., codons 565, 567, 568). (Downloaded from Google on April 27, 2013.)

patients with autosomal dominant hypocalcemia frequently have symptomatic hypocalcemia, such as tetany and seizures, and are very sensitive to vitamin D, the dose of calcitriol must be limited to that which raises serum calcium values to asymptomatic values even if not within the normal range as larger doses lead to hypercalciuria (sometimes even when serum calcium levels remain subnormal), nephrocalcinosis, and functional renal insufficiency. Administration of recombinant human (rh)PTH¹⁻³⁴ (teriparatide) restores calcium homeostasis in this disorder but does not necessarily prevent nephrocalcinosis.^{17,57,58} However, recombinant human PTH¹⁻³⁴ is not routinely administered to children because experimentally, there is an increased incidence of osteosarcoma in young rats receiving very large amounts of this agent, although primates appear to be less susceptible to PTH-induced bone tumor formation than do rodents. Development of stimulatory autoantibodies to the CaSR results in an acquired variant of spontaneous hypoparathyroidism that may be isolated or part of a complex autoimmune endocrinopathy.⁵⁹ Indeed, in perhaps as many as one-third of patients with acquired isolated idiopathic hypoparathyroidism, antibodies directed against epitopes in the extracellular domain of the CaSR may be present. This form of acquired hypoparathyroidism may be reversible, as these antibodies do not destroy the parathyroid glands. In patients with other forms of autoimmune hypoparathyroidism, the antibodies are cytotoxic and accompanied by lymphocytic infiltration, atrophy, and fatty replacement of parathyroid tissue.

In mid-childhood and adolescence, acquired hypoparathyroidism may be a delayed or late manifestation of a genetic variant (e.g., the DiGeorge syndrome), but it may also be the result of destruction of the parathyroid glands by autoimmune disease or surgical removal or operative trauma to the vascular supply of these structures. Unusual causes of acquired hypoparathyroidism in this age group include infiltration by iron (hemosiderosis, excessive blood transfusions) or copper (Wilson hepatolenticular degeneration), granulomatous diseases, or radiation (mantle radiation for Hodgkin/non-Hodgkin lymphoma or radioiodine therapy of hyperthyroidism).⁶⁰

Autoimmune hypoparathyroidism may occur as an isolated disorder or as part of the complex of autoimmune polyendocrinopathy syndrome type I (APS1).⁶¹ Development of an autoimmune endocrinopathy is initiated by failure of recognition of a peptide specific for a target organ by a subgroup of regulatory T cells that ordinarily recognize that peptide. When immunological tolerance for that peptide is lost, clones of CD4 regulatory T cells for the peptide expand; type 1 helper T cells secrete inflammatory cytokines, such as interferon- γ (IFN- γ), whereas type 2 helper T cells stimulate B cell function and lead to autoantibody-mediated inflammation. Loss of immune tolerance may be the consequence of the postinfectious inflammatory state caused by activation of the innate immune system or caused by a gene mutation that depresses immune tolerance and permits expansion of a CD4+ regulatory T cell clone after exposure to quantitatively small amounts of antigen. Genetic variations within the major histocompatibility complex (human leukocyte antigen [HLA]-DQ, HLA-DR) that determine peptide (antigen) presentation to CD4+ regulatory T cells join with genetic abnormalities in immune regulation to induce autoimmune disease. In 30% of patients with isolated idiopathic hypoparathyroidism the disorder is caused by antibodies to the extracellular domain of the CaSR and thus functionally inhibitory but potentially reversible; in approximately 33% of patients serum antibodies to other components of the parathyroid chief cell may be present.⁵⁹ There are several types of autoimmune polyendocrinopathy syndromes but only in type I is hypoparathyroidism a major component.

APS1 (OMIM 240300) is an autosomal recessive disorder with the classic triad of autoimmune polyendocrinopathy,

mucocutaneous candidiasis, and ectodermal dystrophy (APECED).⁶¹ It is characterized by childhood-onset of multiple autoimmune systemic disorders and endocrinopathies including hypoparathyroidism, primary adrenal insufficiency, and chronic mucocutaneous candidiasis, and less often pernicious anemia, hepatitis, primary ovarian and testicular failure, keratitis, retinitis, alopecia, and reversible metaphyseal dysplasia. Autoimmune polyendocrine syndrome 1 is caused by loss-of-function variants of autoimmune regulator (*AIRE*, OMIM 607358).⁶¹ In a Finnish cohort of 91 patients with APS1/APECED, the cardinal manifestations were mucocutaneous candidiasis involving the nails and mouth occurring in 100% of patients—often in the first 2 years of life, hypoparathyroidism, and hypoadrenocorticism both of which illnesses developed in 80% to 90% of affected subjects.⁶² Hypoparathyroidism occurred most often between 2 and 10 years and hypoadrenocorticism developed between 5 and 15 years of age. Almost all females with APECED developed hypoparathyroidism whereas 80% of affected males did so. Hypomagnesemia, often severe and recalcitrant to therapy, was common in patients with hypoparathyroidism caused by APECED. The most frequent presenting manifestations of APECED were mucocutaneous candidiasis—60%, hypoparathyroidism—32%, and hypoadrenocorticism—5%; the disease first became apparent between 2 months and 18 years of age. However, 10% of patients presented with another manifestation of APECED. In addition to hypoparathyroidism and hypoadrenocorticism, other endocrinopathies encountered in APECED included autoimmune oophoritis leading to ovarian failure (70%), orchitis resulting in testicular failure (30%), diabetes mellitus (30%), thyroiditis (30%), and hypophysitis (4%). Besides mucocutaneous candidiasis, dermatological manifestations and complications of APECED in the Finnish cohort included alopecia (40%), vitiligo (30%), and rashes with fever (15%). Keratoconjunctivitis developed in 20% of affected subjects, pernicious anemia in 30%, hepatitis in 20%, and chronic diarrhea in 20%. In a Norwegian population of 36 patients with APECED, 13 had clinical evidence of disease at or before 5 years of age and an additional 15 subjects presented at or before 15 years of age.⁶³ In a Russian population of 46 patients, mucocutaneous candidiasis was present in 70%, hypoparathyroidism in 83%, and adrenal insufficiency in 54%.⁶⁴ Other common problems were alopecia (27%), thyroid dysfunction (20%), and malabsorption syndromes (18%). Unusual findings in this cohort were retinitis pigmentosa and metaphyseal dysplasia—both occurring in 7% of patients. Later manifestations of APECED include esophageal and oral squamous cell carcinoma, asplenia, and interstitial nephritis. The diagnosis of APECED usually requires the presence of two of three of its major diseases (mucocutaneous candidiasis, hypoparathyroidism, hypoadrenocorticism), but occasionally chronic candidiasis, hypoparathyroidism or primary adrenal insufficiency alone may be the only manifestation of an inactivating mutation in *AIRE*.⁶⁴

APS1 is the result of homozygous or compound heterozygous loss-of-function mutations in *AIRE*, a 14 exon gene encoding a 545 aa protein with two zinc-finger motifs that is expressed in nuclei of thymic medullary epithelial cells and in lymph nodes, spleen, and monocytes.⁶¹ *AIRE* also serves as an E3 ubiquitin ligase, an essential component of the ubiquitin-proteasomal system for protein modification and destruction involved in cellular division and differentiation, protein transport, and intracellular signaling. Structurally, *AIRE* contains two plant homeodomains—a sequence of amino acids composed of an octet of cysteines and histidines that coordinate two zinc ions; the first plant homeodomain is essential for the E3 ubiquitin ligase activity of the protein and the second plant homeodomain is required for its transcription-regulating action. *AIRE* also contains a SAND domain, a

structure that permits it to bind DNA. AIRE enables thymic cells (T lymphocytes) to distinguish between normal self-antigens and proteins that are foreign to the host. AIRE does so by inducing the expression of normal self-antigens in the thymus and then eliminating the clonal subsets of T lymphocytes that recognize them thereby establishing self-tolerance. To develop an immune system that is capable of distinguishing between “self” and “foreign” antigens, thymic stromal medullary epithelial cells locally transcribe the majority of protein encoding genes expressed in various peripheral tissues to permit their recognition by developing T cells before the release of these T cells from the thymus and thus prevent an autoimmune response when these proteins are encountered by T cells in the periphery. The autoimmune regulatory gene *AIRE* enables the thymic transcription and expression of these genes by interacting with nuclear transporters (e.g., exportin), chromatin binding proteins (e.g., histones), transcription factors and postinitiation transcription processes (e.g., CREB; complex of DNA-dependent protein kinase-polymerase-topoisomerase), and pre-messenger (m)RNA splicing and processing factors.⁶⁵ *AIRE* does not function as a specific transcription factor. Inasmuch as nuclear *AIRE* binds to the unmethylated tails of histone-3 in chromatin, it has been proposed that *AIRE* activates quiescent genes by attracting to their site the transcription apparatus that enables initiation of transcription, elongation, and pre-mRNA processing. In the Finnish and Russian populations with APS1, the most common loss-of-function mutation in *AIRE* was a homozygous truncating mutation at codon 257 (p.Arg257Ter). Pathogenic mutations in *AIRE* detected in patients with APECED include missense, nonsense (p.Arg139Ter), insertions, and deletions (e.g., 13 base pair deletion—964del13, NT 1094, exon 8) that alter the subcellular distribution of *AIRE* and/or decrease its transcriptional activation capacity and/or its E3 ubiquitin ligase activity.⁶⁴ *AIRE* also stimulates development of thymic FoxP3+ regulatory T cells (Treg) that are able to inhibit autoreactive T cells.⁶¹

Thymic levels of *AIRE* are lower in normal postpubertal estrogenized females than those in normal postpubertal masculinized males, possibly accounting in part for the increased susceptibility of females to the later development of autoimmune disorders.⁶⁶ Despite thymic expression of three copies of *AIRE*, subjects with trisomy 21 (Down syndrome) have decreased thymic tissue levels of *AIRE*, thereby perhaps predisposing this patient population to the development of autoimmune diseases.⁶⁷ When *AIRE* is inactivated by biallelic loss-of-function mutations (e.g., p.Arg257X, p.Cys322del13), self-recognition of T cells is lost; normal self-proteins are not distinguishable from foreign proteins, and thus a destructive (auto) immune response to self-antigens is initiated. (Some variants of *AIRE* are transmitted as autosomal dominant or dominant negative disorders.⁶¹) Patients with mutations of *AIRE* and autoimmune polyendocrinopathy type 1 develop organ-related antibodies to cytoplasmic proteins (e.g., to parathyroid-specific NALRP5 [NACHT-domain, leucine-rich repeat, pyrin domain-containing protein 5, OMIM 609658] that are often demonstrable in patients with hypoparathyroidism). The presence of autoimmune polyendocrinopathy syndrome type 1 is established by the clinical findings, associated endocrinopathies, and measurable autoantibodies to other endocrine tissues (e.g., glutamic acid decarboxylase 65 [type 1 diabetes mellitus], 21-hydroxylase [adrenocortical failure]), and nonendocrine tissues (e.g., retina) and confirmed by genotyping of *AIRE*.⁶⁸ Patients with endocrinopathies attributed to autoimmune polyendocrinopathy type 1 are treated appropriately for the hormone deficiency(ies) present, as well as for the other manifestations of this illness. Patients with hypoparathyroidism require supplemental vitamin D (calcitriol), calcium, and magnesium; recombinant PTH¹⁻³⁴ and PTH¹⁻⁸⁴ have been used in adults for treatment of hypoparathyroidism (although not US Food and Drug

Administration [FDA] approved for use in children because of concern about their oncogenic potential). All asymptomatic first-degree relatives of the patient with autoimmune polyendocrinopathy type 1 should be surveyed for the presence of a pre-symptomatic stage of this illness.

Autoimmune polyendocrine syndrome type II is a polygenic autoimmune disorder manifested by adrenalitis, thyroiditis, and type 1 diabetes mellitus (and in some patients pernicious anemia, hypergonadotropic hypogonadism, hepatitis, celiac disease, myasthenia gravis, alopecia, and vitiligo) and is related to variants in major histocompatibility complexes, for example, DR3-DQ2, DR4-DQ8, DRB1*04, DQB1*02, and others.^{61,69} Hypoparathyroidism does not occur in patients with this disorder. The syndrome of Immunodeficiency, Polyendocrinopathy, and Enteropathy-X-linked (IPEX - OMIM 34790) is associated with the early (neonatal and early childhood) onset of autoimmune enteropathy, insulin-dependent diabetes mellitus, autoimmune thyroid disease, eczema, glomerulonephritis, thrombocytopenia, and hemolytic anemia that occurs only in males because it is the result of loss-of-function mutations in *FOXP3* (OMIM 300292). *FOXP3* is a transcription factor that is essential for normal development of naturally occurring T regulatory cells that maintain self-tolerance.^{61,70} IPEX is a disorder with variable clinical manifestations associated with resistance to the action of PTH whose clinical phenotypes and genetic variabilities have been discussed earlier.

Deficiency of vitamin D intake, aberrant metabolism of cholecalciferol, or decreased biological responsiveness of the VDR may result in hypocalcemia.⁷¹ In the subject with skeletal demineralization caused by marked vitamin D deficiency, serum calcium concentrations may fall precipitously after administration of even small amounts of vitamin D as renewed mineralization of bone matrix caused by accelerated osteoblastic activity consumes calcium and phosphate (the “hungry bone” syndrome). After parathyroidectomy for primary hyperparathyroidism, calcium concentrations often decline rapidly by the same mechanism. Drugs that inhibit PTH secretion (excessive magnesium), osteoclast resorption of bone (bisphosphonates), or renal resorption of calcium (furosemide) may lead to hypocalcemia. Intravenous infusion or rectal administration of phosphate (in enemas), acute cellular destruction by tumor cell lysis or rhabdomyolysis, and acute and chronic renal failure increase serum phosphate levels and lead to reciprocal decline in calcium values. Serum calcium concentrations decline in patients receiving multiple transfusions of citrated blood or during plasmapheresis. In subjects with acute pancreatitis, calcium complexed with free fatty acids generated by pancreatic lipase is deposited in necrotic tissue.⁷² Acute severe illness of diverse pathogenesis is often associated with hypocalcemia; this has been attributed to hypoalbuminemia, hyperphosphatemia, alkalosis, functional hypoparathyroidism, hypercalcitonemia, hypomagnesemia, vitamin D deficiency, decreased calcitriol synthesis, alkalosis and increased serum concentrations of free fatty acids (the latter increase binding of Ca²⁺ to albumin), increased cytokine activity, and administered medications (e.g., aminoglycosides, citrated blood, calcium chelators, fluoride).⁵³

Evaluation

Fig. 20.3 outlines the evaluation of the child/adolescent with hypocalcemia. Hypocalcemia may be asymptomatic until detected by a multiassay chemical profile obtained for another purpose. A prolonged QT interval noted by electrocardiography obtained for evaluation of a functional heart murmur or arrhythmia suggests the presence of hypocalcemia. Roentgenograms obtained for evaluation of abdominal pain or following a head injury may reveal nephrocalcinosis, renal calculi, or calcification of the basal ganglia of the brain, respectively; such

findings merit measurement of the serum calcium concentration. The major symptoms and physical signs of hypocalcemia depend on its rapidity of onset and depth.⁷³ The more quickly is the onset of hypocalcemia and the lower the calcium value achieved, the more substantial are the symptoms. Symptoms may include paresthesias (numbness and tingling of the hands, feet, perioral region), muscular hyperexcitability, including cramping, carpedal spasm (flexion of the elbow and wrist, adduction of the thumb, flexion of the metacarpal/metatarsal-phalangeal joints, and extension of the interphalangeal joints), tetany—particularly during vigorous exercise—occasionally generalized seizures, bronchospasm, and at times neuropsychiatric symptoms. (Laryngospasm is a life-threatening complication of hypocalcemia.) Review of the past medical and family histories may reveal symptoms consistent with or illnesses associated with hypocalcemia (recurrent infections, congenital cardiac anomalies, surgical procedures in the neck, cervical radiation, infiltrative diseases), or may identify related members with hypoparathyroidism, dysmorphic physical characteristics, autoimmune endocrinopathies, or hypomagnesemia.

Physical examination may disclose characteristic abnormalities in children with PHP type IA in whom the phenotype of AHO is present (short stature, round face, subcutaneous hard nodules, brachymetacarpals), the DiGeorge syndrome (atypical face, recurrent infections, cardiac murmur), or familial APS1 (chronic mucocutaneous candidiasis and other ectodermal abnormalities, such as vitiligo, alopecia, keratoconjunctivitis), whereas rachitic deformities in the hypocalcemic child imply the presence of a form of hypovitaminosis D. Most commonly, however, the physical examination reveals no striking abnormality in the hypocalcemic child other than those of increased neuromuscular irritability, such as hyperreflexia, positive Chvostek sign (twitching of the circumoral muscles when tapping lightly over the seventh cranial nerve) or Trousseau sign (carpal pedal spasm when maintaining the blood pressure cuff 20 mm Hg above the systolic blood pressure for 3 minutes) and occasionally cataracts, papilledema, or abnormal dentition. (Tetany also occurs in subjects with hypo- and hypernatremia, hypo- and hyperkalemia, and hypomagnesemia, and a positive Chvostek sign may be found in many normal adolescents.)

Laboratory evaluation of hypocalcemia includes measurements of serum concentrations of total calcium, Ca^{2+} , phosphate, magnesium, PTH, and 25-hydroxyvitamin D, and determination of the urine calcium excretion. In most hypocalcemic patients, urine calcium excretion is low; however, if the urine calcium excretion is inappropriately normal or high, disorders, such as autosomal dominant hypocalcemia type 1 (gain-of-function mutation in *CASR* or *GNA11*) may be considered; these genes may then be analyzed or antibodies to CaSR determined as clinically indicated. Antibodies to the CaSR have been detected in approximately 50% to 80% of patients with autoimmune polyendocrinopathy type 1. The child/adolescent with hypocalcemia, hypocalciuria, hyperphosphatemia, and low or undetectable serum PTH concentration (and normal or only slightly low serum magnesium level) likely has hypoparathyroidism because of a primary defect in PTH synthesis or secretion related to congenital malformation or acquired destruction of the parathyroid glands. Patients with hypoparathyroidism often have low serum concentrations of calcitriol, normal levels of calcidiol, decreased excretion of urinary nephrogenous cyclic AMP, and increased renal tubular reabsorption of phosphate.⁷⁴ The DiGeorge syndrome may be identified by microarray or FISH analysis of chromosome 22q11.2 using probes directed to the deleted segment; occasionally *TBX1* genotyping may be helpful. Analysis of *CASR*, *GNA11*, *PTH*, *GCM2*, *FHL1*, and other genes associated with isolated or syndromic hypoparathyroidism (*TCBE*, *GATA3*) may be indicated in the appropriate clinical context. The diagnosis

of APS1 is based on clinical and laboratory findings and genotyping of *AIRE*. The presence of two of three of its major manifestations (candidiasis and/or ectodermal dystrophy, hypoparathyroidism, hypoadrenocorticism) are accepted criteria for its clinical diagnosis, but isolated hypoparathyroidism, adrenal insufficiency, or chronic mucocandidiasis may occasionally be its only manifestation.⁶⁴ Antibodies to the CaSR or other cellular components of the parathyroid glands, the adrenal glands (side-chain cleavage, 21-hydroxylase, 17 α -hydroxylase enzymes), neurotransmitters (aromatic L-amino acid decarboxylase, tryptophan hydroxylase), and IFNs- α and - ω may be determined; antibodies to IFN- ω are commonly present in patients with APS1/APECED.^{63,75} Genotyping of *AIRE* and identification of the mutation(s) confirm the diagnosis of APECED. There is wide variability in the clinical expression of APECED both between families and among siblings because the phenotype is not directly related to the genotype. In patients with isolated, idiopathic hypoparathyroidism, a search for antibodies to the CaSR and/or to parathyroid tissue is warranted. In hypomagnesemic subjects, magnesium and PTH levels are quite low, and PTH secretion increases rapidly after intravenous administration of magnesium. Primary hypomagnesemia should be considered when hypocalcemia and hypercalciuria coincide and serum PTH and magnesium values are low; urinary magnesium excretion should then be quantitated and *CLDN16* (OMIM 603959) genotyped. Because hypomagnesemia may also be caused by a selective small intestinal defect in magnesium absorption, mutations in the transient receptor potential, cation channel, subfamily M, member 6 (*TRPM6*, OMIM 607009) should be examined as warranted. Primary hypomagnesemia must be differentiated from that caused by the Gittleman and Bartter syndromes (vide infra).

An elevated serum concentration of PTH in a hypocalcemic subject suggests that the patient is either secreting an abnormal PTH molecule or is resistant to PTH or there is a compensatory (secondary) PTH secretory response to hypocalcemia (see Fig. 20.3). Physicochemical characterization of the PTH molecule and analysis of *PTH* (OMIM 168450) enable one to define the abnormality in PTH synthesis, posttranslational processing, secretion, or activity leading to the functionally hypoparathyroid state. Analysis of *GNAS* (OMIM 139320) and its pattern of imprinting permits identification of the specific genetic defect in the majority of patients with clinical PHP type IA and PPHP. Skeletal and renal responsiveness to PTH may be assessed if warranted by measurement of changes in serum calcium, Ca^{2+} , phosphate, cyclic AMP, and calcitriol concentrations and urinary nephrogenous cyclic AMP and phosphate excretion following administration of biosynthetic PTH¹⁻³⁴ (Elsworth-Howard test). In the normal subject and in the patient with primary hypoparathyroidism, urinary cyclic AMP excretion increases 10- to 20-fold and that of phosphate severalfold; in the patient with PHP types IA and IB there is less than a threefold increase in the excretion of cyclic AMP after administration of PTH¹⁻³⁴. The diagnosis of PHP type IB may be established by examining the imprinting patterns of *GNAS* (i.e., the methylation status of the differentially methylated regions of *GNAS*) and analysis of *STX16* (OMIM 603666) and the 5' sequences of *GNAS* as indicated. Acrodystostosis 1 and 2 (OMIM 101800;614613) are skeletal dysplasias with hormone resistance that should also be considered when evaluating a hypocalcemic infant with inappropriately elevated serum levels of PTH. This chondrodystrophy is attributable to inactivating variants of *PRKARIA* (OMIM 188830) or *PDE4D* (OMIM 600129). *PRKARIA* encodes a component of PKA—the major mediator of postreceptor intracellular signal transduction; *PDE4D* encodes a cyclic AMP-specific phosphodiesterase, loss of which alters cyclic AMP function. In the child with vitamin D deficiency, serum levels of calcidiol are low. In patients with decreased renal 25OHD₃-1 α -hydroxylase activity, serum concentrations of calcidiol are normal whereas those of calcitriol are inappropriately

low. Increased concentrations of calcitriol suggest the presence of a defect in the nuclear VDR. The patient with renal failure is recognized by an increased serum creatinine value. Other findings in hypocalcemic subjects include prolongation of the QT interval by electrocardiography and calcification of the basal ganglia by cranial computerized tomography.

Management

The primary goal in the care of the hypocalcemic child and adolescent is to increase serum calcium concentrations to levels at which the patient is asymptomatic and as close to the lower range of normal as possible without inducing hypercalcemia; the secondary goal is to identify the cause of hypocalcemia as quickly as possible to provide disease-specific management.^{55,71,74} The overall aims of management of hypocalcemia in general and hypoparathyroidism specifically are to: (1) ameliorate the signs and symptoms of hypocalcemia, (2) to prevent hypercalcemia by (3) maintaining the serum concentration of calcium at or slightly below the lower range of normal in order (4) to maintain the calcium \times phosphate product below 55 mg/dL and thus (5) to prevent hypercalciuria and (6) calcium/phosphate precipitation and extraskeletal calcifications.^{3,76}

Asymptomatic hypocalcemia (total calcium >7.5 mg/dL = 1.88 mmol/L) may not require immediate intervention. With lower serum calcium levels or when hypocalcemia is symptomatic (tetany, seizures, laryngospasm, bronchospasm), acute management may require the intravenous administration of 10% calcium gluconate (93 mg of elemental calcium/10 mL vial) at a slow rate (not >2 mL [1.86 mg of elemental calcium]/kg over 10 minutes) while closely monitoring pulse rate (and the electrocardiographic QT interval). Acutely, intravenous administration of calcium is intended to ameliorate the more serious consequences of hypocalcemia, such as seizures, bronchospasm, or laryngospasm, not to restore and maintain the eucalcemic state. Intravenously, calcium should not be administered with phosphate or bicarbonate, because these salts may coprecipitate. Extravascular extravasation of calcium is to be avoided because it may precipitate and cause local tissue injury. After the acute symptoms have resolved, calcium gluconate (10 mL = 93 mg of elemental calcium in 100 mL 5% dextrose/0.25 normal saline) may be infused intravenously at a rate sufficient to maintain calcium levels in the asymptomatic low-normal range while the cause of the hypocalcemia is identified and more specific therapy for persistent hypocalcemia prescribed. In the child with marked hyperphosphatemia as a cause of hypocalcemia, in addition to parenteral calcium administration infusion of normal saline sufficient to maintain urine output at or above 2 mL/kg per hour is necessary.⁵⁴ Frequent measurement of serum calcium and phosphate concentrations permits rapid adjustment of fluid and electrolyte therapy. The evaluation should proceed as rapidly as possible and oral therapy begun reasonably quickly.

After stabilization, patients with hypoparathyroidism or PHP may be treated with calcitriol (20–60 ng/kg/d in 2 equally divided doses) and supplemental calcium (calcium gluconate, calcium citrate, calcium carbonate), 30 to 75 mg elemental calcium/kg/d in 4 divided doses daily often with meals—(see Table 20.3) to restore and maintain eucalcemia.⁷³ The serum calcium concentration should be maintained within the low-normal range. Each patient must be carefully monitored to avoid hypercalcemia, hypercalciuria (calcium excretion greater than 4 mg/kg/24 h), nephrocalcinosis, and nephrolithiasis. Basal and periodic measurements of serum concentrations of calcium, phosphate, magnesium, and creatinine and urinary calcium and creatinine excretion (every 3 months) are advisable. Twice yearly, renal sonograms are also suggested to identify the development of nephrocalcinosis in its earliest stage. Children with autosomal dominant hypocalcemia caused by

gain-of-function mutations in *CASR* (OMIM 601199) or *GNA11* (OMIM 139320) are extremely sensitive to vitamin D and its metabolites. Even small doses of calcitriol may lead to hypercalciuria with minimal increase in serum calcium levels; in that instance, addition of hydrochlorothiazide (0.5–2.0 mg/kg/d) may increase renal tubular reabsorption of calcium and lower the calcitriol requirement. Administration of recombinant human PTH^{1–34} has been beneficial in individual subjects with hypoparathyroidism.^{17,57,58} In adults and children with hypoparathyroidism caused by a variety of causes, the use of rhPTH^{1–34} (0.4–0.5 mcg/kg every 12 hours subcutaneously) together with calcium carbonate and vitamin D (1200 mg/day of elemental calcium and 800 IU/day of cholecalciferol in 4 equally divided doses) has proven effective and safe for as long as 3 years.⁵⁸ rhPTH^{1–34} led to acceleration of bone turnover as reflected by increases in serum alkaline phosphatase and osteocalcin and urinary excretion of pyridinoline and deoxypyridinoline, while accrual of bone mineral mass, linear growth, and weight gain were not adversely impacted in treated children. In six children with primary hypoparathyroidism, subcutaneous administration of rhPTH^{1–34} (12.5 mcg twice daily) was well tolerated and allowed withdrawal or lowering of the doses of supplemental calcium and calcitriol.⁷⁷ Continuous infusion of rhPTH^{1–34} has also proven effective in maintaining reasonable serum calcium concentrations in adults with postsurgical hypoparathyroidism with fewer fluctuations in calcium levels and lower urine calcium values when compared with twice daily subcutaneous injections of rhPTH^{1–34}.⁷⁸ However, at present rhPTH^{1–34} is not indicated for routine treatment of children with hypoparathyroidism because of concerns about its link to osteosarcoma. In adults, rhPTH^{1–34} has also been used in the treatment of hypoparathyroidism with substantial decline in supplemental calcium and calcitriol doses and stabilization of serum and urine calcium values over 6 years.³ In addition, bone turnover markers reflected increase in skeletal formation documented by measurement of bone mineral density (BMD) of the lumbar spine. The relationship of rhPTH^{1–34} to bone tumor formation, if any, is unknown at present.

When undertaking total thyroidectomy for treatment of a thyroid malignancy, consider the possible effects of this procedure upon function of the parathyroid glands. Before surgery, serum concentrations of calcium, magnesium, phosphate, and PTH should be recorded. During the operation, efforts to identify and preserve the parathyroid glands and their blood supply should be undertaken.⁵⁶ Physical examination seeking signs of hypocalcemia (Chvostek) and serial measurements of calcium, magnesium, and PTH should begin immediately after completion of surgery and continue as clinically indicated.

Because PHP type IA is associated with resistance to a number of peptide hormones that act through GPCR, periodic assessment of pituitary-thyroid and pituitary-ovarian function and GH secretion is necessary and hormone replacement therapy begun as indicated.⁴⁰ In general, the short stature of PHP type IA reflects the AHO phenotype and not GH deficiency. However, PHP-associated hyposomatotropism may be responsive to exogenous recombinant human GH. Transient hypoparathyroidism of infancy may be the initial manifestation of later onset hypoparathyroidism; thus assess calcium homeostasis in such subjects throughout childhood. Patients with apparently isolated hypoparathyroidism of unknown etiology should be reevaluated periodically to identify the development of autoimmune disorders in the patient or family. Assessment of thymic function is important in those subjects with findings suggestive of the DiGeorge syndrome. The management of patients with vitamin D deficiency or resistance is discussed in subsequent sections.

When hypomagnesemia is symptomatic, administration of magnesium sulfate parenterally may be necessary (50%

solution, 0.1–0.2 mL/kg intramuscularly, repeated after 12–24 hours if needed). The patient with primary hypomagnesemia may require daily parenteral (intramuscular, intravenous) doses of magnesium sulfate to prevent tetany, seizures, and other neurological symptoms (slurred speech, choreoathetoid movements, weakness), and to enable normal growth and development. Calcitriol alone raises serum calcium levels in hypomagnesemic subjects but is ineffective in the prevention of tetany. Continuous overnight nasogastric infusion of magnesium may help alleviate the gastrointestinal side effects of multiple large doses of oral magnesium. More mild and transient forms of hypomagnesemia may be treated with oral magnesium gluconate or tribasic magnesium citrate (see Table 20.3).

HYPERCALCEMIA

Hypercalcemia is present when the serum concentration of ionized calcium is above the normal range for age.¹ The total serum calcium concentration reflects both the serum protein level and serum pH; generally, both total and ionized calcium concentrations should be routinely measured when hypercalcemia is suspected. Hypercalcemia may be considered mild when the total serum calcium concentration is above the upper limit of the normal range and below 12 mg/dL (3.0 mmol/L), moderate with total calcium values of 12 to 14 mg/dL (3.00–3.50 mmol/L), or severe when the total serum calcium value exceeds 14 mg/dL (3.50 mmol/L). Pathophysiologically, hypercalcemia may be the result of excessive intestinal absorption of ingested calcium (e.g., vitamin D excess), increased reabsorption of skeletal calcium caused by enhanced PTH action or a lytic bone lesion, or increased renal tubular reabsorption of calcium caused by a medication, such as a thiazide diuretic. Thus hypercalcemia may be caused by aberrations that are PTH-dependent or -independent.

Hypercalcemia in the Neonate and Infant

Hypercalcemia in neonates and very young infants is defined as total blood calcium concentration above 10.8 to 11.3 mg/dL (2.70–2.83 mmol/L) and Ca^{2+} greater than 5.92 to 6.40 mg/dL (1.48–1.60 mmol/L) (depending on the analytical laboratory). However, substantial symptoms and signs of hypercalcemia (e.g., lethargy, anorexia, emesis, constipation, polyuria, dehydration, gastroesophageal reflux and emesis, constipation, and lethargy and hypotonia or irritability and seizures) may not occur until the total serum calcium level exceeds 12.5 to 13 mg/dL (3.12–4.25 mmol/L).^{1,79} Hypercalcemic infants frequently are polyuric because of renal resistance to antidiuretic hormone and may become dehydrated if fluid intake is restricted. Because of the vasoconstrictive effect of calcium, the hypercalcemic infant may be hypertensive. Hypercalcemia also shortens the S-T segment and can lead to heart block and ultimately asystole. In older infants and young children with chronic hypercalcemia, poor growth and “failure to thrive” are often presenting manifestations. Hypercalcemia also leads to hypercalciuria, nephrocalcinosis, and nephrolithiasis. Hypercalcemia may also be present in patients with phenotypic abnormalities, for example, the Williams-Beuren syndrome (WBS).

Etiology

Causes of hypercalcemia in neonates and infants are listed in Tables 20.4A, 20.4B. Neonatal/infantile hypercalcemia may be iatrogenic in origin—for example, administration of excessive calcium or vitamin D—at times to the mother who secretes cholecalciferol in her breast milk; administration of thiazide diuretics that increase renal tubular absorption of calcium; or purposeful restriction of phosphate. Hypercalcemia, hypophosphatemia, hyperphosphatasemia, and radiographic evidence

of rickets may develop in very premature infants receiving intravenous alimentation deficient in phosphate or in those fed only human breast milk, the phosphate content of which is low. Hypophosphatemia leads to increased synthesis of calcitriol by: (1) stimulating renal tubular 25OHD-1 α hydroxylase activity directly, and (2) inhibiting synthesis of FGF23, thereby removing an agent that depresses activity of 25OHD-1 α hydroxylase; the two pathways additively increase 25OHD-1 α hydroxylase activity, calcitriol synthesis, and intestinal calcium absorption.⁷⁹ The problem may be circumvented by increasing the amount of parenteral phosphate administered to the extent possible or by the use of breast milk fortified with phosphate. (Adequate extrauterine mineralization of the preterm skeleton requires intakes of both calcium and phosphate of approximately 200 mg/kg/d each.) Extracorporeal membrane oxygenation may also be associated with hypercalcemia in neonates, perhaps related to increased secretion of PTH by the neonate.^{1,80}

Hypervitaminosis D may be caused by prolonged feeding of an improperly prepared formula or commercial dairy milk containing excessive vitamin D, iatrogenic prescription of vitamin D, calcidiol, or calcitriol, or increased endogenous production of calcitriol from inflammatory sites.⁸¹ In infants with severe birth trauma, perinatal asphyxia or hypothermia, subcutaneous fat necrosis may develop within the first 6 weeks after birth as manifested by indurated, extremely firm, tender, violaceous nodules on the cheeks, trunk, shoulders, buttocks, arms, and legs.⁸² Hypercalcemia may be present when the lesions first appear or develop as the nodules resolve several weeks later. Histologically, the skin lesions are composed of adipocytes, an inflammatory lymphohistiocytic infiltrate, and multinucleated giant cells in a bed of calcium crystals. The hypercalcemia of subcutaneous fat necrosis has been attributed to reabsorption of precipitated calcium, extrarenal synthesis of calcitriol by local macrophages and resultant hyperabsorption of ingested calcium, and increased osteoclastic activity because of enhanced prostaglandin secretion. The 25OHD-1 α -hydroxylase activity of these inflammatory macrophages is not under the control of PTH, calcium, or phosphate, but is suppressible by glucocorticoids. Excessive production of prostaglandin E and interleukins (IL)-1 and -6 further contributes to hypercalcemia in this disorder by increasing the rate of bone turnover. Hypercalcemia attributable to subcutaneous fat necrosis is managed by the ingestion of a formula low in calcium, avoidance of vitamin D, and administration of fluids, calcitonin, or bisphosphonate (pamidronate), as needed.^{60,82} Administration of loop diuretics (e.g., furosemide) and glucocorticoids should be avoided if possible. Hypercalcemia caused by subcutaneous fat necrosis has also been observed in older children with major trauma or disseminated varicella.⁸¹ Congenital deficiency of lactase and other disaccharidases have also been associated with infantile hypercalcemia, likely because of increased intestinal absorption of calcium promoted by disaccharides.

Neonatal severe hyperparathyroidism (NSHPT) is a potentially lethal form of familial (hereditary) hypocalciuric hypercalcemia (HHC). It is most commonly caused by homozygous or compound heterozygous inactivating mutations of *CASR* that greatly increase the serum concentration of Ca^{2+} needed to suppress PTH synthesis and secretion. However, in several infants with NSHPT, there have been heterozygous inactivating mutations of *CASR* (e.g., pArg185Gln, pArg227Leu) suggesting that the products of these mutations exert a dominant-negative effect on the normal *CaSR*, perhaps by interfering with migration of wild-type receptor to the cell surface or inactivation of wild-type receptor by linking to the mutated *CaSR* once embedded in the cell membrane, or by sequestration of G-proteins.⁸³ In addition, the fetus bearing a heterozygous mutation in *CASR* that has been inherited from an affected father but residing in the womb of a normal mother may be relatively “hypocalcemic” in utero,

TABLE 20.4A Causes of Hypercalcemia**I Neonate/Infant**

- A Maternal disorders
 - 1 Excessive vitamin D ingestion, hypoparathyroidism, pseudohypoparathyroidism
- B Neonate/infant
 - 1 Iatrogenic—excessive intake of calcium, vitamin D, vitamin A
 - 2 Phosphate depletion
 - 3 Subcutaneous fat necrosis
 - 4 Williams-Beuren syndrome (del7q11.23/BAZ1B)
 - 5 Neonatal severe hyperparathyroidism (CASR)
 - 6 Metaphyseal chondrodysplasia, Murk-Jansen type (PTH1R)
 - 7 Idiopathic infantile hypercalcemia type 1 (CYP24A1)
 - 8 Idiopathic infantile hypercalcemia type 2 (SLC34A1)
 - 9 Persistent parathyroid hormone–related protein
 - 10 Lactase/disaccharidase deficiency (LCT)
 - 11 Infantile hypophosphatasia (TNSALP)
 - 12 Mucopolidosis type II (GNPTAB)
 - 13 Blue diaper syndrome
 - 14 Antenatal Bartter syndromes types 1 & 2 (SLC12A1, KCNJ1)
 - 15 Distal renal tubular acidosis
 - 16 IMAGE syndrome (CDKN1C)
 - 17 Postbone marrow transplantation for osteopetrosis
 - 18 Endocrinopathies: Primary adrenal insufficiency, severe congenital hypothyroidism, hyperthyroidism

II Hyperparathyroidism

- A Sporadic
 - 1 Parathyroid hyperplasia, adenoma, carcinoma
- B Familial
 - 1 Hypocalcemic hypercalciuria type 1/neonatal severe hyperparathyroidism (CASR)
 - 2 Hypocalcemic hypercalciuria type 2 (GNA11)
 - 3 Hypocalcemic hypercalciuria type 3 (APS21)
 - 4 Multiple endocrine neoplasia, type I (MEN1)
 - 5 Multiple endocrine neoplasia, type IIA (RET)
 - 6 Multiple endocrine neoplasia, type IIB (RET)
 - 7 Multiple endocrine neoplasia, type IV (CDKN1B)
 - 8 McCune-Albright syndrome (GNAS)
 - 9 Familial isolated hyperparathyroidism 1 (CDC73)
 - 10 Familial isolated hyperparathyroidism 2 (jaw tumor syndrome) (CDC73)
 - 11 Familial isolated hyperparathyroidism 3
 - 12 Jansen metaphyseal dysplasia (PTH1R)
- C Secondary/tertiary
 - 1 Postrenal transplantation
 - 2 Chronic hyperphosphatemia
- D Hypercalcemia of malignancy
 - 1 Ectopic production of PTHrP
 - 2 Metastatic dissolution of bone

III Familial Hypocalciuric Hypercalcemia

- A Familial hypocalciuric hypercalcemia I (CASR)
 - 1 Loss-of-function mutations in CASR
 - a Monoallelic—Familial benign hypercalcemia
 - b Biallelic—Neonatal severe hyperparathyroidism
 - 2 Hypocalcemic hypercalciuria type 2 (GNA11)
 - 3 Hypocalcemic hypercalciuria type 3 (APS21)
- B Familial hypocalciuric hypercalcemia II—Linked to chromosome 19p13.3
- C Familial hypocalciuric hypercalcemia III—Oklahoma variant—Linked to chromosome 19q13
- D CaSR blocking autoantibodies

IV Excessive Calcium and/or Vitamin D

- A Milk-alkali syndrome
- B Exogenous ingestion of calcium or vitamin D or topical application of vitamin D (calcitriol or analog)
- C Ectopic production of calcitriol associated with granulomatous diseases: sarcoidosis, cat scratch fever; tuberculosis, histoplasmosis, coccidioidomycosis, leprosy; human immunodeficiency virus; cytomegalovirus; chronic inflammatory bowel disease
- D Neoplasia
 - 1 Primary bone tumors
 - 2 Metastatic tumors with osteolysis
 - 3 Lymphoma, dysgerminoma
 - 4 Tumors secreting PTHrP, growth factors, cytokines, prostaglandins, osteoclast activating factors
- E Williams-Beuren syndrome (del7q11.23)

V Immobilization**VI Other Causes**

- A Drugs—thiazides, lithium, vitamin A & analogs, calcium, alkali, antiestrogens, aminophylline
- B Total parenteral nutrition
- C Endocrinopathies: hyperthyroidism, hypoadrenocorticism, pheochromocytoma
- D Vasoactive intestinal polypeptide-secreting tumor
- E Acute or chronic renal failure/administration of aluminum
- F Hypophosphatasia
- G Juvenile rheumatoid arthritis - cytokine mediated

CaSR, Calcium sensing receptor; PTHrP, parathyroid hormone–related protein.

(From Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. *Curr Opin Pediatr.* 2010;22:508–515; Davies JH. A practical approach to the problems of hypercalcaemia. In: Allgrove J, Shaw N. (eds). *Calcium and Bone Disorders in Children and Adolescents.* Basel, Karger, *Endocr Dev.* 2009;16:93–114; Benjamin RW, Moats-Staats BM, Calkoglu A, et al. Hypercalcemia in children. *Pediatr Endocrinol Rev.* 2008;5:778–784.)

leading to hyperplasia of the fetal parathyroid glands that persists after birth giving rise to NSHPT. Occasionally, NSHPT may be transmitted as an autosomal recessive disorder by clinically and biochemically normal parents.^{84,85} In subjects with homozygous mutations near the amino terminal of CASR (e.g., p.Leu13Pro), hypercalcemia may not be manifested until midchildhood or even adulthood.⁸⁶ Thus the clinical spectrum of NSHPT ranges from mild (constipation, polyuria) with calcium concentrations ranging from 11 to 13 mg/dL (2.75–3.25 mmol/L) to severe and life-threatening (dysrhythmia, respiratory distress caused by hypotonia, demineralization and fractures of the ribs) when calcium levels exceed 15 mg/dL (3.75 mmol/L).^{79,87} Thus NSHPT may present within the first few days of life to several months of age depending on the degree of hypercalcemia. Search of the family history may identify members with mild hypercalcemia caused by

autosomal dominant HHC. Physical examination often reveals hypotonia, feeding difficulties, and respiratory distress. The serum calcium concentration is frequently markedly elevated (average 14 mg/dL, range 11 to >20 mg/dL; 3.5 mmol/L, 2.75 to 5 mmol/L) as is the PTH value (average 540 pg/mL, range 55 to >1000 pg/mL).⁸⁸ Other findings include: hypophosphatemia, hypermagnesemia, hyperphosphatasemia, and elevated calcitriol values, low renal tubular reabsorption of phosphate and relative hypocalciuria. Radiographically, there is evidence of hyperparathyroid bone disease—osteopenia, metaphyseal widening and irregularity, subperiosteal resorption, varus angulation of the hips, and fractures.

Lowering of the elevated serum calcium concentration present in NSHPT is only modestly affected by induction of diuresis by infusion of sodium chloride and by administration of furosemide; calcitonin and a bisphosphonate

TABLE 20.4B Genetic Variants Associated With Hypocalcemia/Hyperparathyroidism

Gene Protein Chromosome OMIM	Disorder OMIM	Clinical/Biochemical Manifestations	Gene Function/Transmission
CASR Calcium sensing receptor 3q13.3-21.1 601199	Familial hypocalciuric hypercalcemia (FHH) type I, 145980; Neonatal severe hyperparathyroidism (NSHPT) 239200, Adult-onset primary hyperparathyroidism (AOPH)	Hypercalcemia, NSHPT—life-threatening AOPH—nephrolithiasis, low bone mineralization	Calcium receptor regulating synthesis, secretion of PTH, inactivating variant FHH1—AD NSHPT—AD/AR AOPH—AD/AR
GNA11 Guanine nucleotide-binding protein, alpha-11 19p13.3 139313	Familial hypocalciuric hypercalcemia type II, 145981	Hypercalcemia	Encodes G-protein transmitting signal of CaSR to intracellular signal transduction pathways, inactivating variant, AD
AP2S1 Adaptor related protein complex 2, sigma1 subunit 19q13.31 602242	Familial hypocalciuric hypercalcemia type III, 600740	Hypercalcemia, low bone mineralization, impaired cognition	Adaptor-related protein complex 2 interacts with β -arrestin (<i>ARRB1</i> , chr. 11q13.4, OMIM 107940) to form complex facilitating retrograde movement (endocytosis) of the CaSR from the cell surface & its return to the cytoplasm, inactivating variant AD
CYP24A1 Cytochrome P450, family 24, subfamily A, polypeptide 1 20q13.2 143880	Infantile hypercalcemia 1 143880	Hypercalcemia, vomiting, anorexia, inanition, nephrocalcinosis with inappropriate normal/high levels of calcitriol	Encodes 1,25-dihydroxyvitamin D-24-hydroxylase that inactivates calcitriol, AR
SLC34A1 Solute carrier family 34 (Type II sodium phosphate cotransporter), member 1, 5q35 182309	Infantile hypercalcemia 2 616963	Hypercalcemia, vomiting, anorexia, inanition, nephrocalcinosis	Encodes a proximal renal tubular sodium phosphate cotransporter, AR
TRPV6 Transient receptor potential, cation channel, subfamily V, member 6 7q34 606680	Transient neonatal hyperparathyroidism 618188	Skeletal demineralization in utero & postnatally, eucalcemia or hypocalcemia	Encodes a calcium selective channel active in the placental trophoblast, renal tubule, and intestinal tract, AR
Deletion 7q11.23 syndrome 194050	Williams-Beuren syndrome: Infantile hypercalcemia	Hypercalcemia, supravalvular aortic stenosis, peripheral pulmonary arterial stenoses, characteristic face, developmentally challenged, friendly personality, verbal & musical skills	Deletion of 28 (\pm) contiguous genes including <i>ELN</i>
MEN1 Men1 gene 11q13.1 613733	Multiple endocrine neoplasia, type 1 131100	Tumors of the parathyroid, pancreatic islets, intestinal endocrine cells, adenohypophysis	Tumor suppressor, nuclear scaffold protein interacting with several transcription factors, inactivating variant AD
RET Rearranged during transfection protooncogene 10q11.21 164761	Multiple endocrine neoplasia, type 2A 171400; type 2B 162300	Medullary carcinoma of thyroid (2A,2B), pheochromocytoma (2A, 2B), parathyroid adenoma (2A), marfanoid habitus/ganglioneuromas (2B)	Transmembrane receptor tyrosine kinase, activating variant, AD
CDKN1B Cyclin-dependent kinase inhibitor 1B 12p13.1 600778	Multiple endocrine neoplasia, type 4 610755	Parathyroid adenoma, gastric carcinoid, pituitary adenoma	Blocks cell cycle at G0/G1 phase, regulates cell motility, apoptosis, inactivating variant AD
CDC73 Cell division cycle protein 73, S cervisiae, homolog of 1q31.2 607391	Familial isolated hyperparathyroidism—type 1 (145000) & type 2 (145001—associated with the hyperparathyroidism—jaw tumor syndrome)	Parathyroid adenoma/carcinoma, ossifying fibromas of mandible & maxilla, Wilms tumor	Parafibromin—protein intrinsic to the cell division cycle; tumor suppressor, inactivating variant, AD

TABLE 20.4B Genetic Variants Associated With Hypocalcemia/Hyperparathyroidism—cont'd

Gene Protein Chromosome OMIM	Disorder OMIM	Clinical/Biochemical Manifestations	Gene Function/Transmission
<i>GCM2</i> Glial cells missing, drosophila, homolog of, 2 6p24.2 603716	Familial isolated hyperparathyroidism- type 4 617343	Familial isolated hyperparathyroidism with multiple parathyroid adenomas, carcinoma	Encodes transcription factor essential for differentiation of the parathyroid glands, activating variant AD
<i>PTH1R</i> Parathyroid hormone receptor 1 3p21.31 168468	Metaphyseal chondrodysplasia, Murk- Jansen type 156400	Marked growth retardation, genu varum, small mandible, hypercalcemia, hypophosphatemia	G-protein coupled receptor for PTH & PTHrP, activating variant, AD

AD, Autosomal dominant; AR, autosomal recessive; PTH, parathyroid hormone; PTHrP, parathyroid hormone related protein.

(From Stokes VJ, Nielsen MF, Hannan FM, Thakker RV. Hypercalcemic disorders in children. *J Bone Mineral Res.* 2017;32:2157–2170; Hannan FM, Babinsky VN, Thakker RV. Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Mol Endocrinol.* 2016;57:R127–142; Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. *Lancet.* 2018;391:168–178; Marini F, Cianferotti L, Giusti F, Brandi ML. Molecular genetics in primary hyperparathyroidism: the role of genetic tests in differential diagnosis, disease prevention strategy, and therapeutic planning. A 2017 update. *Clin Cases Miner Bone Metab.* 2017;14:60–70; Suzuki Y, Chitayat D, Sawada H, et al. (2018). TRPV6 variants interfere with maternal-fetal calcium transport through the placental and cause transient neonatal hyperparathyroidism. *Am J Human Genet.* 2018;102:1104–1114.)

(e.g., pamidronate/zolendronate) may be required to suppress resorption of bone calcium and reduce serum calcium levels. These agents do not reduce the elevated level of PTH, however, and do not address the marked skeletal demineralization of hyperparathyroidism. The allosteric calcimimetic cinacalcet hydrochloride that acts directly on the CaSR has also been effective in lowering serum calcium concentrations in infants with NSHPT.⁸⁹ (However, cinacalcet is not approved for use in subjects less than 18 years of age.) Subtotal parathyroidectomy may be a requisite lifesaving measure at times. Children with NSHPT who remain hypercalcemic are anorectic, fail to thrive, and at risk for developmental delay.

Secondary hyperparathyroidism in the neonate may be the result of maternal hypocalcemia caused by hypoparathyroidism, PHP, renal tubular acidosis, or vitamin D deficiency.⁷⁹ Maternal hypocalcemia reduces placental transport and net delivery of calcium to the fetus resulting in relative fetal hypocalcemia, leading to hyperplasia of the fetal parathyroid glands and secondary hyperparathyroidism proportional to the maternal calcium deficit. Although only 25% of infants of hypocalcemic mothers are hypercalcemic, most have skeletal changes that reflect PTH excess that vary from severe demineralization with fractures to osteopenia detectable only by bone mineral densitometry. Secondary hyperparathyroidism usually resolves within a few weeks after birth as the infant ingests adequate calcium and phosphate. Normal transplacental maternal-fetal calcium transport is dependent upon three placental trophoblast processes: (1) apical Ca²⁺ entry through Ca²⁺ channels via an electrochemical gradient, (2) intracellular binding of Ca²⁺ to calbindin D9k, and (3) extrusion of Ca²⁺ through the basolateral membrane of the trophoblast. Transient neonatal hyperparathyroidism (OMIM 618188) may also occur in newborns with biallelic inactivating variants of *TRPV6* (OMIM 606680) encoding a permeable calcium selective channel active in the placental trophoblast, renal tubule, and intestinal tract. In this disorder, decreased transplacental transport of calcium from the mother to the fetus leads to in utero fetal hypocalcemia and secondary hyperparathyroidism manifested by skeletal abnormalities, including thin, short bones, some with fractures that resolve within 2 years after birth. Mucopolysaccharidosis type II (OMIM 252500) has also been associated with neonatal secondary hyperparathyroidism; this Hurler-like disorder is characterized by facial abnormalities (asymmetry, flat nasal bridge), hepatosplenomegaly, skeletal deformities (dysostosis multiplex), and developmental delay and is caused

by inactivating mutations in a gene (*GNPTAB*, OMIM 607040) encoding N-acetylglucosamine-1-phosphotransferase, α/β subunits, a lysosomal phosphotransferase required for synthesis of mannose 6-phosphate.⁹⁰ In this disease, maternal calcium concentrations are normal, but placental histology is abnormal suggesting impaired placental transport of calcium and fetal hypocalcemia, leading to compensatory increase in PTH generation by the fetus. In turn, skeletal evidence of PTH excess (osteopenia, fractures) develops; secondary hyperparathyroidism and its adverse effects remit within the first several weeks to months after birth. Transient neonatal distal renal tubular acidosis has also been associated with hypercalcemia because of secondary hyperparathyroidism. Occasionally, neonates with primary congenital hypothyroidism are transiently hypercalcemic.

Murk-Jansen metaphyseal chondrodysplasia (OMIM 156400) is an autosomal dominant chondrodystrophy associated with marked hypercalcemia as a consequence of heterozygous mutations that lead to constitutive, ligand-independent activation of *PTH1R* expressed in the kidney, bone, and growth plate chondrocytes.⁷⁹ Enhanced functional PTH1R leads to accelerated chondrocyte proliferation, decreased rate of chondrocyte maturation, delayed bone formation, and increased bone resorption. Phenotypically, the patient with Murk-Jansen chondrodysplasia manifests short-limbed dwarfism; deformities of the long bones, digits, spine, and pelvis; choanal atresia; highly arched palate; micrognathia; widely open cranial sutures (in infancy); sclerosis of the basal cranial bones; disorganization of the metaphyses (delayed chondrocyte differentiation, irregularly calcified cartilage protruding into the diaphysis); and excessive loss of cortical bone but normal trabecular bone. Although birth length and physical appearance may be normal in affected neonates, there is radiographic evidence of the chondrodysplasia. Affected neonates are often eucalcemic, but later (infancy and childhood) develop hypercalcemia, hypophosphatemia, increased serum concentrations of calcitriol and elevated urinary excretion of nephrogenous cyclic AMP and calcium but low or undetectable serum levels of PTH and PTHrP. (Adults with this disorder are often eucalcemic, as well as very short [adult height 100 cm]; skeletal maturation is delayed; metaphyses of the long bones are long and histologically disorganized; and cranial bones are sclerotic.) Constitutively activating mutations of *PTH1R* in these subjects include most commonly p.His223Arg at the junction of the first intracellular loop and second transmembrane domain and p.Thr410Pro in

the sixth transmembrane domain—sites that are specifically important in conferring ligand-independent activity upon PTH1R.⁸¹ SLK family kinase 3 (*SIK3*, OMIM 614776) is active in the PTH1R intracellular signal transduction pathway; a biallelic inactivating variant of *SIK3* results in a hypercalcemic spondyloepimetaphyseal chondrodysplasia similar to but distinct from that of Murk-Jansen.⁹¹ Excessive secretion of PTHrP and resultant hypercalcemia have also been recorded in infants with neonatal iron storage disease and embryonal renal tumors (Wilms, mesoblastic nephroma).

WBS (OMIM 194050) is a hemizygous contiguous gene deletion syndrome (involving approximately 26–28 genes on chromosome 7q11.23) that is transmitted as an autosomal dominant disorder and whose prevalence is 1/7500 to 1:15,000 live births.^{92,93} The WBS is characterized by intrauterine and postnatal growth retardation, hypercalcemia in infancy in 15% of patients that usually resolves by 2 years of age but may occasionally persist into adulthood, consistent hypercalciuria, supravalvular aortic stenosis in 30% of subjects, narrowing and stenoses of the thoracic aorta and coronary, pulmonary, renal, mesenteric, and celiac arteries, hypertension, microcephaly, “elfin” face (broad forehead, epicanthal folds, stellate iris pattern, esotropia, short nose with full nasal tip, arched upper and prominent lower lips, long philtrum, full cheeks with flattened malar eminences, dental malocclusion), hoarse voice, hyperacusis in childhood associated with nerve deafness, radioulnar synostosis, renal hypoplasia or unilateral agenesis, developmental delay (poor visual-motor integration, attention deficit disorder, mean IQ 58, range 20–106), and short stature. Although most patients with WBS are developmentally challenged, they have unique and proficient verbal skills with a large vocabulary and enhanced auditory memory particularly for names, adept social language skills, and exceptional musical aptitude including the ability to memorize and sing many musical compositions and play many instruments.⁸¹ Children with WBS prefer to be in the company of adults rather than with their peers and are not shy or fearful of strangers.⁹²

Hypercalcemia in subjects with the WBS may be pathogenetically related to partial loss of function of the Williams syndrome transcription factor (WSTF) encoded by *BAZ1B* (Bromodomain adjacent to zinc-finger domain, 1B, OMIM 605681). The WSTF is a nuclear protein that is part of a multimeric, ATP-dependent, chromatin remodeling complex termed WINAC (WSTF including nucleoside assembly complex).⁹⁴ Independently of ligand-binding, the VDR interacts with WINAC. Under usual circumstances, binding of calcitriol to the VDR-WINAC complex (1) represses expression of renal tubular *CYP27B1*, the gene encoding 25-hydroxyvitamin D-1 α -hydroxylase, and (2) enhances transcription of *CYP24A1*, the gene encoding 25-hydroxyvitamin D-24-hydroxylase. Hence haploinsufficiency of WSTF may lead to increased and relatively unregulated synthesis of calcitriol while slowing its rate of degradation; in response to the increase in calcitriol generation, the intestinal absorption of calcium increases and hypercalcemia ensues. Other endocrinopathies associated with WBS include glucose intolerance and hypothyroidism.

WBS is the consequence of deletion of chromosome 7q11.23 caused by unequal meiotic recombination (unequal crossover of genes between chromosome 7 homologs during meiosis); this genetic error occurs with equal frequency in the gametes of both parents and results in the loss of 26 to 28 genes.⁸² Hemizygosities of *ELN* (OMIM 130160) encoding elastin is the attributed cause of the cardiovascular malformations and hypertension observed in WBS, inasmuch as partial loss of *ELN* leads to compensatory increase in the number of rings of smooth muscle and elastic lamellae resulting in arterial thickening and increased risk of obstruction. Functional hemizygosities for general transcription factor II-I (*GTF2I*, OMIM 601679), *GTF2IRD1* (OMIM 604238),

HIP1 (OMIM 601767), and *YWHAQ* (OMIM 605356) may underlie the neurocognitive deficits and strengths that characterize WBS and their distinctive personalities.^{92,95} Haploinsufficiency of LIM domain kinase 1 (*LIMK1*, OMIM 601329) may also be linked to the neurocognitive defects in patients with WBS.⁹⁶ Hemizygous loss of *STX1A* (OMIM 186590) may be pathogenetically related to development of glucose intolerance in WBS subjects.⁹⁷ The WBS phenotype has also been associated with interstitial deletion of chromosome 6q22.2-q23, as well as defects in chromosomes 4, 11, and 22—implying that the syndrome is genetically heterogeneous. The diagnosis of the WBS syndrome is suspected on the basis of the characteristic clinical phenotype (with/without hypercalcemia) and confirmed by demonstration of the microdeletion at chromosome 7q11.23 by microarray or FISH, although a normal chromosome analysis does not entirely eliminate this diagnosis. Specific genotyping may also prove diagnostically useful on occasion. Hypercalcemia is managed by ingestion of a low calcium, vitamin D-free formula; occasionally short-term glucocorticoid therapy may be necessary to restore eucalcemia.

A contiguous gene deletion syndrome characterized by developmental delay and seizures involving a segment of chromosome 7q11.23 distal to the deletion associated with the WBS has been described (OMIM 613729). Duplication of chromosome 7q11.23 results in a syndrome associated with heart defects, diaphragmatic hernia, cryptorchidism, and neurocognitive and behavioral aberrations.⁹⁸ Additional findings in patients with dup7q11.23 include short stature and minor dysmorphic facial features (prominent forehead, long nasal tip, short philtrum, thin lips, retrognathia, folded ear helices). Neurocognitively, there is severe speech delay and often autistic behavior but intact visuocognitive skills—characteristics that are opposite to those present in patients with the WBS.

Although phenotypically normal, infants with hypercalcemia of infancy types 1 and 2 manifest anorexia, impaired growth, developmental delay, febrile episodes, polyuria, and vomiting, leading to dehydration, hypercalciuria, and nephrocalcinosis associated with hypercalcemia in the presence of decreased serum concentrations of PTH.^{97,99} Infantile hypercalcemia type 1 (OMIM 143880) is characterized by elevated or inappropriately normal levels (relative to serum calcium concentrations) of calcitriol most often caused by biallelic inactivating variants of *CYP24A1* (OMIM 126065) encoding 24-hydroxylase, the enzyme that converts 25-hydroxyvitamin D to 24,25 dihydroxyvitamin D and 1,25-dihydroxyvitamin D to 1,24,25-trihydroxyvitamin D, thereby increasing their solubility and subsequent excretion in bile and urine.^{97,100,101} Delaying the degradation of 1,25-dihydroxyvitamin D prolongs its effective biological life. In many patients with infantile hypercalcemia type I, hypercalcemia resolves within the first several years of life but it may persist to older ages.² Inactivating mutations in *CYP24A1* have also been found in adults with hypercalcemia, hypercalciuria, and nephrolithiasis.^{102–104} *CYP24A1* mutations may either decrease the binding of the enzyme to its substrate or interfere with the interaction of heme and enzyme protein; either type of mutation decreases its function.¹⁰⁵ Avoidance of vitamin D, low dietary calcium intake, increased fluids, and occasionally glucocorticoids to reduce intestinal absorption of calcium are therapeutic modalities for this disorder. Infantile hypercalcemia type 2 (OMIM 616963) is the result of biallelic variations in *SLC34A1* encoding solute carrier family 34 (Type II sodium phosphate cotransporter), member 1 (OMIM 182309), a proximal renal tubular sodium phosphate cotransporter, and is manifested by vomiting, anorexia, inanition, hypercalcemia, hypophosphatemia, hypercalciuria, and nephrocalcinosis.^{99,106,107} In this disorder, decreased renal tubular transport of phosphate results in hyperphosphaturia and hypophosphatemia, leading to decreased

synthesis of FGF-23 and consequently to increase in functional activity of 25OHD-1 α hydroxylase (*CYP27B1*) and the ensuing synthesis of calcitriol and hyperabsorption of intestinal calcium. Restricted intake of calcium and vitamin D have also been used in the management of patients with infantile hypercalcemia type 2, but phosphate supplementation may also be considered.¹⁰⁷ As adults, patients with variants of either *CYP24A1* or *SLC34A1* are of normal height and weight and in reasonably good health, although in some subjects, nephrocalcinosis persists. Some affected patients, however, develop progressive renal failure as adults.

Antenatal/neonatal Bartter syndromes type 1 (OMIM 601678) and type 2 (OMIM 241200) are clinically and biochemically similar and are caused by biallelic loss-of-function mutations in genes controlling a transepithelial sodium-potassium-chloride transporter (*SLC12A1*, OMIM 600839) and an inwardly rectifying apical potassium channel (*KCNJ1*, OMIM 600359), respectively, expressed in the renal tubular TALH. Affected fetuses develop polyhydramnios, leading to premature delivery with postnatal salt-wasting and secondary hyperaldosteronism; increase renal and systemic production of prostaglandin E2 inhibits sodium and chloride reabsorption in the TALH and enhances juxtaglomerular renin release resulting in hypokalemic metabolic acidosis, hyperparathyroidism, hypercalcemia, hypercalciuria, nephrocalcinosis, hypomagnesemia, osteopenia, and often death in type 1 antenatal Bartter syndrome.^{108,109} Type 2 neonatal Bartter syndrome caused by variants of *KCNJ1* is manifested by hypokalemia (occasionally transient hyperkalemia), reduced intravascular volume, and increased levels of angiotensin leading to renal and systemic production of prostaglandin E2 that also impair renal tubular reabsorption of sodium and chloride in the TALH. Hypochloremic, hypokalemic alkalosis, hyperprostaglandin E, hypercalcemia, hypercalciuria, and nephrocalcinosis suggest neonatal Bartter syndrome. Replacement of fluid and electrolytes and administration of potassium-sparing diuretics (thiazides) and cyclooxygenase inhibitors (indomethacin) may be effective in ameliorating the biochemical and clinical manifestations of the disease.

Neonatal or infantile hypercalcemia may occur in patients with one of several inborn errors of metabolism.¹ Both congenital lactase deficiency (OMIM 223000) caused by mutations in *LCT* (OMIM 603202) and sucrase-isomaltase deficiency (OMIM 222900) caused by variants of *SI* (OMIM 609845) have been associated with infantile hypercalcemia, likely caused by increased intestinal absorption of calcium promoted by disaccharides. Hypercalcemia may also be encountered in infants with primary oxalosis (OMIM 259900) that may be the result of variants in one of three genes (*AGXT*, OMIM 604285; *GRHPR*, OMIM 604296; *DHDPSL*, OMIM 613597). The "blue diaper syndrome" (OMIM 211000) is the result of inability of the gastrointestinal tract to absorb tryptophan that intestinal bacteria metabolize to an indole that is absorbed and excreted in the urine resulting in indicanuria that is then oxidized to the pigment "indigo blue" thus staining the diaper of the affected neonate and infant. Hypercalcemia, hypercalciuria, and nephrocalcinosis are common but not invariable; the pathogenesis of hypercalcemia in these patients is unknown. This disorder has been associated with a homozygous loss-of-function mutation in *PCSK1* encoding a prohormone convertase/calcium-dependent serine endoproteinase (proprotein convertase, subtilisin/kexin-type 1, OMIM 162150) that converts a multicomponent protein to specific bioactive protein products.¹¹⁰ Variants of *PCSK1* have also been related to defects in insulin synthesis, as well as several endocrinopathies (hypogonadotropism, central hypoadrenocorticism, and central diabetes insipidus).¹¹¹ Hypercalcemia may also occur in patients with the IMAGe syndrome of

intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies (OMIM 614732) caused by heterozygous variants of *CDKN1C* encoding cyclin-dependent kinase inhibitor 1C (OMIM 600586), a regulator of early cell division.

Hypophosphatasia (HPP) is a congenital disorder of bone mineralization resulting in rickets of variable severity that is caused by a decreased osteoblast synthesis of tissue nonspecific alkaline phosphatase (TNSALP)—the consequence of monoallelic or biallelic inactivating mutations in *ALPL* (OMIM 171760) (vide infra).¹¹² Decreased production of alkaline phosphatase leads to a deficit in phosphate ions impairing synthesis of hydroxyapatite resulting in rickets, whereas continued intestinal absorption of calcium can lead to hypercalcemia. The infantile form of hypophosphatasia (OMIM 241500) is caused by biallelic loss-of-function variants of *ALPL*. It becomes clinically apparent after the first month of life but usually before 6 months of age and is often fatal. Initial symptoms are failure to thrive associated with impaired feeding, weakness, and delayed achievement of motor milestones; rachitic deformations of the thorax and limbs are visible. Radiographically, it is characterized by demineralization of the calvarium and peripheral skeleton, rickets, spurs of cartilage and bone extending from the sides of the knee and elbow joints, and premature craniosynostosis, leading to increased intracranial pressure. Also present are vitamin B₆-dependent seizures, hypercalcemia, and hypercalciuria.^{112,113} Defective osteoblast synthesis of TNSALP is the result of loss-of-function missense, nonsense, and donor splice site mutations of *ALPL*. The lethal homozygous or compound heterozygous mutations of *ALPL* are located within or near the enzyme domain and/or the homodimer and tetramer interfaces. Inappropriately low serum bone alkaline phosphatase activity differentiates this illness from other rachitic or osteopenic states (osteogenesis imperfecta congenita [OIC] or achondrogenesis type 1A) in which alkaline phosphatase activity is usually normal or elevated. Increased urine phosphoethanolamine and serum inorganic pyrophosphate (an inhibitor of hydroxyapatite crystallization that also impairs skeletal mineralization) and pyridoxal-5'-phosphate values are consistent with the diagnosis of hypophosphatasia, whereas analysis of *ALPL* identifies the gene mutation(s) itself. The diagnosis of hypophosphatasia can be suspected on prenatal ultrasonography and confirmed by *ALPL* genotyping. The hypercalcemia of infantile hypophosphatasia is managed by hydration, diuretics that act upon the TALH (e.g., furosemide), and administration of bisphosphonates (pamidronate), calcitonin, or glucocorticoids as necessary. Dietary calcium intake should be restricted and vitamin D and its metabolites avoided. Bone marrow transplantation and "stem cell boosts" of transfused donor osteoblasts have also been used to treat affected patients. Administration of a bone-targeted form of TNSALP has been used successfully to treat life-threatening hypophosphatasia and is currently FDA approved for this purpose.^{114,115}

Hypercalcemia and hypercalciuria of unknown pathogenesis occur occasionally in subjects with the IMAGe syndrome (OMIM 614732) caused by gain-of-function variants of *CDKN1C* (OMIM 600856, chr. 11p15.4) encoding a paternally imprinted (maternally transmitted) cyclin-dependent kinase inhibitor critical for normal regulation of the cell's mitotic processes. Craniosynostosis, cleft palate, and scoliosis may also develop in these patients.

Evaluation and Management

After historical review (the family history is explored for members with mineral disorders and maternal status considered in depth, and the infant's intake of calcium, phosphate, and

vitamin D is estimated) and physical examination (nonspecific manifestations of hypercalcemia include—hypertension, subnormal growth, hypotonia, weakness, lethargy, stupor, occasionally seizures; specific findings associated with hypercalcemia include—facial and cardiovascular signs of WBS, subcutaneous nodules consistent with fat necrosis, skeletal deformities suggestive of metaphyseal chondrodysplasia or infantile hypophosphatasia) have been completed, evaluation of the hypercalcemic neonate and infant continues with measurements of total serum calcium and Ca^{2+} , phosphate, alkaline phosphatase, PTH, calcidiol, and calcitriol and determination of urine calcium and creatinine levels (Fig. 20.5). Hypocalciuria in the presence of hypercalcemia suggests the presence of NSHPT. Occasionally, one may encounter a neonate in whom the total calcium concentration is elevated because of hyperproteinemia but the Ca^{2+} value is normal, a state termed *pseudohypercalcemia*.⁷⁹ In infants

with suspected WBS, microarray or FISH analysis searching for deletion of chromosome 7q11.23 should be undertaken. In those in whom the diagnosis of NSHPT is being considered, determination of parental serum and urine calcium and creatinine values is often helpful unless the disorder has been transmitted as an autosomal recessive disease.⁹⁰ Genotyping of *CASR* should be undertaken when NSHPT is suspected.

Treatment of hypercalcemia in neonates and infants must be directed to prevention of progression, identification of cause, and assessment of severity. Use of a formula low in calcium and avoidance of vitamin D (excessive intake or sunlight) are helpful in the majority of neonates with modest hypercalcemia. Infants with significantly elevated serum calcium levels, particularly if long-standing, are often dehydrated. Immediate treatment consists of infusion of 0.9% sodium chloride with 30 mEq/L of potassium chloride (10–20 mL/kg over 1 hour);

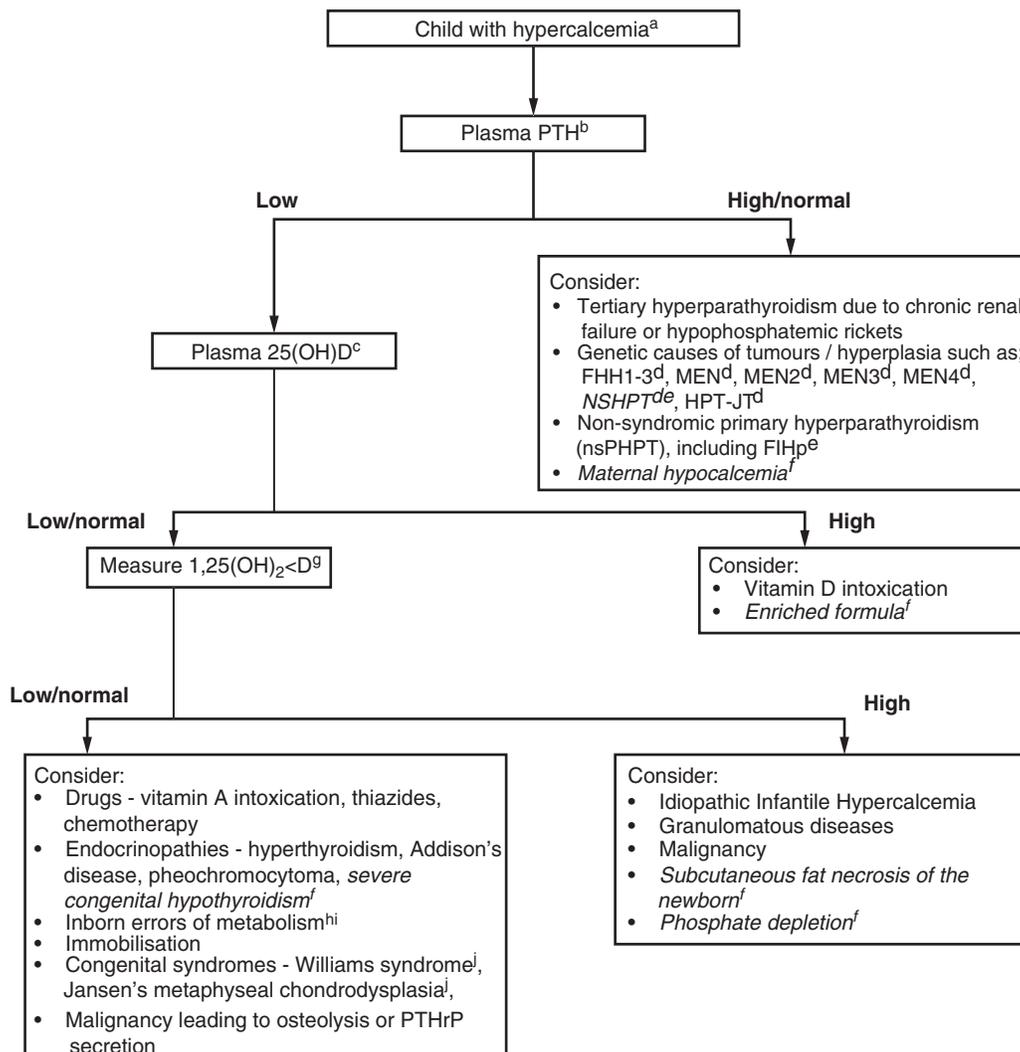


Fig. 20.5 Evaluation of hypercalcemia. a. Hypercalcemia: Serum calcium >10.5 mg/dL (or per laboratory data), b. Parathyroid hormone, c. 25-Hydroxyvitamin D (Calcidiol), d. Familial hypocalcemic hypercalcemia 1-3, Multiple endocrine neoplasia 1-4, Neonatal severe hyperparathyroidism, Hyperparathyroidism-jaw tumor syndrome, e. Familial isolated hyperparathyroidism, f. *Conditions affecting neonates*, g. 1,25-Dihydroxyvitamin D = Calcitriol, h. Inborn errors of metabolism, i. Dysmorphic syndromes (e.g., William's, Jansen's syndromes). (From Stokes VJ, Nielsen MF, Hannan FM, Thakker RV. Hypercalcemic disorders in children. *J Bone Mineral Res.* 2017;32:2157–2170, with permission.)

after dehydration has been corrected and adequate urine flow established and if substantial hypercalcemia persists an intravenous bolus injection of furosemide (1–2 mg/kg) may be administered. Currently, bisphosphonates, analogs of pyrophosphate that adhere to the surface of hydroxyapatite crystals and are incorporated by osteoclasts thereby inhibiting osteoclast-mediated dissolution of bone mineral and matrix, are the agents of choice for the treatment of substantial hypercalcemia in infants. Pamidronate (0.5–2.0 mg/kg in 30 mL normal saline intravenously over 4 hours) has been successfully used in infants with hypercalcemia caused by vitamin D intoxication, subcutaneous fat necrosis, NSHPT, and other causes.^{1,79,116} The effects of bisphosphonate last for several weeks to months and when administered in excess can lead to hypocalcemia, hypophosphatemia, and hypomagnesemia. Other agents that may be considered in the treatment of a hypercalcemic infant include salmon calcitonin (2–4 units/kg subcutaneously every 6–12 hours) to inhibit calcium mobilization from bone. Although the type II calcimimetic—cinacalcet—has been demonstrated to improve function of many inactive mutant forms of the CASR and to be of clinical benefit in adults with familial hypocalciuric hypocalcemia, this agent is not approved for use in children in the United States, a therapeutic trial of this agent may be considered in a patient with NSHPT with appropriate safeguards and permissions. In the newborn with life-threatening NSHPT, total parathyroidectomy may be urgently required.¹¹⁶ Postoperatively, the patients may become hypocalcemic as calcium is avidly deposited in bone (hungry bone syndrome) and require large amounts of supplemental calcium until stabilized.

Infants with hypercalcemia caused by WBS (that often remits by 2 years of age) or with some forms of osteopetrosis may be managed by use of a low-calcium formula (e.g., Calcilo XD—2.9 mg of calcium per 100 calories; free of vitamin D) and withholding of supplemental vitamin D. Infants with “idiopathic” hypercalcemia of infancy caused by inactivating mutations of *CYP24A1* should receive the low-calcium formula and supplemental vitamin D should be withheld. Some children with hypercalcemia caused by inactivating mutations of *CYP24A1* respond to ketoconazole, which inhibits conversion of calcidiol to calcitriol or rifampin, which also inactivates metabolites of vitamin D. Patients with mutations in *SLC34A1* may respond to administration of supplemental phosphate. Mineral homeostasis must be monitored closely in infants receiving a low-calcium formula and from whom vitamin D is being withheld to prevent deficiencies of both nutrients and development of secondary hyperparathyroidism.

Hypercalcemia in the Child and Adolescent

Hypercalcemia is present in the child/adolescent when the serum calcium concentration exceeds plus two standard deviations above the normal mean; for subjects 4 to 18 years of age these values are: total calcium 10.7 mg/dL (2.70 mmol/L); Ca^{2+} 5.52 mg/dL (1.38 mmol/L).¹ Hypercalcemia is most often asymptomatic in the older child and adolescent and identified unexpectedly by routine serum chemical analysis for an unrelated problem. Clinical manifestations of hypercalcemia in the child/adolescent include: anorexia, nausea, vomiting, constipation, abdominal or flank pain, polydipsia, polyuria, renal colic, altered alertness, lethargy, hypotonia, irritability, or seizures.^{1,117,118} Unrecognized, chronic hypercalcemia may impair growth, lead to nephrocalcinosis and nephrolithiasis preceding renal failure, pancreatitis, impaired cognition, or symptoms of mental illness. Long bone fracture through a fibrous cystic “brown tumor” (osteitis fibrosa cystica) may be a presenting complaint. Physical findings are nonspecific and may include hypertension, weakness, hyporeflexia, dull affect, aberrant mentation, or flank tenderness.

Etiology and Pathogenesis

Causes of hypercalcemia in children and adolescents are listed in Tables 20.4A, 20.4B. In the presence of a normal serum protein concentration, hypercalcemia occurs when the “set point” for serum Ca^{2+} is increased because of a loss-of-function mutation in *CASR* or the set point is reversibly increased by lithium, or when the rate of entry of calcium into the extracellular and circulatory compartments from bone, intestinal tract, or kidney exceeds its rate of loss.¹¹⁹ The resorption rate of bone mineral may be increased by excessive secretion of PTH or PTHrP, constitutive activation of PTH1R, excessive intake of vitamin D or metabolites, increased production of osteoclast-activating inflammatory cytokines or localized osteolytic processes, such as metastatic neoplasms, and dissociation of the rates of bone formation and resorption—for example, immobilization. The absorption rate of intestinal calcium may be increased by excessive intake of calcium, by hypervitaminosis D of exogenous or endogenous origin, or by increased secretion of PTH or PTHrP. Augmented renal tubular reabsorption of filtered calcium may occur with administration of calcium-sparing diuretics, such as thiazides, an effect that may “unmask” hypercalcemia in a previously eucalcemic patient with hyperparathyroidism. Thus hypercalcemia may be considered to be PTH mediated or PTH independent.¹⁰³ In the presence of hyperalbuminemia, the total serum calcium concentration but not the Ca^{2+} concentration is increased (pseudohypercalcemia). Venous stasis (e.g., by tourniquet during blood sampling) results in spuriously altered local pH and Ca^{2+} values.

Hyperparathyroidism is most often caused by a single parathyroid adenoma but may also be caused by hyperplasia of all four parathyroid glands as in patients with HHC caused by loss-of-function variants of *CASR*, *GNA11*, or *AP2S1*. Respectively, these genes encode the plasma membrane heptahelical CaSR, its guanosine triphosphate–coupled signal transduction factor (Gq/11 α), and an adaptor-related protein complex that facilitates endocytosis of the CaSR.^{117,120,121} Familial HHC type 1 (HHC1, OMIM 145980) is an autosomal dominant disorder with complete penetrance at all ages characterized by PTH-dependent, usually asymptomatic (total and ionized) hypercalcemia with hypocalciuria (and, therefore absence of nephrocalcinosis or calcium-containing renal calculi), hypermagnesemia, hypomagnesuria, and hypophosphatemia that is caused by heterozygous loss-of-function mutations in *CASR* (OMIM 601199).^{121–124} Consequently, higher serum concentrations of Ca^{2+} are required to activate the CaSR and inhibit synthesis and secretion of PTH. Serum concentrations of PTH may be normal or slightly elevated, but inappropriately high for the Ca^{2+} level; calcidiol and calcitriol values are normal. In children, HHC1 is most commonly suspected initially by the presence of unexpected hypercalcemia (11–13 mg/dL) in a chemistry profile or through family screening of a parent or other relative with hypercalcemia. Although patients with HHC1 are hypercalcemic, they are paradoxically hypocalciuric because inactivation of the CaSR results in enhanced distal renal tubular reabsorption of filtered calcium. PTH levels are normal or slightly elevated. When mutations in *CASR* are biallelic or when a neonate with a monoallelic variant of *CASR* is born to a normocalcemic woman, hypercalcemia may become extreme and even life-threatening (NSHPT). Older subjects with HHC1 may complain of fatigue, weakness, or polyuria; there is a slightly increased incidence of relapsing pancreatitis, cholelithiasis, chondrocalcinosis, and premature vascular calcification in subjects with HHC1, but bone mass and fracture rate are normal. Because of decrease in the number or functional competence of parathyroid chief cell membrane CaSRs, the set point for Ca^{2+} suppression of PTH secretion is reset upward.¹²⁵ The parathyroid glands are slightly hyperplastic.

In renal tubular cells, decrease in the number and activity of CaSRs results in increased renal tubular reabsorption of filtered calcium and relative hypocalciuria (ratio of calcium clearance/creatinine clearance <0.01 in 80% of patients with HHC1); because the renal tubular reabsorption of magnesium is also increased, hypermagnesemia and hypomagnesuria are present; urinary concentrating ability and other measures of renal function are normal; in hypercalcemia of other pathogenesis, urinary calcium excretion is increased and renal concentrating function depressed. Usually, HHC1 requires no therapy, but must be differentiated from mild primary hyperparathyroidism in which hypomagnesemia and hypercalciuria are present. Subtotal parathyroidectomy in HHC1 does not lower calcium levels as the residual parathyroid glands hypertrophy; total parathyroidectomy is unnecessary except in a rare infant with NSHPT. More than 100 nonsense, missense, insertion, and deletion mutations in *CASR* associated with HHC1 or NSHPT have been identified, mostly in the receptor's extracellular Ca^{2+} binding domain; these mutations either decrease receptor affinity for Ca^{2+} or alter intracellular processing of the CaSR (glycosylation, dimerization—e.g., p.Arg66His, p.Asn583Stop) in the endoplasmic reticulum preventing its translocation to the surface of the cell membrane^{122,125,126} (Fig. 20.6). Many of the mutations are located in the extracellular domain of CaSR between codons 39 to 300, a region rich in aspartate and glutamate residues in which Ca^{2+} may nestle. Glycosylation is necessary for both dimerization and trafficking of the CaSR. Missense mutations involving arginine at codon 66 (p.Arg66His/Cys) result in a product that is able to be only partially glycosylated; it can form homodimers in the endoplasmic reticulum but cannot enter the Golgi apparatus and be transported to the cell's plasma membrane.¹²⁶ Experimentally, approximately 50% of the products of inactivating mutations of *CASR* can be “escorted” to the plasma membrane and functionally stabilized by the allosteric calcimimetic cinacalcet proffering a potential therapeutic agent for the management of patients with symptomatic hypercalcemia because of loss-of-function *CASR* mutations, especially those with NSHPT, if it proves safe to do so in children. Mutations in *CASR* tend to be unique to the affected family. In approximately one-third of families with HHC1, no mutations in the coding region of *CASR* have been identified; thus they may have a mutation in a noncoding region of *CASR* or a variant of *GNA11* or *AP2S1*. Although the phenotypes are similar, these are two other genetic causes of hypocalcemic hypercalciuria designated types 2 and 3. Hereditary hypocalcemic hypercalciuria type 2 (OMIM 145981) is caused by loss-of-function variants of *GNA11* (OMIM 139313), encoding a CaSR G-protein transmitting signal ($G\alpha_{11}$) to intracellular signal transduction pathways. Hereditary HHC type 3 (OMIM 600740) is the consequence of inactivating mutations in *AP2S1* (OMIM 602242) encoding a protein complex that interacts with β -arrestin (*ARRB1*, OMIM 107940, chr. 11q13.4) forming a structure enabling movement of the CaSR from the cell membrane and its return to the cytoplasm; as adults, patients with HHC3 may have hypophosphatemic osteomalacia. Autoantibodies against the amino terminal extracellular domain of the CaSR that reversibly inhibit receptor activity have been identified in patients with acquired HHC; this disorder may be responsive to glucocorticoid therapy.¹²⁷ In some patients with primary or uremic secondary hyperparathyroidism, there is reduced expression of *CASR* and an increased set point for suppression of PTH secretion.

Primary hyperparathyroidism is an unusual childhood disorder with an incidence of 2 to 5/100,000 as compared with that in adults of approximately 100/100,000.^{117,128,129} In adults with hyperparathyroidism, females outnumber males 3:1; in children the female/male ratio is closer to one. In older

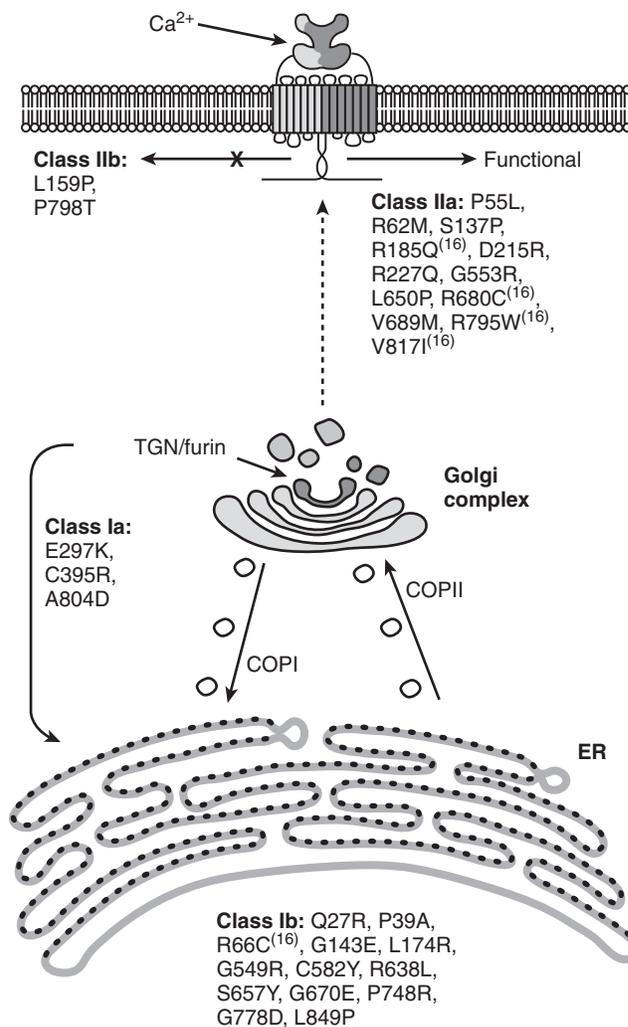


Fig. 20.6 Movement of the calcium sensing receptor (CaSR) through the endoplasmic reticulum (ER) and Golgi complex to the cell membrane before insertion. Inactivating mutations of CaSR may arrest movement of CaSR at various sites along this pathway, including immediately below the cell membrane. The mutations are designated classes Ia, Ib, and IIa, depending on the site of arrested movement as depicted. Class IIa mutants are able to be inserted into the cell membrane but remain subactive. (From White E, McKenna J, Cavanaugh A, Breitwieser GE. Pharmacochaperone-mediated rescue of calcium sensing receptor loss-of-function mutants. *Mol Endocrinol*. 2009;23:1115–1123, with permission.)

children and adolescents, primary hyperparathyroidism is most often a sporadic disease and usually the result of a single parathyroid adenoma. Hyperparathyroidism may occur as an autosomal dominant disorder in patients with familial isolated primary hyperparathyroidism involving variants of *CDC73* (OMIM 607391) and *GCM2* (OMIM 603716) (vide infra). Familial hyperparathyroidism also occurs in patients with variants of the genes responsible for the syndromes of multiple endocrine neoplasia (MEN) type 1 (*MEN1*, OMIM 613733), MEN types 2/3 (*RET*, OMIM 164761), and MEN type 4 (*CDKN1B*, OMIM 600778).^{1,79,116,130}

MEN types 1 and 4 are clinically similar in that both entities are associated with neoplastic lesions of the parathyroid glands, adenohypophysis (somatotropinoma, prolactinoma), and endocrine secreting cells of the intestinal (gastrinoma, insulinoma) or genitourinary tracts. MEN1 is the consequence of

heterozygous loss-of-function mutations in *MEN1* encoding a nuclear scaffold protein that interacts with multiple transcription factors and is a tumor suppressive agent. *MEN2A* is associated with loss-of-function variants of *RET* encoding a transmembrane receptor tyrosine kinase that is associated with parathyroid adenoma, medullary carcinoma of the thyroid, and pheochromocytoma; *RET* variants associated with *MEN2A* involve cysteine residues in its extracellular domain. *MEN2B* is associated with medullary carcinoma of the thyroid (MCT) that is highly aggressive, pheochromocytoma, thickening of corneal nerves, mucosal ganglioneuromas on the tongue, lips, and eyelids, and a marfanoid habitus; genetically, *MEN2B* is related to the *RET* variant p.Met918Thr in exon 16, the site of the receptor's tyrosine kinase domain. *MEN4* is associated with adenomas of the parathyroid gland, adenohypophysis, and gastrointestinal tract that is the consequence of monoallelic inactivating variants of *CDKN1B*, a cyclin-dependent kinase inhibitor that blocks the cell cycle at the G0/G1 phase. (Cyclins are cell proteins that regulate progression of the cell cycle of growth, division, and duplication by activating selected cell cycle-related kinases [phosphorylases].) Familial isolated hyperparathyroidism type 1 (OMIM 145000) and familial isolated hyperparathyroidism type 2 associated with fibrous tumors of the maxilla and mandible (OMIM 145001) are caused by loss-of-function mutations in *CDC73* (OMIM 607391) encoding a tumor suppressor. Familial isolated hyperparathyroidism type 3 (610071) has been mapped to chromosome 2p14-p13.3. *GCM2* (OMIM 603716) encodes a transcription factor essential for differentiation of the parathyroid glands. Gain-of-function variants of *GCM2* result in familial isolated hyperparathyroidism type 4 (OMIM 239200) with development of multiple parathyroid adenomas and occasionally carcinomas. *NSHPT* (OMIM 239200) is the consequence of inactivating mutations of *CASR* (OMIM 601199).

Children and adolescents with hypercalcemia caused by hyperparathyroidism may be asymptomatic or may display personality and behavioral changes—particularly depression, headache, malaise, proximal muscle weakness, anorexia, abdominal cramping, nausea and vomiting, constipation, polydipsia and polyuria or have symptoms reflecting the consequences of this disorder, such as flank pain and hematuria caused by renal calculi (hypercalciuria), abdominal pain (pancreatitis), or pathological fractures (through areas of osteopenia or lesions of osteitis fibrosa cystica).^{88,116} Rarely is it possible to palpate a cervical mass in these patients, although the physical examination may reveal slight weakness of proximal musculature. Hypercalcemia, hypophosphatemia, and elevated serum concentrations of intact PTH are present in the majority of children with hyperparathyroidism. In subjects with primary hyperparathyroidism, hypercalcemia is the result of increased secretion of PTH caused by loss of the normal relationship between the set point of serum Ca^{2+} and PTH synthesis and release and to Ca^{2+} -independent (constitutive) PTH secretion related to the mass of parathyroid tissue.^{117,128} Ultrasonography, magnetic resonance imaging, computed tomography (CT), and radionuclide scans (^{99m}technetium-labeled sestamibi-single photon emission CT scan) have been used to localize the abnormal parathyroid gland(s) before surgical excision. (Sestamibi is methoxyisobutylisonitrile, a lipophilic cation.) Occasionally, the parathyroid tumor may be located ectopically in the thymus, thyroid gland, or mediastinum. Further studies may reveal superiosteal bone resorption, nephrocalcinosis, or nephrolithiasis.

Pathologically, hyperparathyroidism in children is most often because of a chief cell adenoma involving one parathyroid gland, but adenomas in several parathyroid glands and diffuse hyperplasia (particularly in patients with MEN type 1), and rarely carcinoma of the chief cells may also occur. The majority

of parathyroid adenomas are monoclonal in origin; that is, a single mutant cell develops into a tumor. In adolescents, parathyroid tumors may occasionally develop after external radiation of the neck for treatment of lymphoma. In some parathyroid tumors, increased expression of cyclin D1 (encoded by *CCND1*, OMIM 168461, chr. 11q13.3) has been demonstrated. Overexpression of *CCND1* in parathyroid chief cells is at times the result of a somatic chromosome mutation—inversion (rotation) of regions 11p15 and 11q13 in which the promoter region of *PTH* is repositioned to serve as a promoter for *CCND1*, thereby increasing the rate of chief cell division whenever the (hypocalcemic) stimulus for PTH generation is received and leading ultimately to (benign) tumor formation. However, in many parathyroid adenomas, there is increased activity of cyclin D1 without this chromosomal rearrangement. In patients with parathyroid adenomas and carcinomas, overexpression of the retinoblastoma and p53 tumor-suppressor genes, whose products normally inhibit the cell cycle at the G1/S step, has also been demonstrated as have somatic mutations in *CDC73*.¹³¹ In approximately 35% of sporadic parathyroid adenomas, a somatic loss-of-function mutation of the tumor suppressor factor menin—the germline mutation in patients with MEN type 1—can be identified together with loss of heterozygosity on chromosome 11.¹³¹ Germline loss-of-function mutations in *MEN1* and *CASR* and gain-of-function variants in *GCM2* have also been detected in patients with isolated primary hyperparathyroidism in association with multi-glandular involvement; in this instance, patients with *CASR* mutations may not have the typical biochemical findings of HHC1 (see Table 20.4B). Chronic renal insufficiency leads to secondary hyperparathyroidism because of hyperplasia of the parathyroid glands and also to monoclonal parathyroid tumors (tertiary hyperparathyroidism) associated with somatic chromosomal deletions in some instances.

Familial isolated hyperparathyroidism type 1 (OMIM 145000) and type 2 (OMIM 145001—hyperparathyroidism associated with familial benign and malignant parathyroid tumors or multiple ossifying fibromas of the maxilla and mandible, respectively) are both caused by germline mutations in *CDC73* (OMIM 607393, chr. 1q31.2) encoding parafibromin, a protein intrinsic to the cell division cycle. Hyperparathyroidism occurs in 80% of subjects with a mutation in *CDC73* at a mean age of 32 years but may also appear in children before 10 years of age. In these patients, the parathyroid lesion may be an atypical, potentially premalignant cystic adenoma (65%), hyperplasia (20%), or even carcinoma (15%); the parathyroid tumor may be an isolated finding or it may be associated with maxillary and/or mandibular bone tumors composed of ossified fibrous tissue; renal (Wilms tumor, papillary renal cell carcinoma, hamartoma, polycystic kidney), pancreatic (carcinoma), and uterine (tumor) lesions may also develop in these patients. Lesions within the parathyroid glands may develop asynchronously. *CDC73* is a 17 exon gene that encodes parafibromin, a 531 aa nuclear protein that is a component of a complex of accessory factors that modulates the activity of RNA polymerase II and of a histone methyltransferase and hence regulates gene expression and cell proliferation—functioning as both a transcriptional activator and repressor.^{132,133} For a parathyroid tumor to develop, a “second hit” must occur that results in loss of heterozygosity: that is, the germline inactivating mutation of *CDC73* on one allele must be matched by a mutation in or deletion of *CDC73* in the remaining normal allele. When a germline mutation in *CDC73* has been detected, screening of family members for this mutation and longitudinal evaluation of affected subjects is recommended as asymptomatic individuals with atypical adenoma or carcinoma of the parathyroid glands may be so identified. Somatic mutations in *CDC73* have also been identified

in atypical parathyroid adenomas and many parathyroid carcinomas but are unusual in the typical sporadic parathyroid adenoma. Familial isolated hyperparathyroidism type 3 (OMIM 610071) has been linked to chromosome 2p14-13.3 but a specific gene variant in this site has not been identified. Hyperparathyroidism type 4 (OMIM 617343) is the result of variants of *GCM2* (OMIM 603716) whose product is essential for differentiation of the parathyroid glands.¹

The syndromes of MEN are familial autosomal dominant diseases of high penetrance associated with the development of tumors in two or more endocrine glands within a single individual.¹³⁰ There are four defined MEN syndromes—1, 2A, 2B, and 4 (Table 20.5). MEN1 is characterized by development of tumors of the parathyroid glands (adenoma), pituitary (prolactinoma, somatotropinoma), and enteropancreatic unit (gastrinoma, insulinoma, pancreatic polypeptide-oma, carcinoid). In addition, neoplasms of the adrenal cortex, neuroendocrine tumors of the bronchopulmonary tree and thymus, lipomas, angiofibromas, collagenomas, and meningiomas develop in affected subjects. The postmortem incidence of MEN1 is 0.25%; in hyperparathyroid subjects 1% to 18%; and in patients with gastrinoma 16% to 38%. Hyperparathyroidism (because of a solitary parathyroid adenoma or tumors within several parathyroid glands or hyperplasia of all four parathyroid glands) is the most common manifestation of MEN1 occurring in more than 90% to 95% of affected patients; it is the most frequent endocrinopathy in children with MEN1, at times developing before 10 years of age. Unlike other forms of hyperparathyroidism, equal numbers of males and females are affected in MEN1. Pituitary tumors secreting prolactin and/or GH often (30%–40% of MEN1 subjects) develop as do

gastrin- (Zollinger-Ellison syndrome), insulin, and glucagon-secreting tumors of the pancreatic islets and gastrointestinal system (30%–70% of patients); these neoplasms also occur in children and adolescents with MEN1. Hypercortisolemia in patients with MEN1 may be caused by excessive secretion of adrenocorticotropin by a pituitary adenoma or ectopically by a neoplasm or to a primary adrenal tumor. Thyroid neoplasms occur in 25% of patients with MEN1. Nonendocrine tumors, such as lipomas (34%), intestinal and bronchial carcinoids, and other intestinal neoplasms are reasonably common in subjects with MEN1. Indeed, the dermatological manifestations of MEN1, including angiofibroma (85%), collagenomas (70%) are extremely sensitive and specific indicators of this disease. Patients with MEN1 may also develop a schwannoma or a pheochromocytoma, the latter a tumor most often present in patients with MEN types 2A and 2B. Although most of the tumors that develop in MEN1 are benign but functionally hyperactive, those of pancreatic, intestinal, and foregut origin may be malignant.

Germline mutations in *MEN1*, a 10 exon gene that encodes a 610 aa cytoplasmic and nuclear protein termed *menin*, have been demonstrated in the majority of patients with familial and sporadic forms of MEN1. Menin regulates cell growth, division, and demise in two sites and through multiple pathways. In the cytoplasm, menin binds AKT1 (protein kinase B—OMIM 164730, chr. 14q32.33) preventing its translocation to the cell membrane where it is ordinarily activated by phosphorylation enabling AKT1 to exert proliferative and antiapoptotic effects; by binding of menin to AKT1, both the cytosolic level of AKT1 and its kinase activity are depressed, thereby suppressing its effects on cell division and survival.¹³⁴ Menin enters the

TABLE 20.5 Multiple Endocrine Neoplasia Syndromes

Subtype	Gene	Chromosome	Site of Frequent Tumors	Mutations
MEN 1	<i>MEN1</i>	11q13.1	Parathyroid (90%) Enteropancreatic (30%–70%)—gastrinoma, insulinoma, pancreatic polypeptide, nonfunctional Anterior pituitary (30%–40%)—prolactinoma, GH, ACTH, nonfunctional Adrenocortical (40%)—diffuse and nodular hyperplasia, adenoma, carcinoma Intestinal—gastric neuroendocrine (10%), Thymic neuroendocrine (2%) Other: facial angiofibroma (85%), collagenoma (70%), lipoma (30%), meningioma (8%)	Intron 4 N:5168 G⇒A (10%) Codons 83–84 (4%) Codons 119 (3%) Codons 209–211 (8%) Codon 418 (4%) Codon 516 (7%)
MEN 2 MEN 2A	<i>RET</i>	10q11.21	MCT (90%) Pheochromocytoma (50%) Parathyroid hyperplasia (20%–30%) Cutaneous lichen amyloidosis	Codon 634 (Cys⇒Arg - 85%)
MEN 2B ³			MCT (>90%) Pheochromocytoma (40%–50%) Associated: Marfanoid habitus Mucosal neuromas (90%) Medullated corneal nerves	Codon 918 (Met⇒Thr - >95%)
MEN 2C MEN 4	<i>CDKN1B</i>	12p13.1	Megacolon MCT (100%) Parathyroid Pituitary Testicular/Cervical Renal/Adrenal	Codon 618 (>50%)

ACTH, Adrenocorticotropin hormone; GH, growth hormone; MCT, medullary carcinoma of thyroid.

(From Thakker RV, Newey PJ, Walls GV, et al. Clinical Practice Guidelines for Multiple Endocrine Neoplasia type 1 (MEN1). *J Clin Endocrinol Metab*.

2012;97:2990–3011; Marx SJ, Simonds WF. Hereditary hormone excess: genes, molecular pathways, and syndromes. *Endocr Rev*. 2005;26:615–661.)

nucleus with the guidance of two nuclear localization signals in its carboxyl terminus (aa 479–498, 589–608) where it is involved directly in the regulation of transcription, replication, and survival. By binding directly to JunD, menin blocks JunD-mediated inhibition of transcription of activating protein-1 (and consequently cell division); many of the mutations in *MEN1* in patients with MEN1 cluster in exon 4 and interrupt the binding of these two proteins (between menin aa 139–142 and 323–428). By interacting with small mothers against decapentaplegic (SMAD)3 (OMIM 603109, chr. 15q22.3), menin inhibits signaling by transforming growth factor (TGF) β and impairs TGF β -mediated inhibitory control of cell replication. Interaction of menin with the SMAD 1/5 complex inhibits signaling by bone morphogenetic protein (BMP) 2; menin also inhibits the transcription regulating protein—nuclear factor κ B (NF- κ B). Further, menin interacts directly with a histone methyltransferase complex and with genes that regulate DNA repair and cell replication and apoptosis, such as tumor suppressor *TP53* (p53), *CDKN1A*, *CDKN1B*, *GADD45A*, *POLR2A*, *BBC3*, *TP5313*, and *FAS*.¹³⁵ Variants of *CDKN1A* (OMIM 116899), *CDKN2B* (600431), and *CDKN2C* (OMIM 603369), proteins that regulate the cell division cycle, have been associated with nonsyndromic primary hyperparathyroidism.¹

In subjects with heterozygous germline loss-of-function mutations in *MEN1*, unregulated cell growth and tumor formation occur when a second insult leads to loss of *MEN1* on the normal allele within susceptible tissues. As with *CDC73* (vide supra), the “two hit” hypothesis of tumorigenesis in *MEN1* denotes that the patient inherits germline susceptibility to neoplasia superimposed on which is a second insult, leading to loss of heterozygosity for chromosome segment 11q13 and biallelic loss of *MEN1*; the second hit may be deletion of a segment of chromosome 11 that includes 11q13 or a mutation (missense, frameshift) within the wild-type *MEN1* allele itself, an observation that also extends to MEN type 1 tumors with somatic mutations in *MEN1*.¹²¹ Several hundred germline mutations in *MEN1* have been identified in patients with MEN1; 25% have been nonsense mutations, 15% missense mutations, 45% frameshift insertions or deletions, with more than 80% leading to the synthesis of an inactive product because of loss of the nuclear localization signal or the ability to bind to JunD or other downstream factor. Especially susceptible germline mutational “hot-spots” in *MEN1* are the nucleotide 5168 G \Rightarrow A transition that results in a novel splice site in intron 4, codons 83 to 84, 118 to 119, 209 to 211, 418, and 516 where collectively mutations have been identified in 37% of patients with MEN1. On either side of many of these sites are segments of repeat DNA sequences of single nucleic acids or of dinucleotides to octanucleotides; this configuration may lead to increased susceptibility to “replication-slippage” because of misalignment of the nucleotide repeat segments during DNA replication permitting deletion or insertion of nucleotides at inappropriate sites. Mutations within the exonic/intronic splice site coding region of *MEN1* have been detected in approximately 70% of patients with familial MEN1; likely in the remaining 30% of subjects, there is either deletion of one *MEN1* allele or a mutation in one of its noncoding regions.¹³⁶ New mutations of *MEN1* occur sporadically in 10% of patients with MEN1. Familial autosomal dominant, isolated primary hyperparathyroidism has been variably associated with germline mutations (e.g., Val184Glu, Glu255Lys, Gln260Pro) in *MEN1* as well. Somatic mutations in *MEN1* have also been identified in patients with sporadic, isolated tumors of the parathyroid glands, pancreatic islet cells, anterior pituitary, and adrenal cortex. Clinically apparent disease because of mutations in *MEN1* increases with advancing age: at 10 years of age 7% of children with a mutation in *MEN1* have a detectable endocrinopathy (primarily hyperparathyroidism); 52% of

affected 20-year-old subjects manifest one or more tumors; penetrance increases to 87% by 30 years, to 98% by 40 years, and to 100% by 60 years.¹³⁰

There are several forms of MEN type 2—subtypes 2A, 2B, and familial medullary thyroid carcinoma (2C). MCT is the most common neoplasm encountered in MEN 2A occurring in 90% of patients (OMIM 171400) (see Table 20.5) (Thakker et al 2012¹³⁰; Arnold & Marx 2008¹³⁶). It evolves from antecedent parafollicular or C cell hyperplasia within the thyroid gland. These subjects also develop adrenal medullary pheochromocytomas (50%), parathyroid hyperplasia or adenoma (20%–30%), localized cutaneous lichen amyloidosis (suprascapular pruritic deposits of subepidermal keratin), and partial or complete megacolon. Calcitonin, the secretory product of thyroid C cells, is also produced in very large amounts by MCT; its measurement aids in the diagnosis of MCT and in monitoring the response of this tumor to therapy. In addition to MCT and pheochromocytoma, patients with MEN 2B (OMIM 162300) have a Marfanoid habitus, mucosal ganglioneuromas of the lips and tongue, gastrointestinal ganglioneuromas, medullated corneal nerve fibers, and megacolon, but they do not develop parathyroid disease.^{130,136} Familial isolated MCT is a variant of these disorders. Germline heterozygous gain-of-function mutations in the *RET* protooncogene underlie the pathogenesis of the type 2 hereditary MENs. *RET* is a 20-exon gene encoding an 860 aa glycosylated cell membrane tyrosine kinase receptor with extracellular, transmembrane, and intracellular domains that is expressed in tissue of neural crest origin (sympathetic ganglia, adrenal medulla, thyroid parafollicular cells) (Fig. 20.7). The natural ligand of this receptor is glial cell line–derived neurotrophic factor (*GDNF*, OMIM 600837). Constitutively activating mutations of one of six cysteine residues in the extracellular domain of *RET*—codons 609, 611, 618, 620, 630, and particularly codon 634 (Cys \Rightarrow Arg)—are present in patients with MEN 2A. Loss of

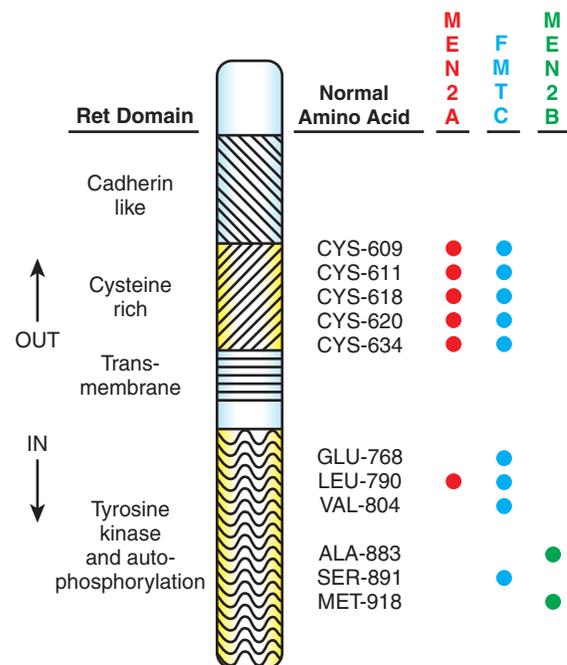


Fig. 20.7 Schematic depiction of the domains of the *RET* protooncogene with sites of activating mutations in *RET* associated with multiple endocrine neoplasia types 2A and 2B and familial medullary carcinoma of the thyroid. (From Marx SJ, Simonds WF. Hereditary hormone excess: genes, molecular pathways, and syndromes. *Endocr Rev.* 2005;26:615–661, with permission.)

but one cysteine residue facilitates receptor homodimerization without ligand binding, thereby activating the RET intracellular tyrosine kinase domain resulting in autophosphorylation of critical tyrosine residues (particularly at codons 1015 and 1062), and subsequent signal transduction. An activating mutation has been identified at codon 918 (Met \Rightarrow Thr) in more than 95% of patients with MEN 2B; this site lies within the tyrosine kinase domain and this mutation permits signal transduction and neural cell transformation and differentiation in the absence of both ligand binding and receptor homodimerization. The p.Met918Thr MEN IIB-associated *RET* mutation often arises de novo from sporadic mutations that occur in the germline of an older father, because this mutation confers a "selective advantage" upon the mutated spermatocyte.¹³⁷ Other missense mutations of *RET* associated with MEN 2B have been identified at codons p.Val804, p.Ala883, and p.Ser904. Missense mutations at *RET* codons 609, 611, 618, 620, and 634 within the extracellular domain and at codons 768, 790, 804, and 891 within the intracellular tyrosine kinase domain have been found in patients with familial MCT. In children, hyperparathyroidism may be an unusual manifestation of the McCune-Albright syndrome (OMIM 174800) because of a postzygotic activating mutation in *GNAS*. Hyperparathyroidism and nephrogenic diabetes insipidus have been noted in subjects with antenatal Bartter syndrome (OMIM 601678) associated with biallelic variants of *SLC12A1* (OMIM 600839), the gene encoding a sodium-potassium-chloride transporter protein.¹⁰⁹

Ingestion of excessive amounts of vitamin D or calcitriol for therapeutic reasons (treatment of rickets, hypoparathyroidism, or other causes of hypocalcemia), megavitamin intake, manufacturing errors in the preparation of vitamin supplements, or inappropriate fortification of milk are significant causes of vitamin D-mediated hypercalcemia.^{103,138} Topical application of creams containing vitamin D or an analogue (e.g., 22-oxacalcitriol) for treatment of psoriasis might also lead to hypercalcemia—particularly if the urinary excretion of calcium is compromised. Patients with granulomatous diseases (noninfectious: sarcoidosis, berylliosis, eosinophilic granuloma, subcutaneous fat necrosis, inflammatory bowel disease; infectious: tuberculosis, histoplasmosis, coccidioidomycosis, candidiasis, cat-scratch disease) and neoplastic disorders (B-cell lymphoma, Hodgkin disease, dysgerminoma) develop hypercalcemia because of associated monocytic (macrophage and other cells) expression of *CYP27B1* and resulting production of calcitriol.¹³⁹ Unlike renal tubular cells in which 25-hydroxyvitamin D-1 α hydroxylase activity is within mitochondria and the transcription of *CYP27B1* is closely regulated by PTH, calcitriol, calcium, and phosphate, in monocytes, this enzyme is microsomal in location and its gene is constitutively expressed and calcitriol synthesis quantitatively determined by the amount of substrate. The monocytic expression of *CYP27B1* is very sensitive to stimulation by IFN- γ and its postreceptor signal transducer nitric oxide, as well as by leukotriene C₄; it is readily suppressible by glucocorticoids, ketoconazole, and chloroquine. In patients with inactivating mutations of *CYP24A1*, calcitriol is unable to be further hydroxylated and excreted.

Patients with acquired immunodeficiency disease may become hypercalcemic by infection with granuloma-forming organisms or by osteoclast-activating cytokines elaborated during the course of this disorder. Elevated serum calcium concentrations have also been recorded in children with congenital hypothyroidism, primary oxalosis, congenital lactase deficiency, trisomy 21, and juvenile idiopathic (rheumatoid) arthritis.¹⁴⁰ In some hypercalcemic children, excessive prostaglandin production may be of pathogenetic significance. Hypercalcemia develops frequently in young subjects with the infantile form of hypophosphatasia, likely a consequence

of the dissociation of the rates of low bone formation and normal bone resorption. By the same pathogenetic mechanism, acute immobilization of the rapidly growing child with a femoral fracture or a spinal cord injury results in decreased bone mineral accretion and uncoupling of the interaction of osteoblasts and osteoclasts with an increased rate of bone resorption, leading to hypercalciuria, hypercalcemia, and "acute disuse osteoporosis."¹⁴¹ Acute disuse osteoporosis and hypercalcemia can even occur in the immobilized hypoparathyroid or vitamin D-depleted individual. Children and adolescents ingesting a ketogenic diet in an effort to manage refractory epilepsy may develop hypercalciuria and hypercalcemia and experience decline in bone mineralization because of dissociation between the rates of bone accrual and bone mineral reabsorption. Hypercalcemia may occur in children receiving excessive amounts of milk and alkali for treatment of gastritis. It may follow successful bone marrow transplantation in infants with osteopetrosis as functional osteoclasts rapidly reabsorb excess bone mineral.

Oncogenic or malignancy-associated hypercalcemia may be the consequence of synthesis and secretion of osteoclast activating agents, such as PTHrP, (rarely PTH), calcitriol, or cytokines (ILs, tumor necrosis factor [TNF], TGF β), or it may be caused by direct invasion and destruction of bone by the neoplasm.^{79,116} Although hypercalcemia occurs in less than 1% of children with cancer, it may develop in patients with acute lymphatic and monocytic leukemias, Hodgkin and non-Hodgkin lymphoma, rhabdomyosarcoma, hepatoblastoma, neuroblastoma, and Ewing sarcoma.¹⁴² When the rate of bone resorption exceeds the renal tubular capacity for excretion of calcium, hypercalcemia ensues. Increased intake of calcium and absorbable alkali (milk or calcium containing antacids, such as calcium carbonate) for peptic ulcer disease or as dietary supplements lead to absorptive hypercalcemia, hypercalciuria, and nephrocalcinosis. Parenteral nutrition with excessive calcium or aluminum or too little phosphate can also result in hypercalcemia. Hypophosphatemia of various etiologies leads to hypercalcemia as the body attempts to maintain the calcium \times phosphate product over 30. Drugs causing hypercalcemia include: thiazide diuretics increase renal tubular resorption of calcium and decrease plasma volume; vitamin D and analogs increase intestinal absorption of calcium; vitamin A and its retinoic acid analogs stimulate bone resorption; lithium increases the set point for PTH secretion—thereby increasing serum calcium concentrations while lowering urinary calcium excretion and thus mimicking HHC1. In the thyrotoxic subject, hypercalcemia is the result of thyroid hormone-mediated stimulation of osteoclast function and subsequent increase in the rate of bone resorption.¹¹⁶ Pheochromocytomas and some islet cell tumors may be associated with hypercalcemia, in some instances because of cosecretion of PTHrP. Hypercalcemia in the patient with hypoadrenocorticism is the consequence of continued mobilization of bone calcium, decreased renal glomerular filtration of this cation and its increased renal tubular reabsorption and is reversed by restoration of the eucorticoid state.

During recovery from acute renal failure, serum calcium levels may increase because of mobilization of calcium from ectopic sites, such as muscle in which it had been deposited during the hyperphosphatemic phase of the illness from which it is released by rhabdomyolysis.¹¹⁶ Hypercalcemia can develop in patients with chronic renal failure caused by a combination of factors including: immobilization, aluminum toxicity, excessive ingestion of calcium-containing antacids or vitamin D or its analogs, and secondary hyperparathyroidism. After renal transplantation, hypercalcemia is often the result of secondary hyperparathyroidism caused by hypertrophy and hyperplasia of parathyroid chief cells that occurred in response to the PTH stimulatory effects of hyperphosphatemia,

hypocalcemia, and decreased synthesis of and response to calcitriol during the period of chronic renal insufficiency. In patients with compromised renal function, mild hypocalcemia and calcitriol deficiency develop when the glomerular filtration rate falls below 80 to 60 mL/min/1.73 m², whereas phosphate retention occurs after the glomerular filtration rate has fallen to 60 to 30 mL/min/1.73 m².¹⁴³ The secretion of PTH rises secondarily in these patients in an effort to increase the synthesis of calcitriol, increase renal tubular reabsorption of calcium, raise calcium levels, decrease renal tubular reabsorption of phosphate, and lower phosphate values. Prolonged, uncontrolled secondary hyperparathyroidism can lead to relatively autonomous parathyroid hyperfunction ("tertiary hyperparathyroidism") and hypercalcemia, primarily in patients with chronic renal failure. Hyperplasia of chief cells is followed by defects in function of the CaSR and loss of effective downregulation of PTH secretion refractory to increased serum concentrations of Ca²⁺. There is an expanded number of monoclonal chief cells in which the expression of CaSR and the number of vitamin D nuclear receptors have declined. Secondary and tertiary hyperparathyroidism have occurred in patients with prolonged nutritional vitamin D deficiency rickets and in subjects with X-linked hypophosphatemic rickets receiving large amounts of phosphate.¹⁴⁴ Secondary hyperparathyroidism (in which by definition serum calcium concentrations are normal) also develops in patients with inadequate dietary calcium, impaired intestinal absorption of calcium (lactose intolerance, ingestion of phytates, malabsorption syndromes caused by pancreatic insufficiency or celiac disease), or excessive calcium loss in the urine or soft tissues. Enhanced but transient secretion of PTH may accompany the administration of GH to adolescents with chronic renal failure, likely the result of superimposing upon a high basal rate of PTH secretion further increase in the rate of bone remodeling related to somatotropin and sex hormones. In acutely ill adults, administration of GH has been associated with hypercalcemia as well.¹⁴¹

Isolated hypercalciuria in the eucalcemic child may be idiopathic or caused by renal medullary or tubular dysfunction or increased intestinal absorption of calcium, including mutations in genes encoding the vitamin D and CaSRs and soluble adenylyl cyclase, demineralizing disorders, such as juvenile idiopathic arthritis, hyperalimentation, metabolic acidosis, excessive protein ingestion, and diabetes mellitus; hypercalciuria with/without hypercalcemia may be observed in patients with familial forms of hypomagnesemia, several types of Bartter syndrome, and distal renal tubular acidosis.

Evaluation

Evaluation of the child with hypercalcemia requires systematic examination of each of its possible causes (see Fig. 20.5). Careful review of the personal and family histories and thorough physical examination precede measurement of serum levels of total and ionized calcium, phosphate, creatinine, intact PTH, and 25-hydroxyvitamin D and of urinary calcium and creatinine excretion; renal ultrasonography is used to detect nephrocalcinosis or renal calculi if hypercalciuria is present. If the serum concentration of PTH is not suppressed in a patient with hypercalcemia, the presence of hyperparathyroidism is to be further considered. If repetitive determinations are consistent with hyperparathyroidism, then its pathogenesis is to be identified. Imaging studies of the neck to be undertaken may include ultrasonography (a parathyroid adenoma is hypoechoic), technetium-99m sestamibi scanning with single-photon-emission CT, dynamic (4D) CT imaging (useful to detect multiple and ectopic parathyroid adenomas), and magnetic resonance imaging (thus avoiding radiation exposure).¹²⁹

When hypercalcemia is mild (total calcium concentration <12 mg/dL), there may be few, if any, symptoms; thus children/adolescents with mild hypercalcemia are often identified unexpectedly by a screening panel of blood chemistries obtained for another purpose. Hypercalcemia may also be detected during studies for renal calculi, abnormal bone mass, pathological fractures, or during screening of families for associated problems. An elevated serum (total or ionized) calcium concentration in a single specimen may also reflect assay variability and must be verified by repeated determinations in a reliable laboratory. Pseudohypercalcemia is the presence of persistently elevated total calcium concentrations whereas the Ca²⁺ is normal and is found in subjects with hyperalbuminemia and other dysproteinemic states. Symptoms attributable to hypercalcemia are independent of its cause and are related to the degree of hypercalcemia and include: intestinal— anorexia, nausea, vomiting, abdominal pain (peptic ulceration, acute pancreatitis), and constipation; urinary—polydipsia, nocturia, and polyuria (calcium acts as an osmotic diuretic whereas hypercalcemia impairs the concentrating function of the distal renal tubule); skeletal—bone pain; nervous system—headache, muscular weakness, impaired ability to concentrate, increased requirement for sleep, altered consciousness (ranging from lethargy and confusion to irritability, delirium, stupor, and coma); on occasion, depression may be the major presenting concern in an adolescent with hypercalcemia.^{116,119} In the toddler and young child, hypercalcemia is manifested by anorexia, constipation, poor weight gain, and impaired linear growth ("failure to thrive"). In a series of 52 children and adolescents with hypercalcemia caused by primary hyperparathyroidism, 80% were symptomatic; the most common symptoms were fatigue/lethargy (35%), depression (14%), headache (35%), nausea (29%), vomiting (23%), and polydipsia (21%).¹⁴⁵ Bone involvement (low bone mass, fractures) was present in 30%. All of the children (*n* = 17) with nephrolithiasis in this series were symptomatic. In another series of 44 children and adolescents (26 girls) with primary hyperparathyroidism, mean age at diagnosis was 13 years (range 6–18 years), overall 37 were symptomatic (anorexia, weight loss, malaise, depression) and there were 18 patients with nephrolithiasis.⁸⁸ At surgery, 29 patients had a parathyroid adenoma and 11 had hyperplastic parathyroid glands—two of whom had MEN. Several features of hyperparathyroidism in children and adolescents differ from those in adults, including heavier and larger adenomas and higher serum and urinary calcium levels but comparable serum levels of iPTH, phosphate, and alkaline phosphatase.

Evaluation of the hypercalcemic child begins with the historical review during which the family/patient is queried not only about symptoms related to hypercalcemia and its consequences (renal calculi) but also about possibly excessive intake of vitamin D, vitamin A and related compounds (such as retinoic acid for treatment of acne), calcium (perhaps to "prevent" osteoporosis), and alkali, or drugs that affect calcium metabolism (thiazide diuretics may "unmask" hyperparathyroidism by increasing renal tubular resorption of calcium thereby raising borderline calcium concentrations into the hypercalcemic range). The family history is explored for members with known disorders of calcium metabolism (HHC1, hyperparathyroidism, renal calculi) or familial neoplasms (galactorrhea as a sign of a prolactinoma, severe peptic ulcer disease as an indicator of a gastrinoma). Except in extreme instances when hypertension (if normally hydrated) or bradycardia, dehydration, decreased muscular strength, or altered consciousness may be present or in the Marfanoid subject with MEN IIB, physical examination of the hypercalcemic child and adolescent is usually normal. Rarely, is a paratracheal (parathyroid) mass palpable in the hyperparathyroid patient. (Subjects with hypercalcemia caused

TABLE 20.6 Laboratory Findings in Hypercalcemic Patients

Disorder	Calcium	Phosphate	Urine calcium	PTH	PTHrP	25OHD	1,25(OH) ₂ D
NSHPT	↑↑↑	↓	↓	↑↑	↓	N	↑
FHH/HHC	↑, ↑↑	↓	↓	↑	↓	N, ↓	↑
Primary hyperparathyroidism	↑, ↑↑	↓	↑	↑, ↑↑	↓	N	↑
Vitamin D intoxication	↑	↑	↑↑	↓	↓	↑↑	↑
WBS	↑	↑	↑	↓	↓	N	↑
Immobilization	↑, ↑↑	↑	↑↑	↓	↓	N	↓
Malignancy	↑↑	↓	↑	↓	↑↑	N	N, ↑, ↓
Granulomatous disease	↑	↑	↑↑	↓	↓	N	↑↑
Subcutaneous fat necrosis	↑	↑	↑	↓	↓	N	↑↑

↑, Increased; ↑↑, greatly increased; ↓, decreased; *FHH*, familial hypocalciuric hypercalcemia; *HHC*, hypocalciuric hypercalcemia; *N*, normal; *NSHPT*, neonatal severe hyperparathyroidism; *PTH*, parathyroid hormone; *PTHrP*, PTH-related peptide; *WBS*, Williams-Beuren syndrome.

(From Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. *Curr Opin Pediatr*. 2010;22:508–515; Benjamin RW, Moats-Staats BM, Calikoglu A, et al. Hypercalcemia in children. *Pediatr Endocrinol Rev*. 2008;5:778–784.)

by subcutaneous fat necrosis have firm to hard, irregular, movable masses scattered about the trunk and extremities. Those with WBS have a typical face whereas those with Jansen metaphyseal chondrodysplasia have characteristic skeletal deformities.)

After confirming the presence of total and ionized hypercalcemia, the urinary excretion of calcium is next measured (Table 20.6). If the PTH concentration is normal or elevated and the calcium excretion is low, it is most probable that the patient has HHC1; this diagnosis can be substantiated by the finding of asymptomatic HHC in one of the parents and further defined by identification of the inactivating mutation in *CASR*. If the patient is hypercalciuric, other causes of hypercalcemia should be sought. With highly sensitive and specific immunoassays for intact PTH¹⁻⁸⁴ in comparison with serum calcium values, separation of patients with hyperparathyroidism from those with other causes of hypercalcemia in whom PTH values are low or subnormal is usually possible. In the absence of secondary hyperparathyroidism (chronic renal insufficiency, malabsorption syndromes, ingestion of thiazide diuretics or lithium), consistently elevated PTH concentrations in the hypercalcemic, hypophosphatemic, hypercalciuric child or adolescent are consistent with primary hyperparathyroidism. Although the diagnosis of primary hyperparathyroidism is usually quite apparent in children and adolescents, there is an occasional patient in whom serum calcium and/or PTH values may not be elevated in a single specimen and in whom repeated measurements of serum and urine calcium and PTH values are necessary before this diagnosis can be established. In 52 children/adolescents with hyperparathyroidism, serum calcium values were normal in 10% and PTH levels in 15%; however, in all subjects, the PTH concentration was inappropriately increased relative to the calcium level.¹⁴⁵ Hypercalcemia, hypophosphatemia, and elevated PTH concentrations were recorded in all 44 children and adolescents (6–18 years of age at diagnosis) with primary hyperparathyroidism in a second series.⁸⁸ Osteitis fibrosa cystica, brown tumors (localized nonneoplastic areas of bone resorption composed of osteoclast-like multinuclear giant cells, fibroblast-like spindle shaped cells, and hemorrhagic infiltrates), and subperiosteal and endosteal bone resorption can be detected radiographically in most children with hyperparathyroidism, whereas cortical (distal radial) BMD is likely to be decreased in these subjects. Nonspecific findings in the hypercalcemic subject of diverse etiology include shortening of the QT interval by electrocardiography because the rate of cardiac repolarization is accelerated by increased calcium levels, bradycardia, and first-degree atrioventricular block, and nephrocalcinosis and renal calculi detected by ultrasonography. Serum concentrations of PTHrP should be measured when clinical and laboratory findings are consistent

with primary hyperparathyroidism, but PTH values are low and humoral hypercalcemia of malignancy is suspected. When PTH concentrations are low in the hypercalcemic patient, metabolites of vitamin D (calcidiol, calcitriol) should be measured and other causes of hypercalcemia sought.

Preoperatively, a parathyroid adenoma may be localized by high resolution ultrasonography, dynamic (4D) single photon-emission computed tomography (SPECT), radionuclide scanning with ^{99m}Tc-sestamibi coupled with SPECT, or magnetic resonance imaging.¹²⁹ The ^{99m}Tc-sestamibi radionuclide is taken up by both the thyroid and parathyroid glands but quickly “washed out” from the thyroid gland; thus two scans obtained 2 hours apart permit differentiation of parathyroid from thyroid tissue. ^{99m}Tc-sestamibi scans may be obtained by conventional two-dimensional or CT techniques, the latter offering a three-dimensional image; lateral views may better locate the enlarged parathyroid gland. Fusion of ^{99m}Tc-sestamibi SPECT and CT methodology has been successful in localizing ectopic parathyroid adenomas. Combining ^{99m}Tc-sestamibi scanning and ultrasonography affords a sensitivity approaching 90% in children with parathyroid adenomas. Rarely is it necessary to undertake selective venous catheterization with sampling of local PTH levels and/or arteriography to identify the site of a parathyroid adenoma. Evaluation for associated endocrine tumors is necessary, if the family history suggests the possibility of MEN 1 or MEN IIA or if there are clinical findings (galactorrhea, excessive growth, hypertension) that suggest the presence of a prolactinoma, somatotropinoma, or pheochromocytoma. Patients at risk for MEN may be screened by determining basal and stimulated serum concentrations of prolactin, GH/insulin-like growth factor 1 (IGF-1), gastrin, glucagon, pancreatic polypeptide, calcitonin, catecholamines, and other substances as warranted. Genetic screening of MEN candidate genes may also be worthwhile. Indeed, it is reasonable to consider screening children with hyperparathyroidism preoperatively for an associated pheochromocytoma or tumors of the maxilla and mandible and for mutations in *RET*, *MEN1*, and *CDC73*.

In the patient with hypercalcemia caused by ingestion of excessive amounts of vitamin D, serum concentrations of calcidiol are markedly elevated; in those receiving exogenous calcitriol or in hypercalcemic patients with granulomatous, chronic inflammatory and lymphomatous diseases serum levels of calcitriol are increased. Other disorders associated with hypercalcemia (see Table 20.4A) should be eliminated by appropriate historical findings and laboratory and genetic studies.

Management

Appropriate management of the patient with hypercalcemia depends on the severity and the cause of the high calcium

levels. It is important to distinguish the patient with pseudohypercalcemia (because of hyperproteinemia) from one with a pathological cause of hypercalcemia so that effective medical and surgical therapy may be initiated promptly. It is equally important to recognize the child/adolescent with HHC1 so that aggressive therapy is avoided. When the calcium concentration is under 12 mg/dL in the asymptomatic subject, treatment may be delayed until the cause of the hypercalcemia is understood; in the interim, it is reasonable to recommend that the patient increase fluid intake, avoid calcium and vitamin D supplements, and discontinue drugs associated with hypercalcemia if relevant. The child with HHC1 often has serum total calcium concentrations between 11 and 13 mg/dL but no clinical symptoms and requires no therapy under usual circumstances. In the absence of HHC1, if the total serum calcium concentration exceeds 12 mg/dL or if the child is symptomatic (anorexia, nausea, altered sensorium, polydipsia, polyuria, dehydration, muscular weakness, bradycardic with a prolonged QT interval), efforts to lower the serum calcium level are usually necessary because of the adverse effects of hypercalcemia on cardiac, central nervous, renal, and gastrointestinal function. In these children and adolescents, diagnostic studies as outlined earlier and treatment to lower the serum calcium concentration should begin simultaneously. The tools of therapy of hypercalcemia are usually used sequentially.

The therapy of severe hypercalcemia involves: (1) hydration—with 0.9% saline (twice maintenance volume over 24–48 hours)—thus restoring intravascular volume, diluting and decreasing serum Ca^{2+} levels, increasing glomerular filtration of Ca^{2+} , decreasing reabsorption of Ca^{2+} in the proximal and distal renal tubules, and promoting calciuresis; hydration alone usually lowers the total serum calcium concentration 1 to 3 mg/dL; (2) calciuresis—achieved by intravenous infusion of the loop diuretic furosemide (1–2 mg/kg slowly) initiated after restoration of extracellular fluid volume with saline further lowers calcium levels by inhibiting resorption of calcium (and sodium) by the TALH—loop diuretics should be restricted to patients who develop fluid overload while receiving saline as these agents may lead to dehydration and decline in the glomerular filtration rate; bumetanide is a similar but more potent loop diuretic whose use may be considered in the hypercalcemic patient; (thiazide diuretics are to be avoided as they increase renal tubular reabsorption of calcium and increase serum calcium concentrations and are clearly contraindicated in the management of hypercalcemia); (3) inhibition of bone resorption—if hypercalcemia does not respond to the earlier measures, a bisphosphonate that inhibits osteoclast function may be used: pamidronate (0.5–2 mg/kg up to 90 mg infused intravenously over 4–6 hours) and zoledronic acid (0.025–0.05 mg/kg up to 4 mg infused intravenously over 30–60 minutes) have proven useful in the acute management of hypercalcemia in children and adolescents.¹⁴⁶ The full hypocalcemic effects of bisphosphonates are established over several days after administration and last for days to weeks; after bisphosphonate infusion, acute systemic effects (fever, myalgia) are often observed and may be managed symptomatically. Salmon calcitonin lowers serum calcium concentrations rapidly but transiently by inhibiting osteoclast function and inducing calciuresis; it is administered subcutaneously (2–4 U/kg every 6–12 hours). Calcitonin and a bisphosphonate may be paired to lower serum calcium levels more rapidly.

In adults with hypercalcemia of malignancy, denosumab, a monoclonal antibody to the receptor activator of nuclear factor κB (RANK)-ligand that impedes osteoclast formation, function, and survival, has been used successfully and is an option in children with hypercalcemia, but experience with this agent in children is limited.^{147,148} The effect of denosumab on bone turnover is rapidly reversible; thus hypercalcemia may recur

after discontinuation of this agent. Although glucocorticoids do not lower calcium levels in patients with hyperparathyroidism or solid tumor malignancies, they are effective in the management of hypercalcemia because of excess vitamin D ingestion or calcitriol production by activated monocytes or hematological malignancies. Rarely, it may be necessary to dialyze (peritoneal or hemodialysis with low- or zero-calcium dialysate) the severely hypercalcemic patient resistant to conventional therapy. During acute treatment of hypercalcemia, Ca^{2+} concentrations may be assessed indirectly by monitoring the (shortened) QT interval by electrocardiography, but serial measurements of total calcium and Ca^{2+} levels are necessary to monitor the efficacy of therapy and to prevent hypocalcemia.

Although in older adults (>50 years) with asymptomatic primary hyperparathyroidism (without bone disease or renal stones), no immediate intervention may be advised at times, in younger adults, children, and adolescents with hyperparathyroidism, surgical intervention by an experienced and expert surgeon is recommended when the diagnosis is established.¹¹⁷ Whereas hyperparathyroidism in youth is most often (>60%) because of an adenoma, hyperplasia of the parathyroid glands is also common (~30%).^{88,145} Before surgical intervention in a child/adolescent with hyperparathyroidism, a parathyroid adenoma should be localized using one (or more) imaging techniques, such as echography with Doppler analysis, magnetic resonance imaging, high resolution and four-dimensional CT or ^{99m}technetium-sestamibi combined with CT (Fig. 20.8). Construction of a three-dimensional virtual model of the neck by pairing iodine contrast with thin-slice CT imaging with color contrast enables clear definition of the cervical vasculature and its normal and abnormal structures.¹⁴⁹ In adults and older adolescents, minimally invasive focused procedures to remove an anatomically identified parathyroid adenoma(s) may be employed using ^{99m}technetium-sestamibi administered 2 hours before surgery, with dissection directed by insertion of a hand held gamma probe, until the adenoma is located and removed. Video assistance is an alternative method for localization of a parathyroid adenoma. A complementary technique for assessing the completeness of the removal of the adenoma intraoperatively is to measure peripheral levels of PTH by rapid immunoassay before and 10 minutes after removal of the adenoma; a 50% decline in PTH levels indicates complete excision; if the PTH concentration does not decline following removal of suspected abnormal parathyroid tissue further exploration is undertaken and additional tissue removed as guided by the serum PTH level. Serum concentrations of PTH achieve normal values 15 to 30 minutes after removal of a single functioning parathyroid adenoma.¹¹⁷ These procedures are cost-effective because they decrease operative time and patient morbidity; many patients return home within hours after leaving the operating suite. These techniques have been applied to the surgical management of the child and infant with a parathyroid adenoma or hyperplasia of multiple parathyroid glands. If there is parathyroid hyperplasia, total parathyroidectomy is performed and autotransplantation of small fragments of one gland to a forearm pocket undertaken. Following removal of a parathyroid adenoma, many patients develop transient hypocalcemia that may be managed by the administration of supplemental oral calcium. When there is severe osteitis fibrosa cystica and marked demineralization, substantial hypocalcemia may occur as a result of the hungry bone syndrome and require more intense management. Other complications of surgery include (transient or permanent) vocal cord dysfunction and the need for further operations because of development of a second adenoma. If permanent hypoparathyroidism develops postoperatively, it is treated by the administration of calcitriol and supplemental calcium as needed. When hyperparathyroidism is caused by hyperplasia

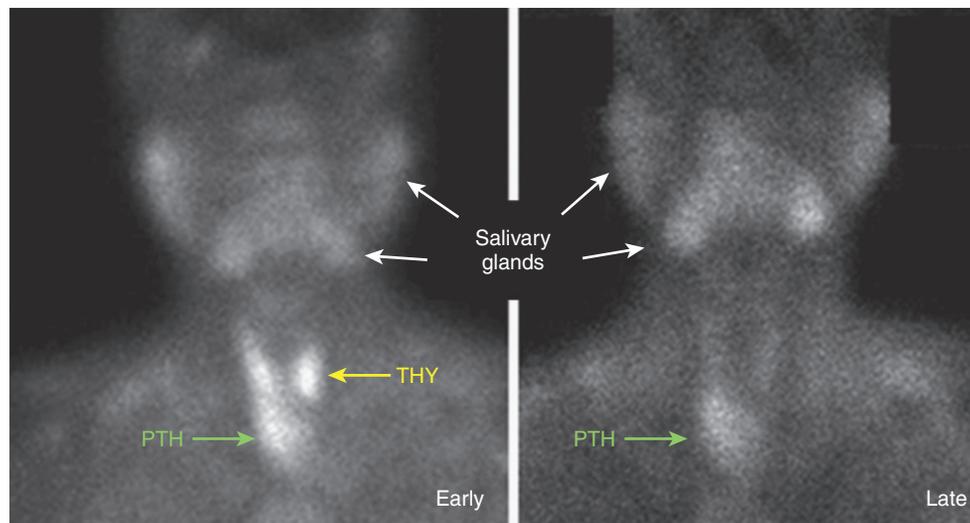


Fig. 20.8 Visualization of a parathyroid adenoma with technetium-99m sestamibi scanning. (From Steenkamp D, Lee SL. Hypercalcemic crisis in a young woman. *Endocrine Today*. 2011:October, with permission from SLACK Incorporated.)

of all parathyroid glands, near total parathyroidectomy is to be considered with implantation of minced parathyroid tissue into a forearm muscular pocket.

Patients with hypercalcemia caused by HHC are usually asymptomatic or only mildly symptomatic (fatigue, weakness) and usually do not require treatment. Neonates with severe neonatal hyperparathyroidism may necessitate urgent total parathyroidectomy if hypercalcemia cannot be controlled by other means (hydration, calciuresis with furosemide, administration of a bisphosphonate or perhaps denosumab). Calcimimetic agents (phenylalkylamines; e.g., cinacalcet) bind to and activate the CaSR on the membrane of the parathyroid chief cell, thereby increasing cytosolic levels of Ca^{2+} and depressing secretion of PTH in adults with mild primary hyperparathyroidism.¹¹⁷ When indicated in a symptomatic older subject with HHC, administration of a calcimimetic agent (cinacalcet) may be considered, although these drugs are approved for administration only to subjects older than 18 years of age.¹²⁴ The use of these agents in children and adolescents with this disorder offers a potential treatment pathway for subjects with diffuse parathyroid hyperplasia. Indeed, this agent has been used successfully in the management of infants with NSHPT.¹⁵⁰ However, in 2013 the FDA suspended the use of cinacalcet in children and adolescents pending further evaluation of its safety in this population. The secondary hyperparathyroidism of chronic renal disease is best managed by lowering serum phosphate concentrations to the extent possible by limiting intake and by administration of oral phosphate binding agents and by maintaining serum Ca^{2+} levels within the low-normal range by the administration of calcitriol or analogue. Parathyroidectomy may be necessary for effective management of refractory secondary and tertiary hyperparathyroidism as manifested by severe renal osteodystrophy, hypercalcemia, and systemic symptoms, such as pruritus and bone pain.¹⁴⁴

Hypercalcemia caused by hypervitaminosis D or excessive production of calcitriol by granulomatous and chronic inflammatory tissues may be treated with glucocorticoids to suppress activity of 25OHD₃-1 α -hydroxylase. Ketoconazole (3–9 mg/kg/d in 3 divided doses) is an antifungal agent that also inhibits renal 25OHD₃-1 α -hydroxylase activity and promptly lowers calcitriol and calcium values in children and adults with such disorders. Side effects of therapy with ketoconazole include nausea, vomiting, abdominal pain, depressed secretion of gonadal steroids, and impaired adrenal

production of cortisol. Therefore careful monitoring of patients receiving ketoconazole is essential. Glucocorticoids ameliorate the hypercalcemia related to excessive IL-1 β production in adolescents with juvenile rheumatoid/idiopathic arthritis. An attempt should be made to prevent hypercalcemia in the immobilized child or adolescent by provision of a low-calcium diet, avoidance of vitamin D, copious fluid intake, and early mobilization. Serum and urine calcium levels should be monitored frequently, and fluids increased still further if hypercalciuria occurs. Once present, hypercalcemia is best treated by mobilization; saline diuresis and/or bisphosphonate administration may be necessary until eucalcemia is restored. Restriction of dietary calcium and limitation of exposure to sunlight may be appropriate in the long-term management of some patients with hypercalcemia not amenable to more specific treatment. Antiprostaglandin agents may be useful in the child with hypercalcemia associated with excessive production of these compounds. Specific treatment of diseases accompanied by hypercalcemia (thyrotoxicosis, hypoadrenocorticism) restores the eucalcemic state.

DISORDERS OF MAGNESIUM METABOLISM

Magnesium (Mg) is a cation essential for control of neuromuscular function and is a required element for enzymatic reactions involving ATP, as well as maintenance of the structures of DNA and RNA. Magnesium regulates the secretion but not the synthesis of PTH and the generation of calcitriol. In serum, magnesium is both complexed to proteins and ionized or free. Approximately 50% of body magnesium stores are deposited within bone adsorbed to the surface of hydroxyapatite. The CaSR recognizes and responds to Mg^{2+} as well as to Ca^{2+} . Serum concentrations of magnesium are maintained within narrowly defined limits (1.6–2.4 mg/dL = 0.66–0.99 mmol/L) by coordinated intestinal absorption and renal tubular reabsorption of this cation. In the proximal renal tubule and thick ascending loop of Henle magnesium reabsorption is primarily passive through paracellular tight junction channels termed claudins. In the intestinal tract and renal distal convoluted tubule, magnesium is actively absorbed/reabsorbed transcellularly through a channel composed of a heterooligomer of TRPM6 (OMIM 607009) linked to TRPM7 (OMIM 605692), a magnesium-permeable cation channel. The paired TRPM6/TRPM7 heterooligomer is expressed on the surface of cells

lining the intestinal tract and the renal distal convoluted tubule enabling transcellular absorption/reabsorption of Mg^{2+} . In the kidney, 10% to 20% of filtered magnesium is reabsorbed in the proximal renal tubule and 65% to 70% is reabsorbed in the TALH by a passive paracellular process; 10% to 20% of filtered magnesium is reabsorbed in the distal convoluted tubule by an active transcellular system.^{151,152}

Hypomagnesemia

Hypomagnesemia (serum total magnesium concentration <1.5 mg/dL = 0.62 mmol/L) may be clinically silent or it may be manifested by fatigue, weakness, paresthesias, heightened neuromuscular irritability (carpal pedal spasm, spasticity, tremors, tetany, seizures) and when prolonged and profound by muscle wasting, weakness, apathy, and an altered state of consciousness; Chvostek and Trousseau signs may be elicited; prolonged PR and QT intervals may be present on an electrocardiogram.¹⁵³ Hypomagnesemia may lead to hypocalcemia by inhibiting the release of PTH and by interfering with its peripheral action. Hypomagnesemia may be the consequence of decreased ingestion or administration, impaired intestinal absorption, or increased loss of magnesium in the stool or urine that may be acquired or congenital (Tables 20.7A, 20.7B). Infants born to mothers deficient in this cation, those with preeclampsia or gestational or type 1 diabetes mellitus and in neonates with LBW—prematurity or intrauterine growth restriction are prone to develop hypomagnesemia.¹⁵⁴ Prolonged nasogastric suctioning, malabsorptive disorders caused by extensive intestinal resection (“short gut” syndrome), intestinal fistulas, or other diseases associated with chronic diarrhea and steatorrhea also lead to infantile hypomagnesemia. Diuretics (furosemide, thiazide) that depress magnesium absorption in the renal loop of Henle, inhibitors of the proton pump (omeprazole), calcineurin inhibitors (cyclosporin A, tacrolimus), platinum derivatives (cisplatin, carboplatin), antibiotics (aminoglycosides, rapamycin), or adrenergic agents that shift magnesium into cells may result in hypomagnesemia as may nephrotoxic agents (e.g., mercury, gentamycin); hypermagnesuria leads to hypomagnesemia.¹⁵³ In infants, children, and adolescents, hypomagnesemia may be primary and caused by a specific defect in the intestinal absorption of Mg^{2+} or in the renal tubular resorption of filtered Mg^{2+} (vide infra) or secondary and caused by gastrointestinal losses (chronic vomiting or diarrhea or malabsorptive states caused by inflammatory bowel disease, bowel resection or fistulas, or pancreatitis); to associated renal tubulopathies (Gitelman and Bartter

syndromes); exposure to alcohol, diuretics, and chemotherapeutic agents; and specific endocrinopathies (e.g., diabetes mellitus, primary hyperparathyroidism, hyperaldosteronism).⁶⁰

Hypomagnesemia may be associated with hypocalcemia and hypercalciuria as in some patients with activating variants of CASR resulting in autosomal dominant hypoparathyroidism (OMIM 601198). Hypomagnesemia occurs in patients with renal tubular disorders, such as Gitelman and Bartter syndromes (vide infra) and as a result of genetic errors in pathways that adversely affect the intestinal absorption or renal tubular reabsorption of magnesium (see Table 20.7B).^{151–153,155} Hypomagnesemia type 1 with secondary hypocalcemia is the consequence of biallelic inactivating mutations of *TRPM6* encoding the ion channel that forms a heterooligomer with TRPM7, a Mg^{2+} - and Ca^{2+} -permeable ion transport channel present in the intestinal tract and renal tubule. Neonates and infants with pathological variants of *TRPM6* have increased neuromuscular irritability (tremors, spasms, and seizures) within the first several months after birth.¹⁵⁶ Pathophysiologically, this disorder is caused by a selective small intestinal defect in transcellular absorption of Mg^{2+} (e.g., the variant p.Ser141Leu impairs linkage of TRPM6 and TRPM7 thus preventing intestinal absorption of Mg^{2+}). Affected newborns present with hypomagnesemic/hypocalcemic tetany and/or seizures leading to myocardial, renal, and arterial calcinosis. Renal excretion of magnesium is normal in subjects with this disease. Hypocalcemia is attributable to decreased secretion of and peripheral insensitivity to PTH. Oral ingestion of large quantities of magnesium is effective therapy for this illness. Monoallelic inactivating mutations in *FXYD2* (OMIM 601814) encoding a gamma subunit of renal Na^+/K^+ -ATPase result in autosomal dominant hypomagnesemia type 2 with hypocalciuria. *FXYD2* encodes a gamma subunit of a Na^+/K^+ -ATPase expressed in the renal distal convoluted tubule. Monoallelic loss-of-function mutations of *FXYD2* result in misrouting of its protein product to the basolateral membrane of the nephron’s epithelial cells, leading to increased urinary loss of magnesium but paradoxically to increased renal tubular reabsorption of Ca^{2+} in the loop of Henle resulting in hypocalciuria.¹⁵⁷ Inactivation of the regulatory transcription factors of *FXYD2* encoded by *HNF1B* (OMIM 189907) and pterin-4- α -carbinolamine dehydratase 1 (*PCBD1*, OMIM 126090) also result in hypomagnesemia. *HNF1B* encodes hepatocyte nuclear factor 1 homeobox B, a transcription factor necessary for normal embryogenesis of the kidney and pancreas.¹⁵⁸ Inactivating mutations in *HNF1B* lead to malformations of the kidney (cysts, renal malformations), hypomagnesemia, and maturity-onset diabetes of the

TABLE 20.7A Disorders of Magnesium Homeostasis

I Hypomagnesemia

- A Congenital
 - 1 Neonates of mothers with diabetes mellitus, eclampsia, magnesium deficiency
 - 2 Intrauterine growth restriction, prematurity
 - 3 Gene mutations—see Table 20.7B
- B Acquired
 - 1 Drugs—loop and thiazide diuretics, antibiotics (amphotericin, gentamycin), sympathomimetic agents, proton-pump inhibitors (omeprazole), digitalis, antineoplastic agents (cisplatin), cyclosporin
 - 2 Nasogastric suction
 - 3 Intestinal malabsorption (celiac disease, intestinal resection)
 - 4 Secretory diarrhea (inflammatory bowel disease)
 - 5 Acute pancreatitis
 - 6 Hypermagnesuria because of diabetes mellitus or alcoholism or following renal tubular necrosis or renal transplantation
 - 7 In association with hypokalemia

II Hypermagnesemia

- A Acquired
 - 1 Renal insufficiency
 - 2 Excessive intake of magnesium salts or laxatives/antacids that contain magnesium
 - 3 Diabetic ketoacidosis, adrenocortical insufficiency, hyperparathyroidism, lithium intoxication

TABLE 20.7B Gene Mutations Associated With Hypomagnesemia

Gene Protein Chromosome OMIM	Disorder OMIM	Gene Function/Transmission
<i>TRPM6</i> Transient receptor potential, cation channel, subfamily M, member 6 9q21.3 607009	Hypomagnesemia 1, intestinal 602014	TRPM6 forms heterooligomer with TRPM7 (chr. 15q21.2, OMIM 605692), a magnesium-permeable cation channel, AR
<i>FXYD2</i> FXD domain-containing ion transport regulator 2 11q23.3 601814	Hypomagnesemia 2, renal 154020	Gamma subunit of renal Na ⁺ -K ⁺ -ATPase, hypermagnesuria & hypocalciuria because of enhanced renal tubular reabsorption of calcium, FXD2 transactivated by HNF1B/PCBD1, AD
<i>CLDN16</i> Claudin 16 3q28 603959	Hypomagnesemia 3, renal 248250	Paracellular magnesium transport channel, hypermagnesuria, hypercalciuria, nephrocalcinosis, amelogenesis imperfecta, AR
<i>EGF</i> Epidermal growth factor 4q25 131530	Hypomagnesemia 4, renal 611718	Activator of TRPM6, Hypermagnesuria, Normocalciuria (variants of EGFR also result in hypomagnesemia)
<i>CLDN19</i> Claudin 19 1p34.2 610036	Hypomagnesemia 5, renal 248190	Paracellular magnesium transport channel, hypermagnesuria, hypercalciuria, nephrocalcinosis, macular colobomata, AR
<i>CNNM2</i> Cyclin M2 10q24.32 607803	Hypomagnesemia 6, renal 613882	Regulator of TRPM7 & its formation of a heterooligomer with TRPM6, AD
<i>HNF1B</i> HNF1 homeobox B 17q12 189907	Hypomagnesemia, congenital tubulointerstitial kidney disease, maturity-onset diabetes of the young, type 5	Transcription factor regulating expression of FXD2 & renal distal tubular reabsorption of magnesium, AD
<i>PCBD1</i> Pterin-4-alpha-carbinolamine dehydratase 1 10q22.1 126090	Hypomagnesemia, maturity-onset diabetes of the young, type 5	Nuclear dimerization cofactor for HNF1B to enhance expression of FXD2-mediated renal distal tubular reabsorption of magnesium, AR
<i>KCNA1</i> Potassium channel, voltage-gated, shaker- related subfamily, member 1 12p13.32 178260	Hypomagnesemia	Voltage-gated potassium channel colocalizes with TRPM6, inactivating variant results in hypermagnesuria by exerting dominant- negative effect on wild-type KCNA1, AD
<i>CLCNKB</i> Chloride channel, kidney, B 1p36.13 602023	Bartter syndrome (type 3) 241200 Bartter syndrome (type 4b), digenic, 613090	Renal tubule chloride channel, AR
<i>SLC12A3</i> Solute carrier family 12 (sodium/chloride transporter), member 3 16q13 600968	Gitelman syndrome—hypokalemic metabolic acidosis, hypocalciuria, hypomagnesemia, 263800	Renal distal convoluted tubular sodium/ chloride cotransporter, AR

AD, Autosomal dominant; AR, autosomal recessive; ATPase; adenosine triphosphatase.

young.¹⁵⁹ *PCBD1* encodes a protein that binds to HNF1B and increases its transcriptional capacity.

Loss-of-function mutations in the genes encoding the claudin-16 and -19 paracellular tight junction transport channels (*CLDN16*, OMIM 603959; *CLDN19*, OMIM 610036) result in renal hypomagnesemia type 3 and type 5, respectively. These diseases often present in infancy and are associated with tetany, hypermagnesuria, hypercalciuria, mild hypocalcemia, nephrocalcinosis, impaired renal function, and secondary hyperparathyroidism. Claudin-16 (also termed *paracellin 1*) is a 305 aa protein with four transmembrane domains and intracellular amino and carboxyl terminals that is expressed within the intercellular tight junctions of renal epithelial cells in the TALH and distal convoluted tubule where it facilitates

paracellular transport and reabsorption of Mg²⁺ and Ca²⁺ from the renal tubule. The first extracellular loop of claudin-16 bridges the intercellular space and is the site of paracellular conductance of ions; a number of missense mutations in *CLDN16* have been identified—particularly at position leucine 151 (p.Leu151Phe, Trp, Pro). Most of the variants of *CLDN16* impair its normal movement to the renal epithelial cell's lateral surface; in other mutations (p.Ala62Val, p.His71Asp), products localize to the tight junctions but are functionally defective.¹⁶⁰ Oral administration of 20 times the normal daily requirement of magnesium has been successful therapy in these subjects. *CLDN19* encodes claudin-19, a 224 aa protein expressed in the interstices between cells of the renal TALH and distal convoluted tubules, biallelic inactivating variants of which give rise

to hypomagnesemia type 5. *CLDN19* is necessary for paracellular transport of both calcium and magnesium.¹⁶¹ *CLDN19* is also expressed in the eye; in addition to ocular anomalies (colobomata, nystagmus, severe myopia), mutations in *CLDN19* result in hypomagnesemia, hypercalciuria, nephrocalcinosis, and renal failure. Biallelic inactivating mutations in *EGF* (OMIM 131530), the gene encoding epidermal growth factor, result in renal hypomagnesemia type 4, a disorder associated with seizures and developmental delay but in which serum and urine levels of calcium are normal.¹⁶² Epidermal growth factor (EGF) is a necessary cofactor for normal renal distal convoluted tubular resorption of Mg^{2+} by TRPM6. An inactivating variant of the EGF receptor (*EGFR*, OMIM 131550) has also been associated with hypomagnesemia, as well as neonatal inflammatory skin and bowel disease (OMIM 616069).¹⁵³ Monoallelic inactivating mutations of *CNNM2* encoding cyclin M2 (OMIM 607803), a protein that is critical for formation of the TRPM6-TRPM7 heterooligomer, result in hypomagnesemia type 6.¹⁶³ Despite hypomagnesemia, urine excretion of magnesium is inappropriately normal; serum and urine calcium values are normal in these subjects. *CNNM2* encodes a protein localized to the basolateral membrane of the cells comprising the renal thick ascending loop of Henle and the distal convoluted tubule where its expression is increased by magnesium deficiency. One function of *CNNM2* may be to “sense” ambient Mg^{2+} concentrations. Hypomagnesemia associated with myokymia (quivering of muscles) has been related to a heterozygous loss-of-function mutation (p.Asn255Asp) in *KCNA1* (OMIM 178260) encoding a renal potassium channel that colocalizes with TRPM6 in the renal distal convoluted tubule and is also critical for Mg^{2+} transport.^{157,164,165}

Hypomagnesemia is encountered in patients with hypokalemic metabolic acidosis in association with the Sesame syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) caused by inactivation mutations in *KCNJ10* (OMIM 602208—encoding a potassium channel), and antenatal Bartter syndrome type 2 (polyhydramnios, prematurity, poor postnatal growth, salt wasting, and hypercalciuria caused by inactivating mutations in a gene encoding a second potassium channel—*KCNJ1* (OMIM 600359). Bartter syndrome type 1 (OMIM 601678) is an autosomal recessive disorder manifested by salt wasting because of impaired sodium and potassium reabsorption in the renal ascending loop of Henle, hypokalemic metabolic alkalosis, hypercalciuria, and at times hypermagnesuria because of variants of *SLC12A1* (OMIM 600839). (Inactivating biallelic variants in *CLCNKB* [OMIM 602023] encoding a chloride channel [OMIM 602023] are present in patients with the classic form of this illness—Bartter syndrome type 3 [OMIM 607364].) Bartter syndrome type 4a is associated with variants of *BSND* (OMIM 606412) encoding barttin, a beta subunit for the chloride transport channels encoded by *CLCNKA* (OMIM 602024) and *CLCNKB* (OMIM 602023). In children and adolescents, the autosomal recessive Gitelman syndrome (OMIM 263800) of systemic alkalosis, hypokalemia, hypomagnesemia, and hypercalciuria is the result of variants of genes that compose the thiazide-sensitive sodium-chloride symporter in the distal renal convoluted tubule—often *SLC12A3* (OMIM 600968). Hypomagnesemia has also been associated with mitochondrial disorders including infantile histiocytoid cardiomyopathy (OMIM 50000), the Kearns-Sayre syndrome (OMIM 530000), and the syndrome of hyperuricemia—pulmonary hypertension—renal failure—alkalosis (OMIM 613845).¹⁵³

The presence of hypomagnesemia is identified by measurement of serum magnesium concentrations, whereas its pathophysiological etiology is determined by concurrent assay of calcium, phosphate, sodium, potassium, chloride, bicarbonate, creatinine, PTH, and vitamin D levels and assessment of its urinary loss and intestinal absorption.¹⁵⁴ In the concomitant presence of

hypomagnesemia and hypercalciuria, variants of *CLDN16*, *CLDN19*, *CLCNKB*, and *CASR* may be considered.¹⁵³ In hypomagnesemic patients without hypercalciuria, variants of *TRPM6*, *CNNM2*, *EGF/EGFR*, *KCNA1*, and *FAM111A* may be sought.

Hypomagnesemic, hypocalcemic seizures are only transiently responsive and sometimes resistant to parenteral administration of elemental calcium alone. Intravenous or intramuscular administration of a 50% solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$ —0.05–0.1 mL/kg or 2.5–5.0 mg/kg elemental magnesium with cardiac monitoring) may be necessary to control convulsions in the hypomagnesemic neonate.^{60,154} In older subjects, symptomatic hypomagnesemia is managed by the cautious intravenous administration of 20% magnesium sulfate (800 mmol/L) (30 mL in 500 mL of normal saline or 5% dextrose) with close clinical and electrocardiographic monitoring and frequent measurements of serum concentrations of Ca^{2+} and magnesium (Di Maio et al 2018⁷³). Oral magnesium supplements may also be helpful (50% $MgSO_4 \cdot 7H_2O$ —0.2 mL/kg/d). In adult patients with chronic hypomagnesemia, oral preparations of magnesium chloride or glycerophosphate (120 mg thrice daily) may be considered as tolerated; excessively large doses of magnesium may lead to diarrhea and should be avoided.

Hypermagnesemia

Hypermagnesemia (magnesium >2.6 mg/dL = >1.05 mmol/L) is recorded in the neonatal period after magnesium sulfate has been administered to the pregnant women with hypertension, preeclampsia, or toxemia of pregnancy. Most neonates with hypermagnesemia are asymptomatic; however, when serum magnesium concentrations are exceptionally high, hypotonia and depression of the central nervous system may be present and when extensive, metabolic bone disease may develop.¹⁵⁴ Thus prolonged (9–10 weeks) administration of intravenous magnesium sulfate to women who have entered labor prematurely has been associated not only with hypermagnesemia but also with marked hypocalcemia and significant osteopenia in the offspring.¹⁶⁶ Hypermagnesemia may also result from its parenteral administration or oral ingestion of magnesium-containing antacids or enemas. Hypermagnesemia may also develop in patients with renal insufficiency receiving magnesium-containing antacids. Serum magnesium concentrations are modestly increased in patients with familial HHC because of loss-of-function mutations in *CASR*. In large amounts, magnesium sulfate suppresses secretion of PTH and decreases renal tubular reabsorption of calcium, factors contributing to hypocalcemia. Clinical manifestations of severe hypermagnesemia (magnesium >4.8 mg/dL = >2 mmol/L) are similar to those of hypercalcemia and include altered mentation, nausea, vomiting, fatigue, and weakness; pulse rate and blood pressure may be low; hyporeflexia may be present. Both hypo- and hypermagnesemia impair the secretion of PTH leading to hypocalcemia. Variants of claudin-10 (*CLDN10*, OMIM 617579) lead to enhanced paracellular transport of calcium and magnesium and hypermagnesemia identified in subjects with the HELIX syndrome (hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, xerostomia—OMIM 617671).¹⁶⁷ The hypermagnesemic neonate and child are most appropriately managed by withdrawal of exogenous magnesium, saline diuresis, or administration of furosemide if necessary; if the patient is also hypocalcemic and osteopenic administration of calcium and calcitriol is indicated.

DISORDERS OF BONE

Congenital and acquired abnormalities of formation of osteoid, the organic portion of bone matrix composed of fibers of collagen type 1, and/or of orderly deposition of hydroxyapatite

crystals into bone matrix result in compromise of bone strength, resulting in skeletal malformations and deformations and increased fracture risk.

Disorders of Bone Formation and Mineralization in the Neonate and Infant

Low Bone Mass

In the fetus, the formation of the organic portion of bone and its ensuing mineralization proceed sequentially, processes controlled by GH; PTH; PTHrP; vitamins A, D, and C; and various cytokines.¹⁶⁸ Approximately 80% of total bone calcium in the full-term neonate is accrued in the last trimester of pregnancy as the in utero rate of calcium deposition increases more than twofold between 28 and 36 weeks' gestation (calcium accrual in the third trimester approximates 100–130 mg/kg/d). In prematurely delivered neonates, gestational age, birth weight, and rate of postnatal weight gain are important determinants of the postpartum rate of bone mass accrual.¹⁶⁹ Thus prematurely delivered, very LBW (VLBW <1500 g), and extremely LBW (ELBW <1000 g) neonates are particularly vulnerable to the development of metabolic bone disease associated with decreased bone mass (defined as postnatal bone mineral content [BMC] that is less than the BMC of the intrauterine fetus at the same postconceptual age with or without roentgenographic evidence of skeletal hypomineralization). After delivery, extremely preterm neonates are unable to maintain the rapid in utero rates of skeletal growth that require synthesis of organic bone matrix and calcium and phosphate deposition into osteoid from the amount of nutrients that can be provided by oral ingestion and gastrointestinal absorption or by parenteral nutrition.^{168,170,171} Postpartum hypocalcemia and decrease in spontaneous movement against the force exerted by the muscular wall of the uterus also depress the rate of bone mineralization, whereas an increased rate of bone resorption further impairs accrual of skeletal mass in premature infants.^{172,173} Malrotation of the intestinal tract or catastrophic necrotizing enterocolitis, leading to intestinal infarction requiring extensive small bowel resection, substantially increases the risk of malabsorption and consequent low bone mass. Parenteral alimentation of VLBW or ELBW neonates restricts administration of fluid, protein, calcium, and phosphate; excessive aluminum in parenteral fluids also adversely affects bone formation. Increased maternal parity, male gender, severe systemic disease (bronchopulmonary dysplasia), immobility, and pharmacological agents (glucocorticoids, methylxanthines, such as theophylline, diuretics, such as furosemide) also adversely impact bone formation in these neonates.^{173,174} Theophylline and furosemide increase urinary excretion of calcium. Prenatal factors that contribute to low bone mass in VLBW and LBW neonates are: intrauterine growth restriction—possibly by reducing in utero placental transport of calcium and decreasing the rate of bone formation; prenatal exposure to large amounts of magnesium sulfate administered repeatedly to the mother in preterm labor leads to hypocalcemia and osteopenia by suppressing PTH secretion through its interaction with the CaSR and by competing with calcium for deposition at bone surfaces, respectively.¹⁶⁶ In this vulnerable neonatal population, decreased calcification of bone matrix results in low BMC and susceptibility to fracture, whereas depressed calcification of the cartilage growth plate can lead to rickets and its characteristics deformities.

Approximately 30% (16%–40%) of preterm infants with birth weights under 1500 g develop metabolic bone disease within 6 to 16 weeks after birth.^{168,175} Metabolic bone disease of prematurity is associated with decreased BMC; thinning of bone cortices; decreased trabecular bone mass; metaphyseal

flaring, fraying, and cupping; and increased frequency of fracture.^{168,176–178} Biochemically, hypophosphatemia caused by phosphate deficiency and hyperalkaline phosphatasia, evidence of the osteoblast's effort to increase bone mineral deposition, are frequently recorded. Some neonates with metabolic bone disease are calcium deficient as evidenced by increase in serum levels of PTH (secondary hyperparathyroidism); in such enterally alimented neonates, administration of calcitriol may increase intestinal absorption of calcium, thereby raising its serum levels and lowering those of PTH.

In preterm neonates, serum levels of total and bone specific alkaline phosphatase, carboxyl terminal propeptide of collagen type I (PICP), and osteocalcin (markers of osteoblast activity and bone formation) are elevated relative to full-term neonates and older infants and continue to rise over the first 10 weeks of life.¹⁷⁹ It has been recommended that in the VLBW infant, if serum alkaline phosphatase values exceed 500 IU/L and serum phosphate levels fall below 5.6 mg/dL (1.8 mmol/L), the renal tubular reabsorption of phosphate should be determined; values greater than 95% indicate that the infant's phosphate stores are low and initiation of phosphate supplementation is indicated.¹⁷⁷ Urinary hydroxyproline and pyridinoline/deoxypyridoline values (markers of osteoclast activity and bone resorption) are also increased in premature babies, although serum concentration of carboxyl telopeptide of collagen type I (ICTP), another marker of bone resorption, decline during the first 10 weeks after premature delivery. Thus intrauterine and postnatal rates of bone turnover in preterm newborns are rapid and persistently elevated through 40 weeks post-conceptual age, a conclusion confirmed by bone histomorphometry.¹⁸⁰ By photon absorptiometry and quantitative ultrasonography (QUS), bone mass of preterm infants appears to decline during the first several weeks after birth.¹⁸¹ Serum levels of alkaline phosphatase and osteocalcin and urinary excretion of pyridinoline and calcium are high and may remain elevated in LBW infants with low bone mass of prematurity relative to values in LBW infants without bone disease for the first year of life, even though radiographic improvement in skeletal mineralization is usually evident by 6 months of age.¹⁷⁷

Because standard roentgenograms may not detect low bone mineralization before deficits of 20% to 30% or greater have occurred in the second month of life, estimation of BMC by dual energy x-ray absorptiometry (DXA) has become the preferred method to assess bone mineralization in infants because of its accuracy, reproducibility, rapidity of performance, and low radiation exposure (2–3 mrems).¹⁷² Mean whole body BMC in the first 2 days of life ranges from 21.7 g in newborns with birth weights of 1001 to 1500 g to 78.8 g in neonates with birth weights of 3501 to 4000 g, whereas whole body BMD varies from 0.146 mg/cm² (1001–1500 g) to 0.234 gm/cm² (3501–4000 g). BMC and BMD increase through the first year of life and beyond as measured by DXA. At this age, bone mass is best correlated with body weight. QUS may also be used to assess bone integrity and strength in preterm and other LBW infants; QUS may be performed at the crib side, there is no exposure to radiation, and it may be repeated as frequently as necessary.¹⁸² QUS measures the speed of sound (SOS) through a bone (humerus, tibia, radius, patella, os calcis, metacarpal, phalanx), a measurement that is correlated with the strength of the bone; mineral content is but one of several skeletal components (elasticity, cortical thickness, microstructure) that collectively contribute to bone strength. QUS also permits calculation of bone transmission time, a measurement that determines the difference in the velocity of sound as it travels through bone and surrounding soft tissue. There is considerable overlap of SOS values at various ages and somatic sizes; however, bone transmission times may discriminate to a greater extent between these parameters. Humeral and tibial

QUS measurements are lower in preterm than term infants and correlate positively with gestational age, birth weight, postnatal length, and weight.¹⁸³ In neonates with intrauterine growth restriction, tibial SOS levels are variable.¹⁸² The relationships between BMD determined by DXA and bone strength estimated by QUS in preterm and term newborns are weak but significant.¹⁷⁰

With prolonged deprivation of calcium, phosphate, and vitamin D, not only does the LBW infant lag in accumulation of bone mass, but clinical and radiographic evidence of rickets (a disorder of bone mineralization, vide infra) also develops—usually between the 6th and 12th postnatal weeks—and fractures may occur in as many as 24% of VLBW infants.¹⁸¹ The LBW neonate at risk for low bone mass and/or rickets is best managed preventively by maintaining serum calcium concentrations between 8 and 11 mg/dL and serum phosphate values between 5.8 and 9 mg/dL and by providing by daily enteral administration of as much of the needed amounts of calcium (140–160 mg/100 kcal), phosphate (95–108 mg/100 kcal of formula), and vitamin D (400–1000 U), as well as protein (for collagen synthesis) and energy (carbohydrates, lipids) as possible. When parenteral administration of nutrients is necessary in the LBW or VLBW neonate, an attempt should be made to administer the maximum amounts of calcium and phosphate safely attainable. The solubility of calcium and phosphate depends not only on their quantities, but also on the forms selected for infusion, that is, calcium chloride, -gluconate, -glycerophosphate, monobasic phosphate, or dibasic phosphate. Using monobasic phosphate and glycerophosphate, it is possible to infuse as much as 86 mg of calcium/kg/dL and 46 mg/kg/dL of phosphate. However, these preparations increase the risk of metabolic acidosis and hypercalciuria.¹⁷² Infusion of parenteral nutrition solutions containing 60 to 80 mg/kg/d of calcium and 40 to 50 mg/kg/d of phosphate permits only 60% to 70% of the estimated in utero bone mineral accretion rates.¹⁷² Therefore enteral feeding should begin as soon as possible in the VLBW and LBW infant using human breast milk fortified with calcium glycerophosphate (calcium 80–120 mg/kg/d, phosphate 45–60 mg/kg/d).^{171,172,184,185} Prepared formulas for feeding of LBW neonates providing 200 mg/kg/d of calcium and 100 mg/kg/d of phosphate can result in up to 90 mg/kg/d of retained calcium and 40 mg/kg/d of retained phosphate.¹⁷¹ The type of prepared formula (sources of protein, fat and carbohydrates) and its lipid and mineral additives determine the rate of intestinal calcium absorption and retention; therefore the choice of formula must be carefully considered before it is selected. Nevertheless, parenteral nutrition, fortified human milk, and available preterm formulas are unable to provide the amounts of calcium and particularly phosphate that would normally accrue to the fetus in utero. Vitamin D 400 to 1000 IU/day should also be provided to the preterm infant either enterally or parenterally. Monitoring of serum levels of calcium, phosphate, creatinine, and alkaline phosphatase and urinary excretion of calcium, phosphate, and creatinine is essential to prevent hypercalcemia and hypercalciuria and nephrocalcinosis. It is also important to avoid hypocalcemia because of the avidity of bone matrix for calcium once remineralization has commenced (hungry bone syndrome). Passive physical activity (daily range of motion with extension/flexion of all joints of each extremity in the supine infant for 4 weeks, beginning after the neonate has been stabilized between 2 and 6 weeks of postnatal age) with or without gentle massage of the prone infant from head-to-toe increases serum levels of markers of bone formation, as well as bone mineralization by DXA and QUS changes consistent with augmented bone strength.^{176,180,181} Although in the prematurely born infant, bone mass may remain low through infancy and early childhood, size-adjusted (height,

lean body mass, fat mass) bone mineralization eventually normalizes in most adult subjects except perhaps at the femoral neck.^{178,186}

Congenital lethal osteogenesis imperfecta (clinical type 2—vide infra) (OMIM 166210) is a prenatal/perinatal disorder characterized by the presence of “beaded” ribs and long bone deformities and fractures demonstrable in utero and apparent at birth, platyspondyly, and subnormal mineralization of the calvarium.^{187,188} This most severe form of osteogenesis imperfecta is usually the result of heterozygous spontaneously occurring loss-of-function mutations in the genes encoding collagen- α 1(I) (*COL1A1*, OMIM 120150) or collagen- α 2(I) (*COL1A2*, 120160). The mutations may be partial gene deletions resulting in decreased synthesis of the triple helix of type I collagen or missense mutations that lead to amino acid substitutions (e.g., arginine, aspartic acid, cysteine) for the glycine residues that are essential for the normal three-dimensional conformation of collagen- α 1(I)/collagen- α 2(I) and synthesis of the triple helix and structural integrity of type I collagen in extracellular matrix of bone upon which hydroxyapatite is deposited. Lethal osteogenesis imperfecta clinical type 2 may rarely be transmitted as an autosomal dominant trait by a parent who is mosaic for a heterozygous mutation in either collagen- α 1(I) or - α 2(I).^{188–190}

Lethal forms of osteogenesis imperfecta transmitted as autosomal recessive disorders are also attributable to inactivating mutations in *CRTAP* (OMIM 605497), *PPIB* (OMIM259440), or *LEPRE1* (OMIM 610339), three factors necessary for hydroxylation of proline-986 in *COL1A1* and proline-707 in *COL1A2*, essential modifications of these proteins.^{191–193} In addition, variants of *CREB3L1*—encoding cyclic AMP response element-binding protein 3-like 1 (OMIM 616215) may lead to a lethal, prenatal/perinatal form of osteogenesis imperfecta. At the often preterm birth, clinical manifestations of lethal osteogenesis imperfecta include intrauterine growth restriction, long bone deformities and fractures, and a malleable calvarial cortex with large fontanelles; death often occurs in early infancy because of respiratory insufficiency. Radiologically, congenital osteogenesis imperfecta is manifested by short, broad, and “crumpled” long bones, angulation of the tibia, and beading of the ribs. The diagnosis of this illness is most often made in utero; it must be differentiated from achondrogenesis type I, thanatophoric dysplasia, and perinatal hypophosphatasia. and confirmed by quantitation of subnormal amounts of collagen synthesized by fibroblasts in vitro and identification of the gene mutation. The lethal mutations of *COL1A1* are clustered at sites at which the collagen monomer binds integrins, matrix metalloproteinases, fibronectin, and cartilage oligomeric matrix protein; other mutations impair posttranslational processing of collagen α 1(I) and thereby interfere with association of collagen chains or propagation of the triple helix configuration.¹⁹⁴ Mutations in *COL1A2* encoding collagen α 2(I) coincide with binding sites for proteoglycans. Indeed, the lethal mutations in *COL1A1* leading to the clinically most severe forms of osteogenesis imperfecta are more functionally incapacitating to the protein than is complete absence of one *COL1A1* allele; haploinsufficiency of *COL1A1* leads to the less clinically severe osteogenesis imperfecta type I.¹⁹⁵ Although administration of the bisphosphonate pamidronate has been helpful in infants with severe manifestations of osteogenesis imperfecta types III and IV, it has been ineffective in the lethal form of osteogenesis imperfecta (vide infra).¹⁹⁶

Extremely low bone mass is present in infants and children with lysinuric protein intolerance (OMIM 222700), an autosomal recessive disorder of renal tubular and intestinal transport of the dibasic amino acids—lysine, arginine, ornithine—that is caused by loss-of-function variants in *SLC7A7* (OMIM 603593) encoding a cationic amino acid transporter subunit.¹⁹⁷ Clinical

manifestations include vomiting, diarrhea, respiratory distress, failure to thrive, developmental delay, hepatomegaly, and cirrhosis. Affected patients have impaired urea synthesis because of decreased hepatic uptake of ornithine but are episodically hyperammonemic with increased urinary excretion of the dibasic amino acids; there is extremely low bone mass because of marked protein deprivation and perhaps because of increase in cytokine-induced bone resorption. Administration of citrulline has been reported to increase growth and bone mass in some of these patients. Variants of *SLC7A7* include deletion of multiple exons, duplications, missense, nonsense, and splice-site mutations dispersed throughout the gene; patients with the largest deletions had the most severe phenotypes.¹⁹⁸ Subnormal bone mineralization may also be observed in infants with infantile hypophosphatasia (OMIM 241500) and in those with muscular disorders restricting movement, such as the Prader-Willi syndrome (OMIM 176270), glycogen storage disease type II (Pompe disease—OMIM 232300), and forms of arthropodiosis.

Increased Bone Mass

Increased bone mass may be generalized or localized; osteosclerosis refers to thickening of trabecular bone and hyperostosis to increase in cortical bone mass.⁸ The infantile “malignant” form of osteopetrosis (OMIM 259700) is one of several autosomal recessive disorders caused by abnormal osteoclast differentiation or function resulting in defective reabsorption of the mineral phase of bone (vide infra). Infantile osteopetrosis is clinically manifested in affected infants by failure to thrive, delayed development, nasal obstruction, loss of sight, hearing and other cranial nerve functions, and intense bone overgrowth, leading to pancytopenia, hepatosplenomegaly at sites of extramedullary hematopoiesis, and increased susceptibility to infection (e.g., mandibular osteomyelitis) and to fractures because of decreased bone strength despite high bone mass. Death often occurs within the first several years of life because of sepsis, anemia, or hemorrhage. Additional physical findings in newborns and infants with the most severe form of osteopetrosis include impaired linear growth, macrocephaly, frontal bossing, nystagmus, delayed eruption of primary teeth, and ecchymoses. The radiographic hallmark of infantile osteopetrosis is relatively uniform increase in bone density of the skull, vertebrae, and axial skeleton with thickened cortical and trabecular bone, “Ehrlemeyer flask” deformities at the distal ends of the long bones in older children, and alternating bands of sclerotic and lucent bone in the iliac wings and metaphyses.⁸ Biomarkers for osteopetrosis are increased serum levels of acid phosphatase and the brain isoform of creatine kinase. Lethal infantile osteopetrosis may be caused by homozygous or compound heterozygous loss-of-function mutations in genes whose transcripts are essential for normal osteoblast function: T cell immune regulator 1 (*TCIRG1*, OMIM 604592) encodes a subunit of the vacuolar proton pump within the osteoclast ruffled border through which hydrogen ions are transported from the cytosol into the subosteoclast resorption lacuna; chloride channel 7 (*CLCN7*, OMIM 602727) encodes a chloride channel within the osteoclast’s ruffled border required for transport of this cation into and hence acidification of the resorption lacuna; osteopetrosis-associated transmembrane protein 1 (*OSTM1*, OMIM 607649) encodes the beta subunit of *CLCN7* necessary for its normal posttranslational processing (vide infra). In selected subjects, bone marrow transplantation providing osteoclast precursor cells has been effective in arresting progression of this disorder, albeit often with substantial residual deficits. Transient but substantial hypercalcemia may occur after this procedure. Osteopetrosis caused by deficiency of carbonic anhydrase II (*CA2*, OMIM 611492) encoding carbonic

anhydrase II may also present in infancy with failure to thrive or fracture with insignificant trauma.

Disproportionate short stature is the cardinal manifestation of pycnodysostosis (OMIM 265800) and is manifested during infancy or early childhood by a relatively large head circumference with open fontanelles and cranial sutures, dysmorphic facial features (frontooccipital prominence, proptosis, bluish sclerae, hypoplastic maxilla, micrognathia, highly arched palate, malocclusion, beaked nose), stubby and clubbed fingers with hypoplastic nails, narrow thorax, pectus excavatum, lumbar lordosis, kyphoscoliosis, and increased fracture risk.⁸ Radiographically, there is marked osteosclerosis that increases with age, open fontanelles and cranial sutures, thin clavicles with hypoplastic lateral ends, erosion and hypoplasia of the distal phalanges and ribs, and dense vertebrae yet normal transverse processes. Histologically, there is decreased osteoblastic and osteoclastic activity. Pycnodysostosis is caused by homozygous or compound heterozygous loss-of-function mutations (stop, missense, nonsense) in *CTSK* (OMIM 601105)—the gene encoding cathepsin K, a lysosomal cysteine protease expressed in osteoclasts whose loss impairs degradation of collagen and the resorption of osteoid, the organic component of bone matrix, but not its mineral component. The Raine syndrome (OMIM 259775) of prenatal generalized osteosclerosis with periosteal bone formation, facial dysmorphism (hypoplastic nose, low set ears, exophthalmos, cleft palate), and hypoplastic thorax often resulting in perinatal death is the consequence of biallelic inactivating variants of *FAM20C* (OMIM 611061) encoding a phosphorylase that acts upon small integrin-binding ligand N-linked secreted glycoproteins (*SIBLING*) that bind calcium and regulate mineralization of osteoid, the loss of which enhances this process.¹⁹⁹

Disorders of Bone Mineralization and Formation in the Child and Adolescent

Bone formation may be impaired because of lack of minerals (calcium and/or phosphate) or because of deficient production of bone matrix. Bone mineralization may be excessive because of increase in the rate of mineral deposition or decrease in the rate of resorption of the mineral phase of bone. Ectopic calcification of extraskeletal tissues may occur when local calcium and phosphate levels are high, whereas extraskeletal ossification may ensue when the regulation of skeletal bone formation is deranged.

Rickets

Rickets in children is a disorder of the cartilaginous growth plate and the failure to deposit sufficient bone mineral (hydroxyapatite) to achieve necessary bone strength enabling upright weight-bearing and ambulation because of deficiency of calcium, phosphate, vitamin D, or one of many cofactors that ensure appropriate assimilation and utilization of these materials. Thus rickets is a childhood disorder of decreased mineralization of cartilage and primary bone spongiosa of the growth plates at the ends of the long bones and of bone matrix collagen type I below the growth plate that leads to impaired skeletal strength and linear growth and bony deformations (craniotabes, metaphyseal flaring, genu valgum, genu varum), and increased fracture risk.²⁰⁰ Rickets may be caused by lack of calcium and/or phosphate because of limited intake or decreased intestinal absorption of these nutrients; the latter may be related to intrinsic abnormalities of intestinal function (malabsorption syndromes, short bowel length) or to inadequate vitamin D intake or its decreased absorption or subnormal functional efficiency; impaired endogenous cutaneous synthesis of vitamin D may be caused by climatic conditions

resulting in decreased exposure to sunlight, the dermal application of potent ultraviolet light wave-blocking pharmaceutical agents, or the wearing of extensive clothing that substantially limits the skin surface area exposed to sunlight; vitamin D deficiency may also be the consequence of failure to process cholecalciferol to its biologically active metabolite, 1,25-dihydroxyvitamin D (calcitriol), accelerated conversion of 1,25-dihydroxyvitamin D to water soluble 1,24,25-trihydroxyvitamin D and its urinary loss, or to impaired functional activity of calcitriol because of an abnormality of its nuclear VDR; decreased activity of alkaline phosphatase also leads to impaired bone formation^{112,201,202} (Tables 20.8A, 20.8B). In the adult, deficiencies of vitamin D, calcium, or phosphate result in osteomalacia, the failure to mineralize the cortex and trabeculae of bone.

During endochondral bone formation in growing children, cartilage matrix is elaborated and subsequently mineralized. When endochondral matrix is not fully mineralized, cartilage accumulates, there is thickening of the growth plate and disorganization of the chondrocytes; the ends of the long bones (particularly those that are weight-bearing) are distorted resulting in rachitic deformities.²⁰⁰ During the processes of modeling

and remodeling of trabecular bone and the periosteal and endosteal surfaces of cortical bone, osteoid is formed by osteoblasts; failure to mineralize bone matrix in these regions results in osteomalacia. During intervals of calcium and/or phosphate deprivation, the actively growing, weight-bearing child with open cartilaginous growth plates develops rickets and osteomalacia, whereas under similar circumstances adults develop only osteomalacia during remodeling as unmineralized bone matrix accumulates. Thus rickets is the expression of defective endochondral mineralization at the growth plate and osteomalacia is the failure of mineralization of bone cortex and trabeculae.

Clinically, rickets is manifested by skeletal abnormalities, such as delayed closure of the fontanelles, craniotabes (reversible compression of the skull's outer table), frontal bossing (expansion of cranial bones), and occasional craniosynostosis in infants; bowing of the forearms in the preambulatory infant and genu varum and/or valgum in the weight-bearing infant and child; delayed tooth eruption with poor enamel formation and propensity to caries; pectus carinatum, prominence of the costochondral junctions, flaring of the lower rib cage, scoliosis

TABLE 20.8A Disorders of Bone Mineralization: Rickets

I Vitamin D Deficiency

- A Decreased intake, endogenous synthesis, retention, or sequestration
 - 1 Maternal vitamin D deficiency—breastfeeding
 - 2 Reduced skin synthesis—sunlight deprivation, sunscreen use, increased skin pigmentation
 - 3 Malabsorption—celiac disease, hepatobiliary dysfunction, short gut syndrome, cystic fibrosis, inflammatory bowel disease, gastric bypass surgery
 - 4 Drugs—anticonvulsants, glucocorticoids, cholestyramine, antiretroviral agents
 - 5 Nephrotic syndrome
 - 6 Obesity
- B Metabolic errors
 - 1 Deficiency of 25-hydroxylase
 - a Loss-of-function mutation of *CYP2R1*
 - b Hepatic dysfunction
 - 2 Deficiency of 25OH-vitamin D₃-1-hydroxylase
 - a Loss-of-function mutation of *CYP27B1*
 - b Decreased renal mass—hypoplasia, chronic renal insufficiency
 - 3 Impaired response to vitamin D
 - a Loss-of-function mutation of *VDR*
 - b Excessive heterogeneous nuclear ribonucleoprotein C1/C2 (*HNRNPC*)

II Calcium Deficiency—Primary

- A Nutritional deprivation
- B Hypercalciuria—hyperprostaglandin E2 syndromes

III Phosphate Deficiency/Hypophosphatemia

- A Transcellular shifts
 - 1 Glucose or insulin infusion
 - 2 Refeeding
 - 3 Respiratory alkalosis; hyperventilation
 - 4 Salicylate poisoning
 - 5 Catecholamines
- B Decreased intestinal absorption
 - 1 Nutritional deprivation
 - a Low birth weight infant
 - 2 Decreased intestinal absorption
 - a Vitamin D deficiency—nutritional, synthetic error, insensitivity
 - b Aluminum, magnesium, or calcium-containing antacids
 - c Malabsorption because of chronic diarrhea, steatorrhea

C Hyperphosphaturia

- 1 X-linked dominant familial hypophosphatemic rickets (*PHEX*)^a
- 2 X-linked recessive hypophosphatemic rickets (*CLCN5*)
- 3 Autosomal dominant hypophosphatemic rickets (*FGF23*)^a
- 4 Autosomal dominant hypophosphatemia with urolithiasis 1 (*SLC34A1*)^b
- 5 Autosomal dominant hypophosphatemia with urolithiasis 2 (*SLC9A3R1*)
- 6 Autosomal recessive hypophosphatemic rickets 1 (*DMP1*)^a
- 7 Autosomal recessive hypophosphatemic rickets 2 (*ENPP1*)^a
- 8 Autosomal recessive hypophosphatemic rickets with hypercalciuria (*SLC34A3*)
- 9 Hypophosphatemic rickets with hyperparathyroidism (*KL*)
- 10 Hypophosphatemic rickets with Fanconi renal tubular syndrome (*SLC34A1*)^c
- 11 Oncogenic hypophosphatemic rickets/osteomalacia (*FGF23*, *sFRP4*, *MEPE*, *FGF7*)
- 12 FGF23 excess of uncertain pathogenesis (McCune-Albright syndrome [*GNAS*]^a, Jansen type metaphyseal chondrodysplasia [*PTH1R*]^a, Osteoglophonic dysplasia [*FGFR1*]^a, Linear nevus sebaceous syndrome^a, Iron polymaltese^a)
- 13 Renal tubular acidosis/Fanconi syndrome
 - a Heritable—cystinosis, tyrosinemia, hereditary fructose intolerance, galactosemia, idiopathic
 - b Acquired—nephrotic syndrome, vitamin D deficiency, renal vein thrombosis, renal tubular insult because of cadmium, lead, bismuth, outdated tetracycline, 6-mercaptopurine, valproic acid, ifosfamide

D Other causes

- 1 Hyperparathyroidism
- 2 Medications—diuretics (thiazides, acetazolamide, loop diuretics); antiretroviral agents (tenovir, adefovir); antineoplastic drugs (ifosfamide, cisplatin); aminoglycosides, imatinib

IV Hypophosphatasia (*ALPL*)

- A Perinatal, infantile, childhood, adult forms
- B Odontohypophosphatasia
- C Pseudohypophosphatasia

V Inhibitors of Mineralization

- A Aluminum—parenteral
- B Bisphosphonates
- C Fluoride

^aAssociated with increased expression of FGF23.

^bMonoallelic.

^cBiallelic.

(From Gattineni J, Baum M. Genetic disorders of phosphate regulation. *Pediatr Nephrol*. 2012;27:1477–1487; Hori M, Shimizu Y, Fukumoto S. Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology*. 2011;152:4–10; Imel EA, Econs MJ. Approach to the hypophosphatemic patient. *J Clin Endocrinol Metab*. 2012;97:696–706.)

TABLE 20.8B Genetic Disorders of Bone Mineralization: Rickets

Gene Chromosome OMIM	Pathophysiology	Mutation—Clinical Manifestations
Disorders of vitamin D metabolism		
<i>CYP2R1</i> —Cytochrome P450, subfamily IIR, polypeptide 1 11p15.2 608713	Hepatic 25-hydroxylase—enzyme that converts vitamin D3 to 25OH ³ (calcidiol)	Biallelic loss-of-function mutation leads to vitamin D hydroxylation-deficient rickets type 1B (also termed <i>vitamin D-dependent rickets type 1B</i>), AR
<i>CYP27B1</i> —Cytochrome P450, subfamily XXVII, polypeptide 1 12q13.1-q13.3 609506	25OH ³ -1 α hydroxylase—enzyme that converts 25OH ³ to 1,25(OH) ² D ³ (calcitriol)	Biallelic inactivating mutations result in vitamin D-dependent rickets type 1A, AR
<i>VDR</i> —Vitamin D receptor 12q12-q14 601769	Vitamin D receptor—transcription factor that transduces the effects of calcitriol on gene activation or repression	Biallelic loss-of-function mutations lead to insensitivity to calcitriol & vitamin D-dependent rickets type 2A, AR
<i>HNRNPC</i> —Heterogeneous nuclear ribonucleoprotein C 14q11.2 164020	Encodes a ribonucleoprotein that regulates gene transcription by reciprocally & transiently occupying the VDRE in the upstream promoter region of vitamin D-responsive target genes	Overexpression in vitamin D-responsive tissues leads to prolonged occupancy of the VDRE that interferes with interaction of the VDR/RXR with the VDRE resulting in vitamin D-dependent rickets type 2B
<i>CYP3A4</i> —Cytochrome P450, subfamily IIIA, polypeptide 4 7q22.1 124010	Encodes a hepatic CYP450 enzyme that oxidatively metabolizes & inactivates calcidiol & calcitriol	Gain-of-function monoallelic variant resulting in rapid catabolism of calcitriol requiring high doses of calcitriol for treatment of rickets (vitamin D-dependent rickets type 3) (AD)
Disorders of phosphate metabolism		
<i>SLC34A1</i> —Solute carrier family 34 (sodium phosphate cotransporter), member 1 5q35 182309	Encodes NPT2a—sodium/phosphate cotransporter expressed on the apical membrane of the proximal renal tubule; under the inhibitory control of PTH	Loss-of-function mutation results in hypophosphatemic rickets with nephrolithiasis, type 1, AD; Fanconi syndrome type 2, AR
<i>SLC34A2</i> —Solute carrier family 34 (sodium phosphate cotransporter), member 2 2p15.31-p15.2 604217	Encodes NPT2b—sodium/phosphate cotransporter expressed in the small intestine, lung, and testes	Loss-of-function mutations associated with pulmonary alveolar microlithiasis & testicular microlithiasis, AR
<i>SLC34A3</i> —Solute carrier family 34 (sodium phosphate cotransporter), member 3 9q34 609826	Encodes NPT2c—sodium/phosphate cotransporter expressed on the apical membrane of the proximal renal tubule	Loss-of-function mutation results in hereditary hypophosphatemic rickets with hypercalciuria, AR
<i>SLC9A3R1</i> —Solute carrier family 9, member 3, regulator 1 17q25.1 604990	Encodes NHERF1—a renal tubular sodium/hydrogen exchange regulatory factor that binds NPT2a anchoring it to the luminal membrane of the proximal renal tubule; phosphorylation by PTH leads to its dissociation from & endocytosis of NPT2a	Loss-of-function mutations result in hypophosphatemic rickets with nephrolithiasis, type 2, AD
<i>CLCN5</i> —Chloride channel 5 Xp11.23-p11.22 300008	Encodes a proximal renal tubular exchanger of chloride & hydrogen ions	Loss-of-function mutations result in X-linked recessive hypophosphatemic rickets, hypercalciuria, nephrocalcinosis, XLR
<i>PHEX</i> —Phosphate-regulating endopeptidase homolog, X-linked Xp22.2-p22.1 300550	Ectoenzyme expressed on the cell membrane of osteoblasts; its physiological substrate may be MEPE & pASARM, its phosphorylated degradation product that coats hydroxyapatite & hinders mineral deposition	Loss-of-function mutation leads to X-linked hypophosphatemic rickets; associated with increased expression of FGF23; X-linked dominant
<i>DMP1</i> —Dentin matrix acidic phosphoprotein 1 4q22.1 600980	Noncollagenous, serine-rich, bone matrix protein; a small integrin-binding ligand, N-linked glycoprotein (SIBLING) expressed in osteocytes	Loss-of-function mutations lead to increased osteocyte synthesis of FGF23, hyperphosphaturia, & autosomal recessive hypophosphatemic rickets, AR
<i>ENPP1</i> —Ectonucleotide pyrophosphatase/phosphodiesterase 1 6q23.2 173335	Ectoenzyme expressed by chondrocytes, bone, & plasma cells that hydrolyzes ATP to pyrophosphate, an inhibitor of bone mineralization	Loss-of-function mutations result in autosomal recessive hypophosphatemic rickets with increased expression of FGF23, AR
<i>ANKH</i> - ANK, mouse, homolog of 5p15.2-141 605145	Transmembrane-spanning cell-surface protein that regulates pyrophosphate secretion	Inactivating mutations result in mild hypophosphatemia & joint ankylosis, developmental delay, deafness, & dentinogenesis imperfecta; AR
<i>FGF23</i> —Fibroblast growth factor 23 12p13.2 605380	Product of osteoblast & osteocyte that depresses renal tubular reabsorption of phosphate & inhibits synthesis of calcitriol	Gain-of-function mutation that decreases the rate of degradation of FGF23 resulting in autosomal dominant hypophosphatemic rickets, AD; excessive ectopic synthesis by neoplasms leads to hypophosphatemic rickets; loss-of-function mutation leading to decreased FGF23 synthesis results in autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR

TABLE 20.8B Genetic Disorders of Bone Mineralization: Rickets—cont'd

Gene Chromosome OMIM	Pathophysiology	Mutation—Clinical Manifestations
<i>GALNT3</i> —UDP-N-Acetyl-alpha-D-Galactosamine: polypeptide N-acetylglucosaminyl transferase 3 2q24.3 601756	Encodes an enzyme that is essential for O-glycosylation of Thr178 of FGF23 during posttranslational processing; failure of this step leads to degradation of FGF23 before its secretion	Loss-of-function mutation leads to autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR
<i>KL</i> — α -Klotho 13q13.1 604824	Coreceptor with FGFR1(IIIc) for FGF23 that converts FGFR1(IIIc) into the specific FGF23 receptor enabling signal transduction	Loss-of-function mutation leads to autosomal recessive hyperphosphatemic familial tumoral calcinosis, AR; Translocation to (9;13)(q21.13;q13.1) results in excessive Klotho formation leading to hypophosphatemic rickets & hyperparathyroidism
Hypophosphatasia <i>ALPL</i> —Alkaline phosphatase, liver 1p36.12 171760	Tissue nonspecific alkaline phosphatase—ectoenzyme expressed on the cell membrane of osteoblasts, removes organically bound phosphate; major substrates are pyrophosphate, phosphoethanolamine, pyridoxal-5'-phosphate	Loss-of-function mutations lead to hypophosphatasia & rickets of variable severity & onset: perinatal, infancy, transitional, childhood, adult, odontohypophosphatasia, pseudohypophosphatasia; AR, AD

AD, Autosomal dominant; AR, autosomal recessive; ATP, adenosine triphosphate; MEPE, matrix extracellular phosphoglycoprotein; ρ ASARM, phosphorylated acidic serine aspartate-rich MEPE-associated motif.

(From Farrow EG, White KE. Recent advances in renal phosphate handling. *Nature Rev/Nephrol*. 2010;6:207–217; Roizen JD, Li D, O'Lear L, et al. CYP3A4 mutation causes vitamin D-dependent rickets type 3. *J Clin Invest*. 2018;128:1013–1918.)

and kyphosis; flaring of the metaphyses of the long bones, and tibial or femoral torsion (Fig. 20.9).²⁰³ Radiographically, rickets is characterized initially by epiphyseal widening and loss of the defined zone of provisional calcification followed by cupping, splaying, and fraying of the metaphyses of long bones (particularly the distal ulna, distal femur, and proximal tibia), demineralization, deformation of the long bones, and fractures. “Looser zones” are radiolucent lines with dense borders that are pseudofractures located in the femoral neck and shaft. The Thacher radiographic scoring system for assessment of the severity of rickets provides a reasonably objective and useful method for determining the extent of rickets and its response to treatment.²⁰⁴ Histologically, as a consequence of impaired calcification within the cartilage growth plate, the pattern of chondrocyte differentiation and maturation is disrupted and disorganized, while osteoid seams are widened.²⁰⁵ In subjects deprived of phosphate, it is the trabeculae that are primarily undermineralized.

Impaired mineralization of osteoid may be caused by dietary deficiencies or depressed intestinal absorption of calcium, phosphate, or vitamin D, inadequate amounts of these nutrients in fluids used in total parenteral nutrition, metabolic errors in the metabolism or action of vitamin D, defects in renal tubular conservation of phosphate or calcium, or abnormalities of alkaline phosphatase generation and function (see Tables 20.8A, 20.8B). Broadly, rickets may be considered to be calciopenic (usually related to nutritional deprivation of vitamin D or rarely to a defect in its metabolism to the active metabolite—calcitriol—or in its cellular action or to decreased intake of calcium or its excessive loss in urine) or phosphopenic (related to renal phosphate wasting because of primary renal tubular defects in phosphate reabsorption or to generation of excessive amounts of phosphatonins—compounds that inhibit renal tubular reabsorption of phosphate). Thus nutritional rickets may be caused by decreased intake of vitamin D (or inadequate exposure to sunlight) or calcium or to marginal intakes of both nutrients.²⁰⁶ Dietary deficiency of phosphate is unusual given its wide availability, but this nutrient may

be deficient in parenteral fluids. Bone mineralization may also be directly impaired by abnormalities of alkaline phosphatase generation or by agents, such as aluminum or fluoride.²⁰⁵

Calciopenic Rickets. Although dietary calcium deficiency is a rare cause of rickets, there is a substantial incidence of vitamin D deficiency and rickets in children—ranging from 2.9 per 100,000 Canadian children to 7.5, 24, and 95 per 100,000 children in the United Kingdom, United States, and Africa, respectively.²⁰⁷ Vitamin D insufficiency/deficiency may arise from decreased nutritional intake in the presence of suboptimal exposure to sunlight, intestinal malabsorptive syndromes (celiac disease, “short-guts,” cystic fibrosis, inflammatory bowel disease, food allergies), impaired renal synthesis of calcitriol caused by chronic renal disease or to variants of *CYP27B1* (OMIM 609506), or medication or drug exposure (phenytoin, carbamazepine, phenobarbital, glucocorticoids—the latter by increasing activity of renal vitamin D-24-hydroxylase). In the neonate, vitamin D status is related to gestational age and intra-uterine growth and the maternal intake of this nutrient and whether maternal dress permits adequate exposure to sunlight and endogenous synthesis of cholecalciferol. Although obesity is associated with lowered serum concentrations of calcidiol caused by its solubility in adipose tissue and hence sequestration in lipids, obesity does not impair the processes of formation or mineralization of bone.^{207,208} The more darkly pigmented is a child’s skin and the higher the altitude at which she or he lives, the greater is the risk of vitamin D deficiency in the absence of supplementation. In addition, the amount of ultraviolet light that reaches the earth’s surface is influenced by the latitude at which the child dwells, season of the year, time of the day, the amount of time spent outdoors and the clothing worn when outside, cloud cover, and intensity of air pollution. Nutritional rickets caused by deficiency of vitamin D or decreased intake of calcium results in defects of chondrocyte differentiation and function and impaired deposition of calcium into cartilaginous matrix and bone osteoid.²⁰⁹ In serum, more than 90% of cholecalciferol and its metabolites

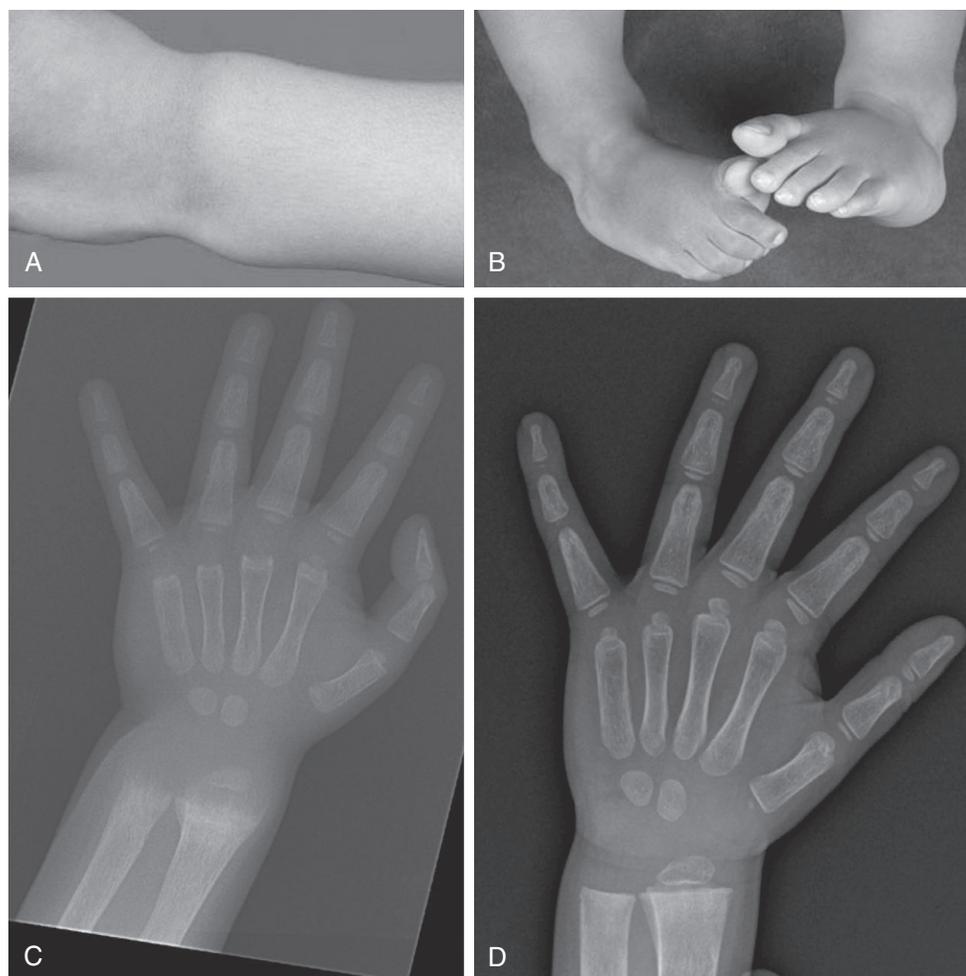


Fig. 20.9 Clinical and radiographic manifestations of vitamin D-deficient rickets in a two year old child. Note flaring of the distal metaphyses of the left wrist (A) and both ankles (B), roentgenographic demonstration of widening of the metaphyses of the radius and ulna © with increased mineralization after treatment with cholecalciferol for three months (D). (From Girgis CM, Clifton-Bligh RJ, Hamrick MW, et al. The roles of vitamin D in skeletal muscle: form, function, and metabolism. *Endocr Rev.* 2013;34:33–83. Images are courtesy of Associate Professor Craig Munns, Childrens' Hospital Westmead, Sydney, Australia.)

(calcidiol, calcitriol) are bound by serum proteins, primarily by vitamin D binding protein (DBP) encoded by *GC* (group-specific component, OMIM 139200). Congenital absence of DBP caused by biallelic deletion of the long arm of chromosome 4 is associated with extremely low but measurable plasma concentrations of total calcidiol and calcitriol without evidence of rickets or osteomalacia—presumably caused by levels of free calcitriol that are adequate for osteoblast-mediated bone formation.²¹⁰ As a consequence of vitamin D deficiency secondary hyperparathyroidism develops resulting in impaired renal tubular reabsorption of filtered phosphate and hypophosphatemia. Although the proximate defect in the vitamin D-deficient subject is calciopenia, impaired growth and development of the cartilaginous growth plate is caused by hypophosphatemia that inhibits expression of *CASP9* (OMIM 602234) encoding caspase 9 that, in turn, impairs phosphate-regulated apoptosis of hypertrophic chondrocytes enabling their continued proliferation.

Thus calciopenic rickets is most often caused by deficiency of vitamin D, which depresses intestinal absorption of dietary calcium and renal reabsorption of filtered calcium. As determined by the measurement of serum concentrations of calcidiol (25OHD), vitamin D deficiency is present when values are below 12 to 15 ng/mL.^{211,212} Calcidiol concentrations between 15 and 19.5 ng/mL indicate vitamin D “insufficiency,” whereas those 20 ng/mL or more are adequate or sufficient. (However, it is critical to assess the validity and variability of

a specific 25OHD/calcidiol assay when interpreting the data provided by that assay as there may be significant quantitative differences between individual reagent sets.²¹³ In addition, because significant concentrations of C-3 epimers of calcidiol are often present in infants that increase the apparent concentration of this analyte, it is important that measurements of serum calcidiol values in children less than 1 year of age be done with an assay that accurately quantifies calcidiol in the presence of C-3 epimers.)

Neonates born to severely vitamin D-deficient mothers may display signs of rickets at birth, including fractures and hypocalcemic seizures; softening of the outer table of the skull in infants and very young children permits its reversible indentation (craniotabes). Clinical manifestations of rickets in preambulatory infants include bowing of the forearms, craniotabes, frontal bossing, and delayed closure of the cranial fontanelles. In children with rickets, anterior bowing of the femora, lateral or medial deviation of the knees leading to genu varum or valgum (bowed legs or “knock knees,” respectively) or a “wind-swept” deformity involving opposite leg deformities, flaring (widening) of the metaphyses of the long bones with markedly enlarged wrists, prominence of the costochondral junctions (rachitic rosary), and indentation of the lower anterior thoracic wall (Harrison’s groove) are noted. Short stature and suboptimal weight are also present frequently. Tooth eruption may be delayed and tooth enamel hypoplastic—predisposing to dental caries. Muscular weakness manifested by hypotonia and delay

in walking may be observed. Hypocalcemic tetany and seizures may occur in a severely vitamin D–deficient infant without gross clinical or radiographic signs of rickets. When vitamin D deficiency is extreme and prolonged in an adolescent hypocalcemic seizures and fractures may occur.²¹⁴

Deficiency of vitamin D is often observed in darkly skinned children and adolescents with limited exposure to sunlight, particularly during and at the end of the winter months, and/or in those ingesting vegetarian diets, or who are being treated with anticonvulsant or antiretroviral drugs.²¹² Because vitamin D has many extraskeletal sites of action because of the multicellular expression of *VDR*, it has been suggested that vitamin D deficiency may play a role in the development of autoimmune, infectious, and systemic disorders (e.g., increased susceptibility to infection—particularly pneumonia—because of both the weakness of the thoracic wall and of the lack of the stimulatory effect of vitamin D on the immune system; asthma and its response to treatment; atopic dermatitis, type 1 diabetes mellitus, inflammatory bowel disease, and cardiovascular disorders).²⁰⁷ Radiographically, decreased bone mineralization with cortical thinning and thin stress fracture lines, as well as cupping, widening, and irregularity (fraying) of the distal metaphyses of the long bones are observed in the rachitic subject.^{9,202} Areas of osteitis fibrosa cystica associated with secondary hyperparathyroidism may sometimes develop.

After introduction of cod liver oil as a dietary supplement in 1918 (and the later fortification of infant formulas and milk and other foods with irradiated ergosterol) and the discovery in 1919 that exposure to sunlight prevented development of rickets, nutritional deficiency of vitamin D became relatively unusual in North America only to reemerge several decades later. Because the fetus is supplied with vitamin D by the transplacental transfer of maternal calcidiol, deficiency of vitamin D occurs predominantly in neonates who are born to mothers with low stores of vitamin D (women of color or those who are vegetarian or poorly nourished, or who have limited exposure to sunlight). Rickets develops in infants and young children who ingest a diet low in vitamin D (little or no milk, meat, eggs, or fish) without supplemental vitamin D who have limited exposure to sunlight because they are confined indoors because of illness, the climate, or parental choice or because they wear clothing that protects the entire body from sunlight. Vitamin D deficiency is more common in breastfed infants because human breast milk contains little vitamin D; black infants are at greater risk than white infants in part because of their increased skin pigmentation resulting in decreased endogenous cutaneous synthesis of cholecalciferol (because melanin absorbs ultraviolet light).²⁰⁵ Normal human breast milk contains only 25 IU/L of vitamin D, therefore it is recommended that all breastfed infants receive supplemental vitamin D 400 IU/day.²¹⁵ Inasmuch as most commercial infant

formulas contain 400 IU/L of vitamin D, it is also recommended that formula-fed infants who consume less than 1 L of formula daily also receive a supplement of 400 IU/day of vitamin D. Infants more than 1 year of age, children, and adolescents should ingest or receive supplemental vitamin D 600 IU daily.

Although definitions of hypovitaminosis D vary between individual studies, subtle forms of vitamin D deficiency or “insufficiency” are likely prevalent throughout the North American population, particularly in the winter months when there is little sunlight exposure and the ultraviolet light requisite for endogenous synthesis of vitamin D is limited.^{9,212,216} Although clinically apparent vitamin D deficiency is unusual in US youth, vitamin D insufficiency may be relatively frequent.^{216,217} In addition to skin pigmentation and northern latitude, low serum calcidiol values have been attributed to meager consumption of milk and multivitamins, large intake of phosphate-containing soft drinks, and increase in fat mass into which vitamin D is deposited and perhaps not readily bioavailable. Using a reliable assay for 25-hydroxyvitamin D, such as those employing liquid chromatography-tandem mass spectrometry, calcidiol concentrations below 12 to 15 ng/mL in children clearly indicate vitamin D deficiency whereas values between 12 to 20 ng/mL are consistent with vitamin D insufficiency.^{207,212} Serum levels of calcidiol that exceed 100 ng/mL are indicative of vitamin D excess. Dietary deficiency of calcium may accentuate the adverse effects of borderline vitamin D stores.²⁰⁶

Vitamin D deficiency may also be the consequence of intestinal malabsorptive disorders, such as celiac or inflammatory bowel diseases, biliary obstruction, gastric and intestinal resection, or pancreatic exocrine insufficiency (e.g., cystic fibrosis), or ingestion of calcium binding agents, such as cholestyramine, or by increased hydroxylation of vitamin D to water-soluble forms that increase its urinary loss by anticonvulsant drugs, such as phenytoin.⁹ Therapeutic doses of glucocorticoids are also associated with lowered serum levels of calcidiol.²¹⁸ In the majority of infants and children with rickets caused by vitamin D deficiency, serum concentrations of total calcium are borderline-normal or low, phosphate levels are low, and alkaline phosphatase activity and PTH concentrations increased (Table 20.9). Secondary hyperparathyroidism develops as the intestinal absorption of calcium is reduced and its serum levels reduced; PTH increases urinary phosphate loss and lowers serum phosphate concentrations, while enhancing the rates of bone resorption and turnover. PTH also increases synthesis of calcitriol, which may increase the rate of calcidiol catabolism and further deplete the body’s store of vitamin D.²⁰⁵ Typically, in patients with vitamin D deficiency serum concentrations of calcidiol are low, whereas calcitriol values may be normal, high, or low depending on whether vitamin D deficiency is modest, moderate, or severe. Serum concentrations of

TABLE 20.9 Laboratory Data in Rickets of Varying Pathogenesis

Type	Calcium	Alkaline Phosphate	Phosphatase	Calcidiol	Calcitriol	PTH	FGF23
Calcium deficiency	N,↓	↓	↑↑	N	↑	↑	N,↓,↑
Phosphate deficiency	N,↑	↓↓	↑↑	N	↑	N,↓,↑	↑
Vitamin D deficiency	N,↓	N,↓	↑↑	↓↓	N,↓	↑	N,↓
Loss-of-function <i>CYP2R1</i> (25-hydroxylase)	↓	↓	↑	↓	↓	↑	N,↓
Loss-of-function <i>CYP27B1</i> (25OHD-1 α -hydroxylase)	↓↓	↓↓	↑↑↑	N	↓↓↓	↑↑↑	N,↓
Loss-of-function <i>VDR</i> (resistance to calcitriol)	↓↓	↓↓	↑↑↑	N	↑↑↑	↑↑↑	N,↓
Loss-of-function <i>PHEX</i> (X-linked hypophosphatemic rickets)	N,↓	↓↓	↑	N	N,↓	N,↑	↑
Hypophosphatasia	N,↑	N,↑	↓	N	N	N,↓	

↓, Low type; ↑, high type; *FGF2*, fibroblast growth factor 2; N, normal; *PTH*, parathyroid hormone; *VDR*, vitamin D receptor.

(From Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers*. 2017;3:17055; Minisola S, Peacock M, Fukumoto S, et al. Tumour-induced osteomalacia. *Nat Rev*. 2017;3:17044.)

osteocalcin are low; serum levels of PICP (a marker of bone formation) and ICTP, and urinary excretion of amino terminal telopeptide of collagen type I (NTx)—both markers of bone resorption—are substantially increased in subjects with vitamin D deficiency rickets indicating increased collagen turnover in this disorder.²¹⁹ With treatment, these values increase transiently and then fall to age-appropriate norms before radiographic healing of the rachitic lesions is complete.

Prevention of vitamin D deficiency is its most effective management. Because the amount of vitamin D in human breast milk is approximately 5 to 80 IU/L and vitamin D supplementation of the breastfeeding mother may be inadequate to ensure normal calcidiol levels in her infant (unless she is receiving approximately 4000 IU per day of vitamin D₃), it is important that all breastfed infants receive a supplement of vitamin D₃ daily (400 IU/day); by extension, this recommendation is also appropriate for infants who are not receiving adequate amounts of vitamin D₃ in their prepared formulas or diet or have suboptimal exposure to sunlight. In the active, lightly dressed white child with skin that normally tans with exposure to sunlight and who plays outdoors 30 minutes thrice weekly or 10 to 15 minutes daily between 1000 to 1500 hours during spring, summer, and fall, endogenous vitamin D synthesis is usually sufficient to achieve satisfactory serum levels of calcidiol.^{205,212} A yellow- or brown-skinned child requires 30 to 45 minutes of outdoor play whereas a black-skinned child requires 60 to 150 minutes of sunlight exposure to achieve serum calcidiol concentrations comparable to those of the white-skinned child. The latitude in which the child lives, season of the year, time of day, local environmental pollutants, amount of clothing, and use of sunscreen affect the time required for sunlight exposure to evoke adequate vitamin D synthesis in the individual child. Between November and February, little or no vitamin D can be synthesized above 35 degrees latitude (Atlanta, GA, USA), but vitamin D insufficiency is also prevalent in lower latitudes.⁹

In obese adolescents, serum concentrations of calcidiol are inversely and PTH values are directly related to fat mass.^{220,221} In a study of 236 adolescent candidates for bariatric surgery, more than 50% were vitamin D deficient as defined by low serum concentrations of calcidiol.²²² Although 43% of these subjects were Caucasian and 15% African American, 82% of the vitamin D-deficient patients were African American compared with 37% of the Caucasian adolescents. Thus obesity is a potential risk factor for subnormal serum concentrations of 25-hydroxyvitamin D; however, bone mineralization by DXA is normal in obese children and adults.^{209,220,223,224} Nevertheless, the normal bone mineralization of obese children and adolescents may not be sufficient to meet the skeletal strength requirements that their higher body mass may need. Therefore obesity is not only nonprotective against fractures in this population but may also increase fracture risk because of the high mechanical load carried by the obese child/adolescent. After diet- and exercise-induced weight loss, abnormalities in serum levels of calcidiol and PTH are reversed, indicating that the aberrations seen in obese children are the consequence and not the cause of obesity.²²¹ It is of interest that the adipocyte and osteoblast are derived from a common mesenchymal stem cell under the direction of different stimuli.²²³ Osteoblastogenesis results when the stem cell progresses along the WNT-Frizzled- β -catenin signal transduction pathway, whereas adipogenesis occurs after stimulation of the stem cell by peroxisome proliferator-activated receptor gamma (PPAR- γ). Whether this dichotomy in cell differentiation plays a role in modulating the effects of obesity on bone mineralization in children is unknown at present. The site of fat deposition may also influence bone mineralization; in adults, abdominal and bone marrow fat may exert adverse effects upon this process.

Evaluation of the child with nutritional rickets includes historical review of dietary intake and sunlight exposure and illnesses encountered, physical examination (documenting deformities of the chest and extremities), roentgenograms of the skeleton (widening of the epiphyses, lucent areas of unmineralized osteoid extending into the metaphyses), and laboratory assessment: complete blood count (hemoglobin, hematocrit), comprehensive chemical measurements (including total calcium, magnesium, phosphorus, bone alkaline phosphatase, creatinine, calcidiol, PTH); spot urinary determination of calcium, creatinine. Nutritional rickets is effectively treated in most subjects by oral doses of vitamin D (cholecalciferol or ergocalciferol): neonates—2000 IU/day for 6 weeks; infants below 1 year of age—2000/day for 6 weeks; children over 1 year of age—2000 IU/day for 8 weeks with the subsequent maintenance dose of 600 to 1000 IU/day.^{212,225} At the beginning of treatment, elemental calcium (30–75 mg/kg/d in 3 divided doses per day) must also be administered to the vitamin D-deficient child receiving vitamin D to avoid the hypocalcemia that accompanies rapid remineralization of bone matrix (the hungry bone syndrome). An alternative therapeutic regimen for treatment of vitamin D-deficient rickets has been the administration of a single oral (or intramuscular) dose of 150,000 to 600,000 units of vitamin D₃ depending on patient age and other individual circumstances.^{9,205} During treatment periodic determinations of total and/or bone-specific alkaline phosphatase values are an effective tool for monitoring the efficacy of treatment as levels decline progressively as the rachitic lesions heal. To avoid hypocalcemia or hypercalcemia, hypercalciuria, and nephrocalcinosis, serial determinations of serum values of calcium, phosphate, and creatinine and urinary excretion of calcium and creatinine should be determined. Sequential radiographs document reversal of rachitic changes (see Fig. 20.9). After completion of therapy and radiographic evidence of healing of the rickets, the vitamin D dose may be lowered to 400 to 600 IU/day; calcidiol values should then be measured prospectively to be certain that the child continues to receive adequate amounts of vitamin D.²⁰⁷

Rickets resulting primarily from low dietary intake of calcium has occurred in infants who ingest low-calcium containing formulas and in children from developing countries receiving a diet with 200 mg (or less) of elemental calcium per day despite normal intake of phosphate and adequate endogenous stores of vitamin D as determined by serum calcidiol levels.²⁰⁵ Calcium deficiency rickets also occurs in the United States if infants are weaned to low-calcium containing foods after completion of breastfeeding. Calcium deficiency rickets may also develop as a consequence of impaired intestinal absorption of dietary calcium because of intrinsic factors or that it has been bound by ingestion of high fiber and phytate containing cereals.²⁰⁶ Calcium deficiency is best addressed by its prevention, ensuring adequate intake of this element according to established guidelines for growing infants, children, and adolescents (recommended calcium intakes: 400–600 mg/day in infants; 700 mg/day in children below 4 years of age; 1000–1300 mg/day in older children and adolescents). When present, calcium deficiency rickets may be effectively treated by ensuring an intake of 1000 to 1500 mg of elemental calcium daily for 6 months with provision of normal amounts of vitamin D by sunlight exposure or supplementation. Codeficiencies of calcium and vitamin D may occur in some children.

Dietary phosphate deficiency is unusual because this anion is present in large amounts in most foods. Hypophosphatemia may be caused by decreased intestinal absorption of phosphate (e.g., starvation, premature infants drinking human breast milk without supplemental phosphate, older infants ingesting a formula very low in phosphate content, subjects ingesting large amounts of aluminum-containing antacids as aluminum

and phosphate coprecipitate in the intestinal tract, patients with the short bowel syndrome, or vitamin D deficiency), administration of parenteral nutrition with fluids low in phosphate, intravenous infusion of iron, increased renal excretion of phosphate in patients with depressed renal tubular reabsorption of this anion, and movement of phosphate from the extracellular fluid space to the intracellular compartment (vide infra).²²⁶ In very premature infants receiving long-term parenteral nutrition, development of metabolic bone disease is frequent and related not only to deficiencies of calcium, phosphate, and vitamin D but also to excess aluminum in the infusates. Infants receiving large amounts of aluminum containing antacids over prolonged periods for treatment of gastroesophageal reflux may also have low bone mass. Aluminum lowers the rate of bone formation by several mechanisms: administered orally aluminum binds intestinal phosphate, thereby impeding its absorption leading to phosphate depletion; administered intravenously during total parenteral nutrition or during hemodialysis, aluminum inhibits osteoblastic function and prevents mineralization of osteoid; it also impairs the secretion of PTH and decreases 25OHD-1 α hydroxylase activity.²²⁷ Patients requiring total parenteral nutrition should receive as much calcium and phosphate as can be administered safely, as well as supplemental vitamin D. Periodic measurements of serum levels of calcium, phosphate, alkaline phosphatase, PTH, and calcidiol, and serial skeletal radiographs and estimations of skeletal mineralization are recommended in patients receiving total parenteral nutrition; if metabolic bone disease develops in spite of these efforts, serum aluminum levels should be measured and if elevated (>100 mcg/L) a search for the source of the aluminum should be initiated and that product eliminated from the infusate if possible. Cadmium, fluoride, and saccharated ferric oxide are also able to impede normal bone mineralization.²²⁷

Metabolic and functional defects of vitamin D lead to rarer forms of rickets (see Tables 20.8A, 20.8B).^{212,228} Biallelic loss-of-function mutations in *CYP2R1* (OMIM 608713), the hepatic 25-hydroxylase enzyme that converts cholecalciferol (vitamin D₃) to 25-hydroxycholecalciferol (calcidiol), result in vitamin D hydroxylation-deficient rickets type 1B (also termed *vitamin D-dependent rickets type 1B*—OMIM 600081). Rickets caused by a defect in 25-hydroxylation has been described in two brothers of Nigerian origin in whom a homozygous loss-of-function mutation (p.Leu99Pro) in *CYP2R1* eliminated the hydroxylase activity of this 501 aa protein.²²⁹ The deleterious homozygous mutation (p.Gly42_LeudelinsArg) in *CYP2R1* has also been identified.²³⁰ 25-Hydroxylase deficiency transmitted as a dominant disorder not because of an identified mutation in the coding exons or intronic regions of *CYP2R1* has been reported also.²³¹ Vitamin D 25-hydroxylation-deficient rickets type 1B is manifested by clinical and radiological signs of rickets in association with hypocalcemia, hypophosphatemia, hyperalkaline phosphatasia, high PTH, and high calcitriol, and very low calcidiol values; heterozygous carriers of a deleterious mutation in *CYP2R1* have reduced levels of calcidiol but do not develop rickets.²³² Affected subjects respond to administration of calcidiol (as well as calcitriol) with improvement in clinical symptoms, normalization of biochemical abnormalities, and increase in vertebral bone mineralization.²³⁰

Vitamin D hydroxylation-deficient rickets, type 1A ([OMIM 264700]—vitamin D-dependent rickets type 1A [VDDR1A] or pseudovitamin D-deficiency rickets type 1A [PDDR1A]) is caused by homozygous or compound heterozygous inactivating mutations of *CYP27B1* (OMIM 609506) encoding the enzyme in the renal proximal tubule that catalyzes 1 α -hydroxylation of carbon-1 of calcidiol converting it to 1,25-dihydroxyvitamin D₃ (calcitriol), the biologically active metabolite of vitamin D.²³³ Although apparently normal at

birth, bone deformities (bowing of the forearms), growth retardation, muscle weakness or spasms, delayed motor milestones (walking), and/or hypocalcemic seizures develop between 6 and 30 months of age in patients with VDDR1A; physical signs of rickets (metaphyseal prominence, rachitic rosary, craniotabes), and hypoplasia of dental enamel is often present; biochemically, hypocalcemia, hypophosphatemia, hyperphosphatemia, markedly elevated serum levels of PTH, normal serum concentrations of calcidiol and inappropriately low calcitriol levels are recorded; radiographs reveal rachitic deformities of the long bones.^{232,234} The diagnosis of VDDR1A is established by the presence of normal serum concentrations of calcidiol whereas calcitriol values are extremely low and do not increase after administration of vitamin D or calcidiol and is confirmed by identification of the mutation in *CYP27B1*. Treatment goals are restoration of eucalcemia without hypercalciuria resulting in increased muscular strength and healing of the rachitic lesions permitting normal growth. The clinical, biochemical, and radiographic manifestations of VDDR1A resolve completely and reasonably rapidly following treatment with physiological amounts of calcitriol (10–20 ng/kg/d). Lifelong therapy is necessary, but effective and compliant treatment leads to normalization of all biochemical parameters, normal linear growth and adult height, and normal bone mineralization. The dose of calcitriol often needs to be increased during pregnancy; however, gestation is uncomplicated and the offspring of affected mothers are normal.²³⁴ VDDR1A is an autosomal recessive disease found with high frequency in a Quebec French-Canadian population but occurs in all races and in diverse geographic regions. *CYP27B1* encodes a 508 aa mitochondrial cytochrome P450 hydroxylase with conserved sites that binds ferredoxin and heme and uses electrons from reduced nicotinamide adenine dinucleotide phosphate and oxygen to convert calcidiol to calcitriol.²²⁸ Many loss-of-function missense, nonsense, splicing, and duplication or deletion/frameshift mutations in *CYP27B1* lead to inactive or truncated protein products that are unable to bind either substrate (calcidiol) or heme, the latter defect preventing electron transfer and inhibiting catalysis. The most common mutation in *CYP27B1* in the Quebec French-Canadian population at risk for VDDR1A is deletion of guanine at nucleotide 958 (exon 2, codon 88) (c.958delG) that changes the reading frame resulting in premature termination of translation and an inactive product (the Charlevoix mutation). A second common mutation in this population is duplication of a 7-base pair sequence in exon 8; however, this mutation is also found in patients of Asian and Hispanic ethnicity.

Biallelic loss-of-function mutations in *VDR* (OMIM 601769) encoding the VDR result in resistance to the physiological effects of calcitriol and vitamin D-resistant rickets type 2A (OMIM 277440).^{228,233} Clinical and biochemical manifestations of resistance to calcitriol include severe infantile-onset bony deformities characteristic of rickets (bowing of the limbs, widening of the distal ends of the long bones, frontal bossing, beading of the costochondral junctions of the rib cage), growth retardation, varying severity of alopecia, hypocalcemia, hypophosphatemia, hyperalkaline phosphatasia, and extraordinarily high serum concentrations of calcitriol (300–1000 pg/mL) and PTH; serum levels of 25-hydroxyvitamin D are normal whereas those of 24,25-dihydroxyvitamin D are often low (see Table 20.9).²³⁵ The high calcitriol values reflect the combined stimulatory effects of hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism on the activity of 25-hydroxyvitamin D₃-1 α -hydroxylase together with decrease in its rate of catabolism because of depressed activity of calcitriol-dependent 1,25 α -dihydroxyvitamin D₃ 24-hydroxylase. Radiographs demonstrate signs of rickets, including metaphyseal cupping and fraying and tibial and

femoral bowing. Genotyping reveals homozygous or compound heterozygous mutations in *VDR* (Fig. 20.10). Intravenous infusions of calcium (0.4–1.4 g/m²/d) have been helpful in restoring normal growth and healing of the rickets but mandate careful monitoring, often in hospital, to avoid cardiac arrhythmias, hypercalciuria, nephrocalcinosis, and related complications. Oral administration of high doses of calcitriol (1–6 mcg/day in 2 divided doses) together with elemental calcium (1–3 g/day) have been used in the treatment of these patients who also require close follow-up and periodic measurements of serum and urinary calcium values.²¹² During treatment, serum values of calcium, phosphate, alkaline phosphatase, creatinine, and PTH, urinary calcium and creatinine excretion, skeletal radiographs, and renal ultrasounds for development of nephrocalcinosis are monitored serially. In patients refractory to oral therapy, continuous intravenous or intracaval administration of large amounts of calcium (0.4–1.4 g of elemental calcium/m²/d) normalizes calcium, phosphate, alkaline phosphatase, and PTH values, heals rickets, and increases growth rate in selected children. If this mode of therapy is used, careful monitoring for catheter sepsis and cardiac arrhythmia, as well as hypercalcemia, hypercalciuria, and nephrocalcinosis is mandatory. After healing of the rickets by parenteral calcium, maintenance therapy with large doses of oral calcium (3.5–9 g of elemental calcium/m²/day) is appropriate. In younger infants with vitamin D-resistant rickets before development of florid rickets, high doses of oral calcium

may ameliorate the rachitic process. These clinical observations suggest that the major defect in patients with vitamin D-resistant rickets is lack of calcium. With treatment, growth may normalize in patients with vitamin D resistance, but alopecia if present is unlikely to resolve.²³⁶ An alternative therapeutic management of vitamin D resistance has been the administration of cinacalcet, a calcimimetic that activates the CaSR in the parathyroid gland thereby lowering the secretion of PTH; short-term (2 months) administration of cinacalcet to a 3-year-old child with vitamin D resistance enabled relatively long-term resolution of the clinical, biochemical, and radiographic manifestations of this disease with maintenance therapy of thrice weekly infusions of calcium.²³⁷ Interestingly, although binding of calcitriol to the VDR is essential for expression of its function in most tissues and hair is dependent on the VDR for normal function of skin keratinocytes and outer root-sheath cells and physiological cycling of the hair follicle, the hair follicle cycle does not require calcitriol; thus maintenance of the normal pattern of hair follicle cycling between phases of rest and growth is likely a ligand-independent function of the VDR achieved by interacting with β -catenin.²³⁸

Although striking in infancy and early childhood, clinical manifestations of vitamin D resistance may vary, and patients with milder defects in *VDR* may not be identified until adolescence or adulthood. In addition, spontaneous remission of the rachitic process may occur, most often between 7 and 15 years of age or when the patient enters puberty.²³⁶ Indeed, after

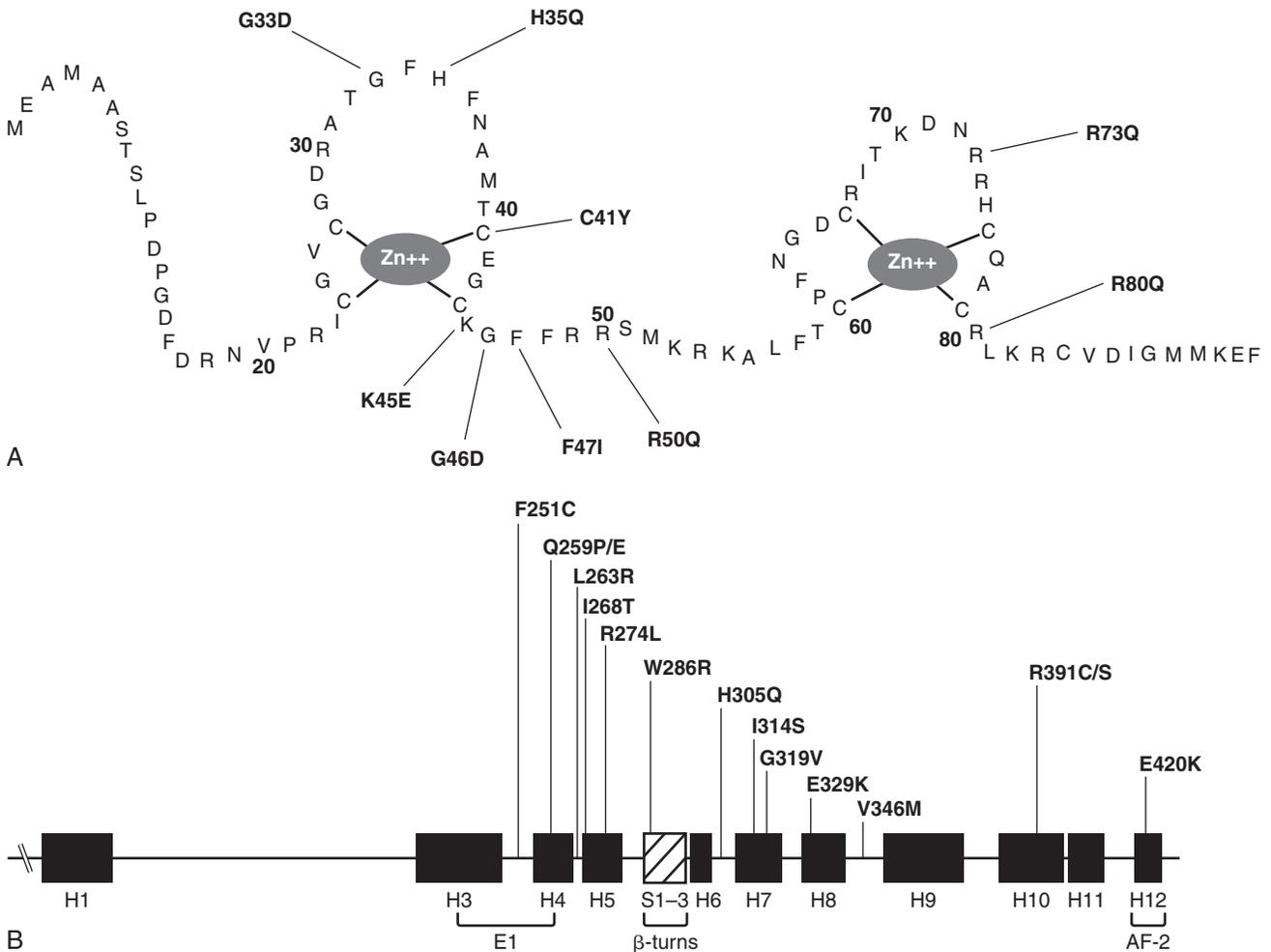


Fig. 20.10 Mutations in the vitamin D receptor (VDR) in patients with end-organ insensitivity to vitamin D. (From Malloy PJ, Feldman D. Genetic disorders and defects in vitamin D actions. *Endocrinol Metab Clin North Am.* 2010;39:333–346.)

puberty in many patients with vitamin D-resistant rickets, serum levels of calcium, phosphate, and alkaline phosphatase normalize and calcium supplementation is no longer needed.²³⁹ Apparently, the skeletal requirement for calcium declines after completion of bone growth, and vitamin D-independent mechanisms of intestinal calcium absorption develop. In a study of 17 patients with vitamin D-resistant rickets between 1.5 and 37 years of age, biochemical parameters improved, the need for supplemental calcium declined, and BMD by DXA normalized in the latter half of the second decade of life, whereas serum calcitriol values remained elevated.²³⁹ Examination of intestinal calcium absorption revealed that the fractional absorption of calcium, while ingesting a low calcium diet, was substantially lower than control subjects in young patients; in patients 18 to 26 years of age, the fractional absorption of calcium was significantly higher than control values; in adult patients, the fractional absorption of calcium was similar to control data. Nevertheless, the growth impairing effects of severe childhood rickets persisted into adulthood in the majority of patients. The VDR is composed of DNA-, ligand-, and retinoic X receptor (RXR)-binding domains and a transactivation domain to which many comodulators of VDR function are recruited. The VDR and RXR bind as a heterodimeric transcription activating complex. Loss-of-function mutations have been found in each VDR domain: thus the mutated VDR may be unable to bind calcitriol because of decreased receptor number or affinity for ligand, incapable of forming heterodimers with RXR or of translocating to the targeted gene in the nucleus, unable to bind to the vitamin D response element (VDRE) or to initiate gene transcription once bound to the VDRE. More than 30 distinct heterozygous mutations in VDR have been identified scattered through the entire VDR protein but with a large number of variants in the ligand binding domain.²²⁸ In general, patients with vitamin D-resistant rickets without alopecia may be more responsive to treatment.

Vitamin D-dependent rickets, type 2B (VDRR2B, OMIM 600785) is a rare, second form of vitamin D-resistant rickets with a phenotype similar to that of patients with a mutated VDR but whose vitamin D nuclear receptor is intact. Overexpression of *HNRNPC* (OMIM 164020), encoding a nuclear ribonucleoprotein C that binds to the VDR/RXR-vitamin D response element (5') upstream of the vitamin D targeted gene and impairs target gene transcription, has been observed in a patient with VDRR2B.²⁴⁰ The mechanism by which overexpression of an otherwise normal ribonucleoprotein occurs and the relationship of this observation to the pathogenesis of VDRR2B are unknown. A variant of *CYP3A4* (OMIM 124010) encoding a hepatic CYP450 enzyme that rapidly oxidatively metabolizes and thereby inactivates calcidiol and calcitriol also results in decreased functional activity of these metabolites of vitamin D, leading to rickets in affected patients who require high doses of calcitriol for therapeutic management. (This disorder has been designated *vitamin D-dependent rickets type 3*.)

Phosphopenic Rickets. Serum concentrations of phosphate decline with age through childhood and adolescence; thus between 0 and 5 days serum phosphate levels range between 4.8 and 8.2 mg/dL; 1 and 3 years—3.8 to 6.5 mg/dL; 4 and 11 years—3.7 to 5.6 mg/dL; 12 and 15 years—2.9 to 5.4 mg/dL; 16 and 19 years—2.7 to 4.7 mg/dL.²³² Acute hypophosphatemia is associated with irritability, confusion, paresthesias, muscle weakness, and ileus; chronic hypophosphatemia leads to rickets in the growing child. Hypophosphatemia in childhood may be caused by hereditary or acquired disorders (see Tables 20.8A, 20.8B and see Fig. 20.12).²²⁶ Hypophosphatemia may be the consequence of the movement of extracellular phosphate into cells (during treatment of diabetic ketoacidosis

with insulin as the intracellular phosphorylation of carbohydrates increases, acute respiratory alkalosis caused by hyperventilation, sepsis), impaired intestinal absorption of phosphate (vitamin D deficiency, chronic ingestion of magnesium or aluminum containing antacids, chronic diarrhea and/or steatorrhea), or increased renal phosphate wasting (secondary to increased secretion of PTH or primary caused by intrinsic abnormalities in the mechanisms that control renal tubular reabsorption of this anion). Hypophosphatemia is observed in nutritionally deprived subjects, such as the LBW neonate or older patient who is dependent on parenteral alimentation, those with severe anorexia, or those who are addicted to alcohol. Hypophosphatemia and metabolic bone disease may occur in infants and children receiving an amino acid based elemental formula with decreased bioavailable phosphate. Hypophosphatemia occurs in many critically ill patients, especially those with sepsis. Rapid remineralization of osteopenic bone is accompanied by hypophosphatemia and hypocalcemia (hungry bone syndrome). Most frequently, chronic hypophosphatemia is the result of hyperphosphaturia—either because of a primary or secondary defect in the regulation of renal tubular phosphate reabsorption in the proximal (where 60%–70% of the filtered load of phosphate is reabsorbed) or distal (where 10%–15% of filtered phosphate is reabsorbed) renal tubule.^{226,241} Thus increased secretion of PTH resulting either from primary hyperparathyroidism or secondary to vitamin D deficiency or other cause can lead to hyperphosphaturia and hypophosphatemia. The Fanconi syndromes are characterized by multiple functional renal tubular defects including abnormalities of phosphate reabsorption. Renal tubular defects that specifically impede the processes through which phosphate is reabsorbed by the renal tubules include abnormalities of transcellular transport of phosphate and excessive production of phosphaturic substances other than PTH, such as FGF23 and other phosphatonins and their cofactors.

In developed countries where severe vitamin D deficiency rickets is not a common problem, X-linked dominant hypophosphatemic rickets (XLHR, OMIM 307800) caused by inactivating variants of phosphate-regulating Endopeptidase homologue (*PHEX*, X-linked—OMIM 300550) is the most frequent form of rickets encountered (1:20,000 births). X-linked dominant hypophosphatemic rickets affects hemizygous males and heterozygous females similarly because of random inactivation of the X chromosome, albeit with substantial inter- and intrafamilial variability in clinical expression. X-linked dominant hypophosphatemic rickets is usually manifested within the first year of life, initially with bowing of the ulna and radius. Physical findings in children with XLHR include short stature, genu varum or valgum that develops when the infant begins to walk, flaring of the metaphyses, rachitic rosary, frontal bossing; increased frequency of dental decay and/or periradicular abscesses in teeth free of caries; bone, muscle, and joint stiffness, aching and occasionally pain. Craniotabes, tetany, and muscular weakness are not found in patients with XLHR as they are in those with vitamin D deficiency. Although they are no longer growing, adults with XLHR have osteomalacia, bone pain, increased fracture rate, and frequent dental abscesses, nephrocalcinosis, and hearing impairment; they may also develop stenosis of the spinal canal and progressive ankylosis of the vertebrae; osteotomies, decompressive laminectomies, and replacement of hip and knee joints are frequently necessary in this population.²⁴² Enesopathy—calcification of tendons, ligaments, and joint capsules—is common in adults with XLHR but may also occur in children with this disorder. The clinical expression of XLHR may vary widely between affected families and between affected children in the same family.²⁴³

Hypophosphatemia, hyperphosphaturia, normocalcemia, hyperalkaline phosphatasia, normal or just slightly elevated

concentrations of PTH, normal calcidiol values, and inappropriately low levels of calcitriol for the degree of hypophosphatemia are present in these patients within the first several months after birth.²³² Because phosphate induces differentiation, maturation, and apoptosis of hypertrophic chondrocytes in the cartilage growth plate of long bones, hypophosphatemia is associated with disorganized overgrowth of chondrocytes. Thus radiographically, distal femoral and proximal tibial metaphyseal flaring is a prominent finding as is bowing of the femora and tibiae in association with knee pain. Children with XLHR who have knee pain but normal radiographs may have bony abnormalities identifiable by magnetic resonance imaging. The axial skeleton may have a dense appearance.

PHEX encodes a zinc metallopeptidase that regulates serum concentrations and urinary excretion of phosphate.²⁴³ *PHEX* is a 22 exon gene that encodes a 749 aa integral membrane protein with a very long extracellular (702 aa) and short transmembrane (27 aa) and intracellular (20 aa) domains. The extracellular domain has 10 conserved cysteine residues and a pentapeptide motif (His-Glu-Phe-Thr-His) characteristic of zinc metallopeptidases that may either convert propeptides to active forms or degrade and inactivate their substrates. *PHEX* is expressed on the surface of osteoblasts and osteocytes, as well as by teeth, muscle, lung, liver, testis, and ovary; *PHEX* transcription by osteoblasts is downregulated by calcitriol. In most patients with XLHR, inactivating mutations of *PHEX* have been found primarily in the extracellular domain and include frameshift deletions (16%), duplications, insertions (8%), deletional-insertional, splice site (15%), nonsense (27%), and missense (34%) mutations, many in exons 15 and 17; a mutation has also been identified in the 5'-untranslated region (A \Rightarrow G transversion 429 bp upstream of the ATG initiation site) (Fig. 20.11).²⁴⁴ Because *PHEX* is a glycosylated protein, failure of glycosylation leads to its sequestration within the endoplasmic reticulum thus impeding its movement (trafficking) to the cell membrane; other mutations interfere with the catalytic function of the protein or its three-dimensional conformation. Mutations in *PHEX* arise spontaneously in more than 20% of patients with XLHR. There is no correlation between the location or type of mutation in *PHEX* and the clinical manifestations or severity of the disease.^{243,244}

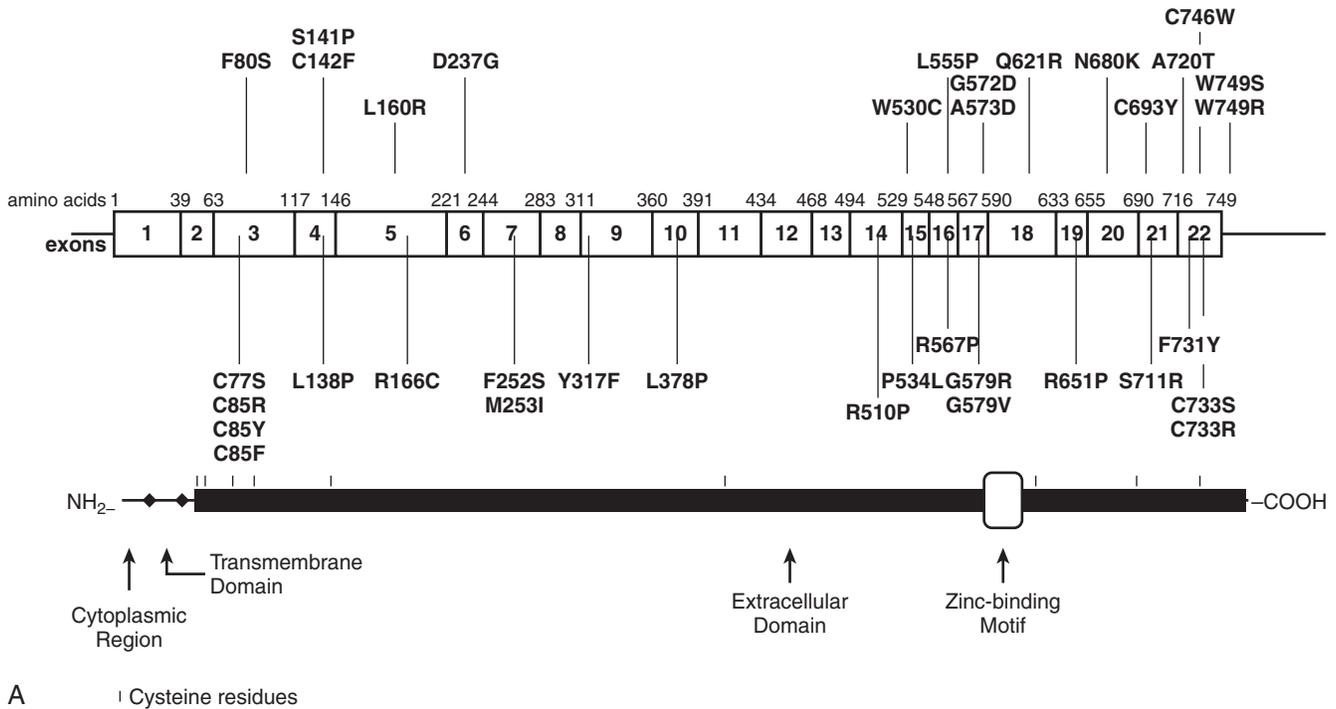
PHEX is a neutral endopeptidase among whose principle substrates are the bone-derived SIBLING family of proteins that include matrix extracellular phosphoglycoprotein (MEPE, OMIM 605912), dentin matrix acidic phosphoprotein 1 (DMP1, OMIM 600980), osteopontin/secreted phosphoprotein 1 (OMIM 166490), integrin-binding sialoprotein (OMIM 147563), and dentin sialophosphoprotein (OMIM 125485), all of whose genes are clustered on the long arm of chromosome 4. SIBLING proteins are secreted into extracellular matrix during mineralization, bind to calcium and regulate biomineralization.^{245,246} MEPE is a 525 aa noncollagenous protein secreted by osteoblasts that is present in extracellular bone matrix, teeth, and brain. As well as affecting biomineralization, MEPE inhibits renal tubular uptake of phosphate and depresses synthesis of calcitriol and is thus a phosphatonin, agents with these dual properties. MEPE and other SIBLING proteins contain an acidic serine aspartate-rich MEPE-associated motif (ASARM = minihibin) within their carboxyl terminal regions that is released by cathepsin B and its serine residue may then be phosphorylated; phosphorylated (p)ASARM binds avidly to hydroxyapatite. *PHEX* binds to MEPE and prevents its proteolytic digestion by cathepsin B, thereby inhibiting release and phosphorylation of ASARM. In the absence of normal *PHEX*-MEPE binding, ASARM is released from MEPE by cathepsin B; in patients with XLHR, serum ASARM levels are increased. Serine-phosphorylated pASARM coats the surface of hydroxyapatite by interacting with its calcium atoms and thus impairs

further deposition of calcium and phosphate and thereby inhibits mineralization. pASARM is also a substrate for the enzymatic activity of *PHEX*, which proteolytically cleaves the peptide between serine-glutamate and serine-aspartate residues; by doing so, *PHEX* destroys pASARM and prevents pASARM-mediated inhibition of bone mineralization. Although nonphosphorylated ASARM also binds (weakly) to hydroxyapatite and inhibits mineralization and can be enzymatically cleaved by *PHEX*, the role of nonphosphorylated ASARM in the mineralization process is as yet incompletely understood. Interestingly, ASARM peptides also impair renal tubular reabsorption of phosphate as pASARM increases expression of *FGF23* (OMIM 605380) in bone cells.²⁴⁵⁻²⁴⁷ Inasmuch as ASARM motifs are present in all SIBLING proteins, pASARM is also derived from osteopontin providing another source of a peptide that is able to bind to and inhibit mineralization of hydroxyapatite; osteopontin-derived pASARM is also a *PHEX* substrate.^{245,248} Accordingly, in XLHR, the primary pathophysiological abnormalities appear to be the failure of mutated, bioinactive *PHEX* to: (1) bind to MEPE, thereby inhibiting cathepsin B-mediated proteolytic cleavage of MEPE and release of ASARM; (2) destroy the pASARM derived from MEPE, thereby permitting pASARM to coat hydroxyapatite and inhibit mineralization; (3) degrade ASARM peptides thus enabling them to depress renal tubular reabsorption of phosphate; and (4) suppress expression of *FGF23* (vide infra).²⁴⁹

Although serum levels of total and ionized calcium are normal in patients with XLHR, hypophosphatemia is marked because of its urinary wastage because of substantially decreased renal tubular reabsorption of filtered phosphate and limited intestinal absorption of this anion. Serum concentrations of PTH and calcidiol are normal, but calcitriol values are inappropriately low for the degree of hypophosphatemia; serum alkaline phosphatase activity is increased as are serum levels of *FGF23* (Fig. 20.12).²⁵⁰ Many of these biochemical abnormalities are present in the affected patient within the first several months after birth. Because the serum ionized calcium concentration is normal, secondary hyperparathyroidism does not occur unless the patient with XLHR receives excessive amounts of supplemental phosphate. Normally, phosphate induces differentiation and death of hypertrophic chondrocytes in the cartilage growth plate by activating the mitochondrial apoptotic pathway that is caspase 9 dependent.^{233,251} Hypophosphatemia leads to delayed loss and increased numbers of hypertrophic chondrocytes and expansion of the growth plate characteristic of rickets. Bone histomorphometry in XLHR reveals unmineralized osteoid accumulating along the trabeculae within cancellous bone (i.e., osteomalacia) and hypomineralized periosteocytic lesions characteristic of this disorder.

FGF23 (OMIM 605380) is a potent phosphaturic agent produced by osteoblasts and osteocytes whose serum concentrations are elevated in patients with XLHR and related inversely to those of phosphate. Pathophysiologically, *FGF23* is responsible for the increased urinary excretion of phosphate characteristic of this disease. Patients with either congenital or acquired disorders that are associated with increased *FGF23* synthesis usually have hypophosphatemia together with normal or low levels of calcitriol, the latter leading to secondary hyperparathyroidism.²⁵² Inasmuch as expression of *FGF23* is downregulated by *PHEX*, inactivating variants of *PHEX* lead to increased production of *FGF23*.^{232,245} In addition, *FGF23* suppresses 1 α -hydroxylation of calcidiol and thus decreases endogenous synthesis of calcitriol impeding intestinal absorption of dietary calcium. *FGF23* inhibits phosphate reabsorption by the renal tubule and when present in excess leads to hyperphosphaturia and consequent hypophosphatemia.²⁵³ *FGF23* is synthesized as a 251 aa protein; after removal of the 24 aa signal sequence, mature *FGF23* circulates as both 227 and 154 aa

PHEX Missense Mutations



PHEX Frameshift Mutations

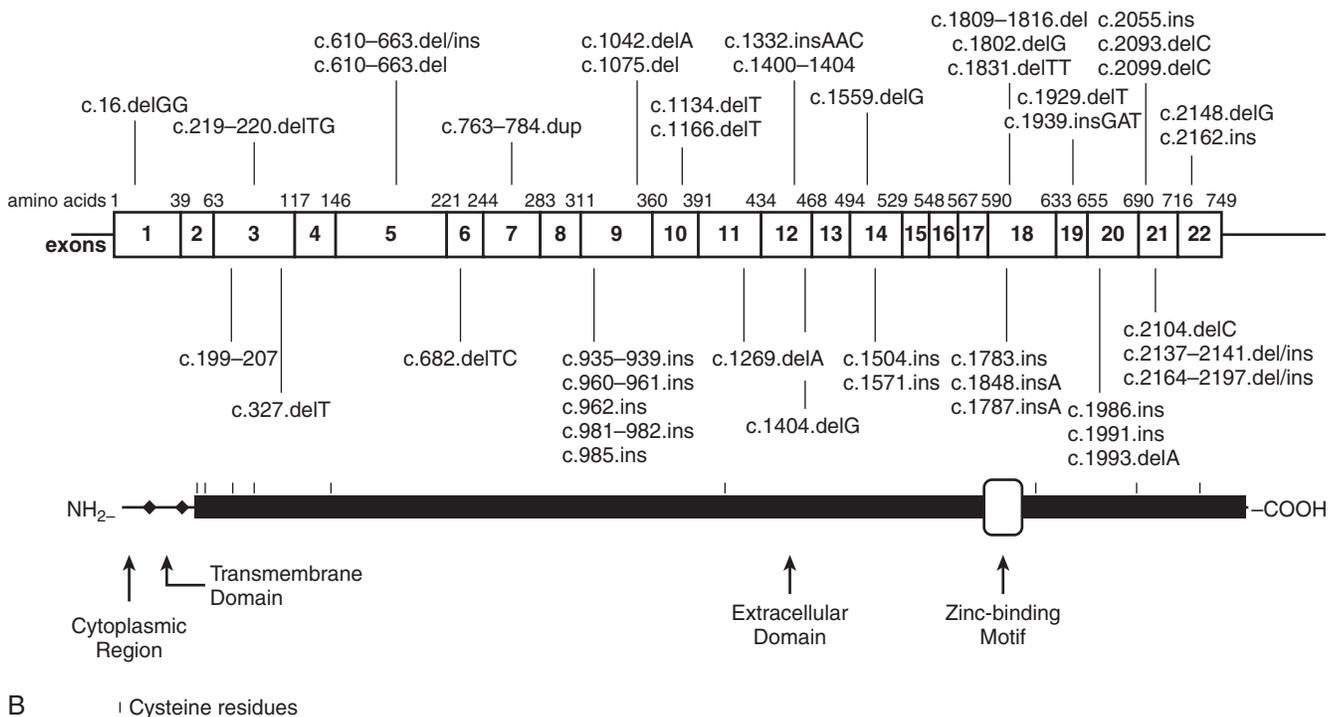


Fig. 20.11 Missense (A) and frameshift (B) mutations in PHEX resulting in X-linked recessive hypophosphatemic rickets. (From PHEXdb, McGill University, Montreal, Quebec, www.phexdb.mcgill.ca/Missense.html and www.phexdb.mcgill.ca/Frameshift.html.)

glycosylated peptides. Acting through fibroblast growth factor receptor 1 (FGFR1) and its coreceptor (α -klotho), FGF23 inhibits sodium-dependent phosphate uptake by renal proximal tubular cells and depresses 25OHD-1 α hydroxylase activity and thus synthesis of calcitriol; thus FGF23 is also a phosphatonin, a family of phosphaturic agents, as is MEPE and serum

frizzled related protein-4 (sFRP4).^{241,254} FGF23 inhibits phosphate reabsorption by downregulating expression of *SLC34A1* and *SLC34A3* that, respectively, encode type II (a and c) sodium-phosphate cotransporters (NPT2a, NPT2c) in the apical membranes of the proximal renal tubule by facilitating their internalization and removal from the renal tubule brush border

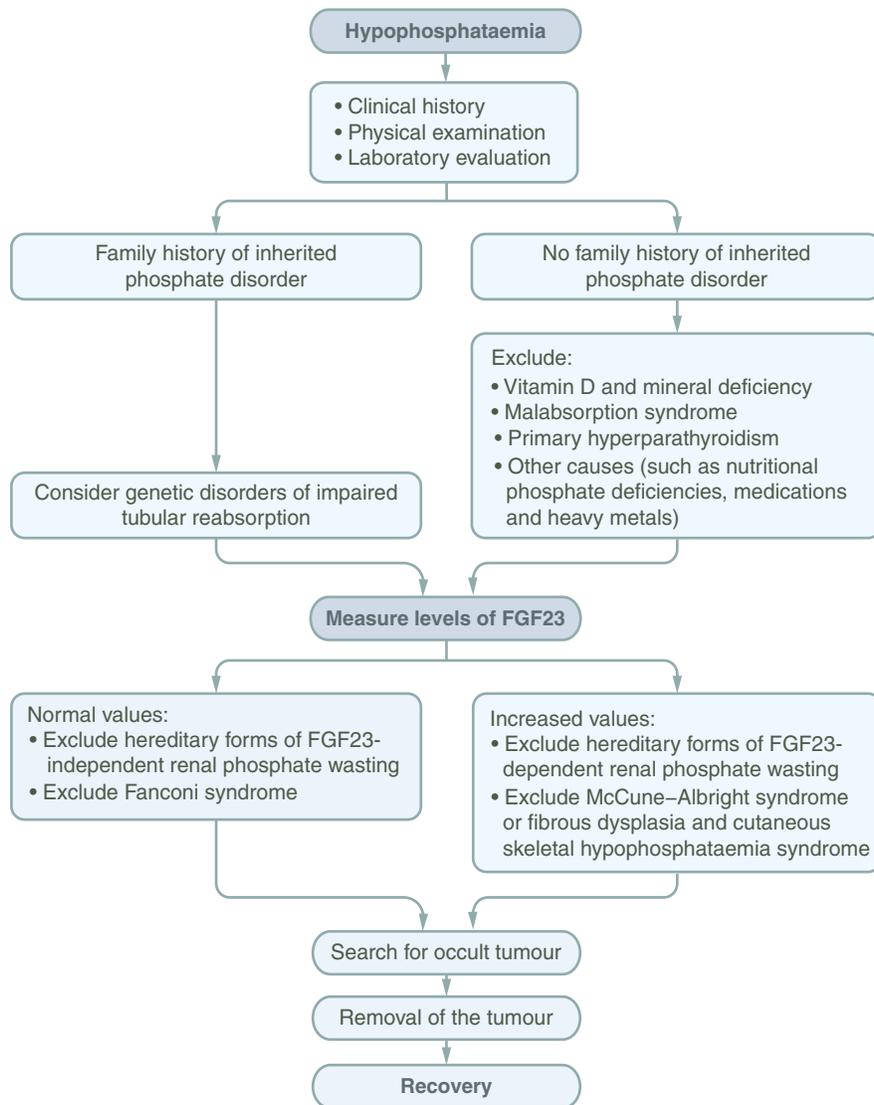


Fig. 20.12 Evaluation of the hypophosphatemic child. (From Minisola, S., Peacock, M., Fukumoto, S., et al. (2017). Tumour-induced osteomalacia. *Nat Rev*, 3, 17044. With permission.)

thereby depressing reabsorption of filtered phosphate.²⁴¹ FGF23 also downregulates expression of *CYP27B1* encoding 25-hydroxyvitamin D-1 α hydroxylase and thus decreases synthesis of calcitriol and induces *CYP24A1* which accelerates inactivation of vitamin D metabolites. *FGF23* is expressed and FGF23 is secreted primarily by osteoblasts and osteocytes; it is coexpressed with *PHEX* in these cells, and *PHEX* inhibits expression of *FGF23*.^{245,246} Although FGF23 is inactivated by cleavage between amino acids Arg179 and Ser180, it is not a biological substrate for *PHEX*.²⁵⁵ Rather, FGF23 is cleaved by subtilisin-like proprotein and furin-like convertases. Inasmuch as *PHEX* decreases expression of *FGF23*, in the absence of *PHEX*, the expression of *FGF23* is increased.²⁴⁵

The diagnosis of XLHR is established when the typical family history (if the patient is not the initial mutant), clinical findings (deformities of the lower extremities, flaring of the metaphyses), roentgenographic (rachitic changes) and laboratory data (hypophosphatemia, hyperphosphaturia, inappropriately low serum level of calcitriol, elevated FGF23 values, normal serum concentrations of PTH, calcium, creatinine, and 25OHD) are present and when other causes of hypophosphatemia and hyperphosphaturia have been excluded (see

Tables 20.8A, 20.8B, 20.9 and see Fig. 20.12). The renal excretion of phosphate may be assessed by measuring the tubular reabsorption of phosphate (TRP) ($1 - \frac{\text{urine phosphate} \times \text{serum creatinine}}{\text{serum phosphate} \times \text{urine creatinine}}$); lower limit of normal ranges between 75% and 85%.²³² In the presence of hypophosphatemia, the TRP is normally greater than 95%. Normally, serum FGF23 values are highest in young infants and decline during childhood and adolescence to adult values (approximately 30 pg/mL using an immunoassay that detects intact FGF23). Concentrations of FGF23 increase with phosphate loading and decline in response to phosphate deprivation.²⁵⁶ However, increased serum concentrations of FGF23 are present in patients with many forms of hypophosphatemic rickets including XLHR, autosomal dominant and recessive hypophosphatemic rickets, the disorders of McCune-Albright, Raine syndrome, opismodysplasia, osteoglyphonic dysplasia, and Jansen type metaphyseal chondrodysplasia, the linear nevus sebaceous syndrome, and tumor-induced rickets/osteomalacia (vide infra).^{232,257} Inasmuch as variants of other genes also result in hypophosphatemic rickets (vide infra), analysis of *PHEX* is occasionally required to confirm the diagnosis of XLHR. Rarely, somatic and germline mosaicism for a mutation

in *PHEX* may mimic autosomal dominant transmission of hypophosphatemic rickets.²⁵⁸

The goals of therapy in XLHR are to correct or minimize rickets/osteomalacia as assessed by resolution of skeletal deformities and radiographic abnormalities and normalization of alkaline phosphatase levels. Restoration of serum phosphate levels is not a treatment goal because doing so increases the risk of secondary hyperparathyroidism and nephrocalcinosis. Traditionally, patients with XLHR have been treated with calcitriol (20–40 ng/kg/d) administered in 2 daily doses with the larger dose given at night when PTH secretion tends to increase and a phosphate preparation (in doses ranging from 40–60 mg/kg/d beginning at a lower dose to avoid diarrhea administered in 4 to 6 divided daily doses depending on age, size, compliance, and response to therapy).²⁴³ Infants and young children may tolerate a phospho-soda solution more readily than other preparation; when able, most older children prefer a chewable phosphate tablet; acidic potassium phosphate products are preferred as they acidify the urine and increase the solubility of calcium phosphate. Calcitriol is available as an oral solution (1 mcg/mL) and as 0.25 and 0.5 mcg capsules. If hypercalcemia or hypercalciuria develops (urine calcium excretion more than 4 mg/kg/d), the dose of calcitriol should be lowered; if the rachitic process exacerbates, addition of an agent that increases renal tubular resorption of calcium (e.g., amiloride) may be added cautiously to the therapeutic program. However, compliance with the intense treatment process is problematic and complications (nephrocalcinosis) frequent. Repeated clinical and laboratory evaluations are necessary to avoid hypercalcemia, hypercalciuria, and nephrocalcinosis, and secondary hyperparathyroidism as high doses of phosphate may lead to counterproductive secondary (and sometimes tertiary) hyperparathyroidism. At least quarterly, clinical evaluation and measurement of serum and urine calcium levels, phosphate, alkaline phosphatase, creatinine, and intact PTH concentrations should be undertaken. Renal sonography before treatment and at 12-month intervals during therapy to identify an early stage of nephrocalcinosis and yearly skeletal radiographs to assess the degree of healing of the rickets are recommended. Development of nephrocalcinosis (and compromise of renal function in some patients) is directly related to the amount of phosphate the patient received as this agent increases counterproductively synthesis of FGF23; hyperoxaluria is also implicated in the pathogenesis of nephrocalcinosis. In a few children with XLHR, short-term administration of cinacalcet, a calcimimetic agent that suppresses secretion of PTH, has been useful in ameliorating the secondary hyperparathyroidism that may occur during treatment of these children. Comanagement of a patient with XLHR with an experienced orthopedist is important, as the orthopedist may prescribe braces or in patients with extreme and progressive deformities perform corrective surgery, such as femoral and tibial hemiepiphysectomies. Defects in the mineralization of dentin lead to dental abscesses and early tooth loss; thus these patients must practice dental hygiene and be monitored by an experienced dentist.

In 2018 the management of XLHR was altered remarkably when it was reported that neutralization of FGF23 with a recombinant human immunoglobulin (Ig)G1 monoclonal antibody to FGF23 (burosumab—administered subcutaneously every 2 to 4 weeks) was therapeutically effective in children with this disorder in whom conventional therapy had been withdrawn.^{259,260} Clinical (decrease in pain, increase in physical activity, increase in growth), radiographic (decline in Thacher rickets severity score), and biochemical improvement (increase in serum levels of phosphate [because of increase in its renal tubular reabsorption] and calcitriol and decline in alkaline phosphatase values) were recorded during administration of burosumab. Whether burosumab will become first-line

therapy for children with XLHR or will be used after a trial of calcitriol/phosphate is yet uncertain. Another agent that might prove useful in the treatment of patients with XLHR is the poly-arginine peptide hexa-D-arginine; in an experimental murine model of XLHR, hexa-D-arginine enhanced degradation of FGF23 into inactive fragments and suppressed FGF23 production thereby improving the primary bone disorder.²⁶¹ Also experimentally, inhibition of CYP24A1, the enzyme responsible for inactivation of calcidiol and calcitriol, substantially improved the skeletal defects in a mouse model of XLHR without altering serum levels of calcium or phosphate.²⁶²

Before the introduction of burosumab, the natural history of patients with XLHR has been that although their birth length was normal, growth rate slowed during the first several years of life leading to progressive shortening of height. Many children with XLHR were significantly short by 5 years of age, although some patients (particularly girls) with minimal involvement grew normally. Treatment of the older child with XLHR with calcitriol and phosphate improved growth rate, in part because of correction of the deformities of the lower extremities. During puberty, gain in height was normal in boys (+28.2 cm) and girls (+24.2 cm) with XLHR; thus the compromised adult stature in XLHR is related to impaired early childhood and preadolescent growth. In a series of 19 closely monitored children with XLHR, initiation of treatment with calcitriol and phosphate at a mean chronological age of 4.2 months (range 7 weeks to 6 months, $n = 8$) resulted in greater adult stature (-0.2 vs. -1.2 SDs) than when treatment began at a mean age of 2.1 years (range 1.3–8.0 years, $n = 11$) with no difference in complication rate (secondary hyperparathyroidism, nephrocalcinosis, craniosynostosis) between the two groups.²⁶³ Despite close adherence to treatment, many children with XLHR have heretofore remained stunted in height primarily because of short lower limbs and retain mild to moderate radiographic signs of rickets and mildly elevated serum alkaline phosphatase activities, although the extent of lower limb deformities is partially ameliorated by good compliance.²⁶⁴ Human GH increases glomerular filtration rate, renal tubular resorption and serum concentrations of phosphate, and the rate of accrual of bone mineral; it accelerates height velocity and the rate of increase in limb length of children with XLHR and may modestly increase adult stature.²⁶⁵ Many untreated adults with XLHR are hypophosphatemic; serum alkaline phosphatase activity is increased and osteomalacia is present on bone biopsy; these patients experience frequent dental abscesses, degenerative hip disease because of deformities of the lower limbs, nephrocalcinosis, and hearing impairment.²⁴² Occasionally, a female with XLHR may be clinically well despite isolated hypophosphatemia. Roentgenographic manifestations of XLHR in adults include degenerative changes in large joints, thickening of the spinous processes and fusion of the vertebrae, and stenosis of the spinal canal. Bone mineralization determined by single and dual photon absorptiometry tends to be normal in adults with XLHR (despite the histomorphological abnormalities), suggesting that most of these subjects are not at increased risk for osteoporotic fractures. However, in approximately 25% of adults with XLHR, there is clinical evidence of osteomalacia, such as progressive lower limb deformities, bone pain, fractures, enesopathies, and pseudofractures. Treatment of selected adult XLHR patients with calcitriol and phosphate may be beneficial. Many adults require osteotomies, replacement of hip and knee joints, and decompressive laminectomies for spinal stenosis.²⁴² Burosumab, the monoclonal antibody to FGF23, may also prove useful in the long-term management of adults with XLHR, although its role in the treatment of adults with this disorder is as yet uncertain.^{243,266}

X-linked recessive hypophosphatemic rickets (OMIM 300554) is associated with hypercalciuria, nephrocalcinosis

and renal failure; it is the consequence of loss-of-function mutations in *CLCN5* (OMIM 300008) encoding a voltage-gated hydrogen/chloride transmembrane exchange transporter expressed in the renal tubule and is independent of FGF23; p. Ser244Leu is a common variant of *CLCN5* associated with this disorder. Inactivating variants of *CLCN5* have also been identified in patients with Dent disease 1 (OMIM 30000—with amino aciduria, proteinuria, glycosuria, hypercalciuria, and nephrocalcinosis) and other renal tubulopathies.

Congenital disorders associated with hypophosphatemic rickets and increased FGF23 production may be related to variants of one of several autosomal or X-linked genes. Children with autosomal dominant hypophosphatemic rickets (ADHR, OMIM 193100) because of activating variants of *FGF23* itself have clinical, biochemical, and radiological evidence of rickets similar to those in patients with XLHR, although in some subjects the extent of disease expression may be minimal or even decline with age; patients with ADHR also manifest muscle weakness as a consequence of hypophosphatemia.^{249,252} Normally, FGF23 is inactivated by cleavage between aa Arg179 and Ser180 by subtilisin-like proteases, but gain-of-function variants of *FGF23* (p.Arg176Gln, p.Arg179Trp) prevent proteolytic cleavage of the intact protein and hence prolong its biological activity leading to persistent hyperphosphaturia and hypophosphatemia with normal or slightly low serum calcium concentrations and low or normal serum levels of calcitriol.^{241,250,257} Because iron deficiency enhances expression of *FGF23*, it is a stimulus to the development of clinical manifestations of ADHR.²³² Treatment involves administration of calcitriol and phosphate with close serial monitoring both for safety and because hyperphosphaturia may occasionally resolve spontaneously. Excessive ectopic synthesis of FGF23 by neoplasms also leads to hypophosphatemic rickets (vide infra).

Hypophosphatemic rickets with hyperparathyroidism (OMIM 612089) is a disorder characterized by onset of hypophosphatemia and diffuse parathyroid hyperplasia in infancy.²⁶⁷ Pathogenetically, it may be the result of excessive production of α -klotho (*KL*, OMIM 604824), the coreceptor with FGFR1(IIIc) for FGF23 that converts FGFR1(IIIc) into the specific FGF23 receptor that stimulates the FGF23 intracellular signaling pathway and its bioactivity. α -Klotho excess appears to enhance the production and bioactivity of FGF23 resulting in hyperphosphaturia and hypophosphatemia and also to mediate parathyroid gland hyperplasia and enhanced synthesis of PTH.²⁵³ In one patient with this disorder, excessive production of α -klotho has been attributed to a spontaneously occurring balanced translocation between the long arms of chromosomes 9 and 13 (–t[9;13][q21.13;q13.1]) possibly involving a promoter region that permitted excessive expression of *KL* and synthesis of α -klotho.²⁶⁷

Mutations in two genes result in autosomal recessive hypophosphatemic rickets (ARHR) that is clinically, biochemically, pathogenetically, and histomorphometrically similar to XLHR but is distinguished by its mode of transmission and development of osteosclerosis at the base of the skull and in calvarial bones. ARHR 1 (OMIM 241520) is caused by loss-of-function biallelic mutations in dentin matrix acidic phosphoprotein-1 (*DMP1*, OMIM 600980), a noncollagenous, serine-rich, bone matrix calcium-binding SIBLING protein synthesized by osteocytes, that also impairs expression of *FGF23*.²³² Without DMP1, osteocytes fail to mature and hydroxyapatite nucleation is hindered; a secondary increase in FGF23 synthesis by osteocytes leads to hyperphosphaturia, hypophosphatemia, and defective bone mineralization.²⁴⁵ In subjects with either ARHR1 or XLHR, bone biopsy reveals a distinct area of nonmineralized osteoid around each osteocyte. Paradoxically, some older patients with ARHR1 develop excessive bone mineralization of the basal and calvarial cranial bones not dissimilar to

that observed in patients with osteopetrosis.²⁴⁹ Type 2 ARHR (OMIM 613312) is the result of biallelic inactivating mutations in ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*, OMIM 173335), an enzyme that hydrolyzes extracellular ATP to cyclic AMP, releasing phosphate to form pyrophosphate, an inhibitor of bone matrix mineralization.^{250,257} (ENPP1 and TNSALP together regulate circulating levels of pyrophosphate. Thus the bone disease caused by deficiency of ENPP1 resembles that of hypophosphatasia [vide infra].) Inactivating variants of *ENPP1* result in secondary hypersecretion of FGF23 by an unclear mechanism that leads to hyperphosphaturia and hypophosphatemia, rickets, and ectopic calcifications.²⁵⁷ (Loss-of-function variants of *ENPP1* also result in autosomal recessive generalized arterial calcification of infancy [OMIM 208000].) ENPP1 increases renal expression of *KL* and is required for synthesis of α -klotho in the setting of a high phosphate load, an observation of possible pathophysiologic relevance in patients with ARHR2.²⁶⁸ Increased FGF23 secretion and hypophosphatemic rickets have also been recorded in subjects with osteoglophonic dysplasia (OMIM 166250—craniosynostosis, rhizomelic shortening of the limbs, noncalcifying bone lesions) associated with monoallelic activating mutations in *FGFR1* (OMIM 136350); Jansen type metaphyseal chondrodysplasia (OMIM 156400) caused by constitutively activating mutations in *PTH1R* (OMIM 168468); fibrous dysplasia/McCune Albright syndrome (OMIM 174800) in association with mutations of *GNAS* (OMIM 139320); the Schimmelpenning-Feuerstein-Mims/linear nevus sebaceous syndrome (OMIM 163200) related to postzygotic somatic gain-of-function variants of *NRAS* (OMIM 164790), *HRAS* (OMIM 190020), or *KRAS* (OMIM 190070); and opismodysplasia (a spondylo[epi]metaphyseal dysplasia with delayed ossification, micromelia, platyspondyly, vertebral hypoplasia severe short stature—OMIM 258480) caused by biallelic inactivating variants of *INPPL1* (OMIM 600829) encoding an inositol-1,4,5-trisphosphatase that hydrolyzes inositol-1,4,5-trisphosphate to inositol-4,5-bisphosphate, an important transducer of intracellular signaling.^{232,249,257,269,270} Family with sequence similarity 20, member C (*FAM20C*, OMIM 611061) encodes a phosphorylase whose substrates are the SIBLING family of proteins including DMP1. Loss-of-function mutations in *FAM20C* result in elevated levels of FGF23, leading to hypophosphatemic rickets or the often fatal disorder termed *Raine syndrome* that is associated with congenital sclerotic osteomalacia with cerebral calcifications (OMIM 259775). Raine syndrome is characterized by congenitally increased calcification of all bones (particularly those of the skull resulting in proptosis and midfacial hypoplasia) in association with periosteal formation of bone—a feature not found in patients with osteopetrosis or other sclerosing bone disorders and cerebral calcifications. Many patients with Raine syndrome die in infancy. *FAM20C* encodes a kinase (dentin matrix protein 4) that normally directly phosphorylates DMP1 that then suppresses transcription of *FGF23*; *FAM20C* also phosphorylates the Ser180 residue of FGF23, thereby inhibiting its glycosylation and promoting cleavage and inactivation of FGF23; hence, loss of *FAM20C* results in decreased suppression and inactivation of FGF23 resulting in its prolonged functional activity.^{232,271}

Tumor-induced osteomalacia/rickets is an acquired disorder because of excessive synthesis of one of several phosphatonins by a tumor of mesodermal origin. Many of the mesenchymal neoplasms synthesize and secrete FGF23, resulting in marked hypophosphatemia and impaired bone formation (tumor-induced osteomalacia).^{249,252,272} Other tumors express a form of *FGFR1*, the receptor for FGF23.²⁵² These neoplasms have also synthesized frizzled related protein-4, MEPE, and FGF 7, all of which can increase urinary phosphate excretion and suppress renal synthesis of calcitriol, albeit perhaps not to the same

extent as FGF23.^{254,273} The identification of an FGF23-secreting mesenchymal tumor is dependent on the sensitivity of the FGF23 assay used. Although unusual in children, an 11-year-old girl had significant bone pain and functional limitation associated with biopsy-proven hypophosphatemic osteomalacia/rickets and markedly elevated serum levels of FGF23.²⁷⁴ Following removal of a benign fibrous tumor from a small exostosis on a distal ulnar metaphysis, serum FGF23 concentrations normalized within 7 hours postoperatively and phosphate levels were normal 2 weeks later. Clinical symptoms abated and radiographic and histomorphometric abnormalities resolved within 1 year after surgery. In an 11-year-old boy with severe bone pain, weakness progressing to confinement to a wheelchair, hyperphosphaturia and hypophosphatemia, elevated levels of FGF23 (1874 RU/mL) declined to normal values (43 RU/mL) within 48 hours after removal of an FGF23-containing hemangiopericytoma from his left iliac wing.²⁷² This lesion had not been identified by routine roentgenograms, CT or magnetic resonance imaging, or technetium bone scan; the tumor was demonstrated only by gradient recall echo magnetic resonance imaging (Fig. 20.13). Within 2 weeks after surgery, the lad was walking without assistance, and several weeks thereafter he resumed normal activity.

Although increased production of FGF23 is the most common pathogenetic mechanism of hypophosphatemia, it may also result from disorders that are not associated with increased FGF23 synthesis (see Table 20.8B). ADHR with nephrocalcinosis/nephrolithiasis/osteoporosis type 1 (OMIM 612286) is caused by monoallelic inactivating mutations in *SLC34A1* (OMIM 182309) encoding the sodium-phosphate cotransporter NPT2a (also designated *NaPi-IIa*). Haploinsufficiency of NPT2a in the brush border apical membrane of the proximal renal tubule whose expression is under the inhibitory control of PTH and FGF23 impedes phosphate reabsorption. This disorder is characterized by diminished phosphate reabsorption, hyperphosphaturia, hypophosphatemia, increased synthesis of calcitriol with augmented absorption of intestinal calcium, hypercalcemia with suppression of PTH secretion, hypercalciuria, formation of renal calculi, and osteopenia.²³² Autosomal recessive Fanconi syndrome with hypophosphatemic rickets (OMIM 613388) (also designated Fanconi renal tubular syndrome 2) is caused by biallelic inactivating mutations in *SLC34A1* as is infantile hypercalcemia type 2 (OMIM

616963).^{275,276} Autosomal dominant hypophosphatemia with urolithiasis type 2 (OMIM 612287) is attributable to heterozygous loss-of-function mutations in *SLC9A3R1* (OMIM 604990) that encodes NHERF1—a renal tubular sodium/hydrogen exchange regulatory factor; one of its functions is to bind NPT2a and anchor it to the luminal membrane of the proximal renal tubule; another is to modulate PTH1R signaling in response to PTH. Loss of NHERF1 function leads to its dissociation from NPT2a and subsequent endocytosis of NPT2a resulting in diminished renal tubular reabsorption of phosphate, hyperphosphaturia, and hypophosphatemia.^{250,277} Patients with mutations in *SLC9A3R1* are also osteopenic; serum levels of PTH and FGF23 are normal in these subjects. The pathogenesis and clinical and laboratory characteristics of patients with hypophosphatemic rickets with nephrocalcinosis/nephrolithiasis/osteoporosis type 2 are similar to the type 1 form of this disorder.²³² Hypophosphatemia leads to increased synthesis of calcitriol, intestinal calcium absorption, mild hypercalcemia, hypercalciuria, and nephrolithiasis.²⁷⁸ Patients with mutations in *SLC9A3R1* are also osteopenic. Biallelic loss-of-function mutations in *SLC34A3* (OMIM 609826) encoding NPT2c, the renal tubular phosphate transporter, result in hypophosphatemic rickets with hypercalciuria (OMIM 241530) independently of FGF23.²⁷⁵ This 599 aa protein with eight transmembrane domains is located in the brush border of juxtamedullary proximal renal tubular cells. Loss of activity of NPT2c leads to hyperphosphaturia, hypophosphatemia, increased synthesis of calcitriol with augmented absorption of intestinal calcium, hypercalciuria, formation of renal calculi, and calcium-deficient rickets. Hypophosphatemia leads to increased synthesis of calcitriol and consequently enhanced absorption of ingested calcium, hypercalciuria, and nephrocalcinosis.²³² Heterozygous carriers of inactivating mutations in *SLC34A3* also have moderately increased serum concentrations of calcitriol, hyperphosphaturia, and hypercalciuria but do not have identified metabolic bone disease. Patients with variants of *SLC34A1*, *SLC9A3R1*, or *SLC34A3* may be treated successfully with oral phosphate alone in conjunction with hydration and avoidance of a high-sodium diet, while administration of calcitriol is contraindicated.

Deleterious variants of chloride channel 5 (*CLCN5*, OMIM 300008) result in Dent disease 1 (OMIM 300009) as a result of progressive renal proximal tubule malfunction leading to renal

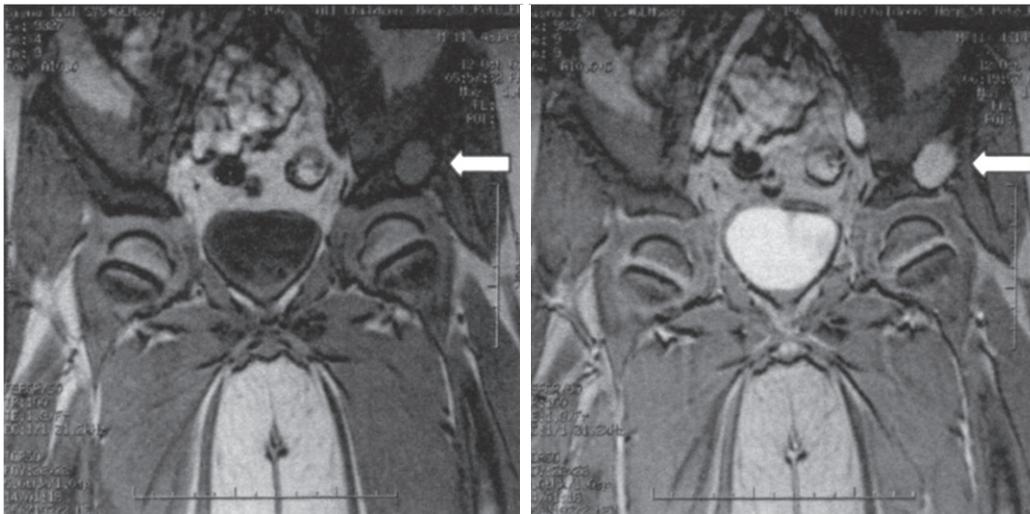


Fig. 20.13 Demonstration by gradient recall echo magnetic resonance imaging of an FGF23-producing hemangiopericytoma in the left iliac wing of an 11-year-old boy with tumor-induced rickets. (From Shulman DI, Hahn G, Benator R, et al. Tumor-induced rickets: usefulness of MR gradient echo recall imaging for tumor localization. *J Pediatr*. 2004;144:381–385.)

failure associated with amino aciduria, proteinuria, hyperphosphaturia, hypophosphatemia, hypercalciuria, glycosuria, and uricosuria and variable associations with metabolic bone disease (hypophosphatemic rickets—OMIM 300554) and nephrocalcinosis (OMIM 310468).²³² Mutations in different domains of this 12 exon, 746 aa transmembrane chloride channel result in varying clinical and biochemical manifestations; the p.Ser244Leu mutation in *CLCN5* has been associated with X-linked recessive hypophosphatemic rickets. The clinical features of Dent disease 2 (OMIM 300555) are similar to those of Dent disease 1, but this disorder is the result of inactivating variants of *OCRL1* (OMIM 300535) encoding a phosphatidylinositol 4,5-bisphosphate-5-phosphatase that affects endocytosis and possibly disrupts the cellular movement of the product of *CLCN5*. The hypophosphatemia of Dent disease patients often can be corrected by oral phosphate supplements. Increased urinary phosphate excretion because of acquired and heritable disorders of the proximal renal tubule is characteristic of the metabolic bone disease that accompanies various forms of the Fanconi syndrome of renal tubular acidosis, glycosuria, and amino aciduria (heritable: cystinosis, tyrosinemia, galactosemia, oculo-cerebral-renal syndrome, fructose intolerance, Wilson disease; acquired: renal transplantation, nephrotic syndrome, renal vein thrombosis, mercury, lead and copper poisoning, outdated tetracycline).²⁷⁹ In addition to hypophosphatemia, acidosis contributes to the pathogenesis of bone disease in Fanconi syndrome by increasing the solubility of the mineral phase of bone and increasing urinary loss of calcium and by impairing conversion of calcidiol to calcitriol. In patients with autosomal recessive Fanconi-Bickel syndrome (OMIM 227810), mutations in *SLC2A2* (OMIM 138160) lead to decreased synthesis of a glucose transporter (*GLUT2*) resulting in impaired utilization of glucose and galactose and consequent accumulation of glycogen in the liver and kidney together with a proximal renal tubular nephropathy. In transgenic mice, deletion of *Slc2a2* results in decreased expression of *Npt2c* in proximal renal tubules.²⁸⁰ Patients with a specific heterozygous missense mutation in *HNF4A* (OMIM 600281) (p.Arg76Trp, c.226C⇒T) also develop the Fanconi-Bickel phenotype as do mice in which *HNF4A* has been eliminated.^{281,282}

Hyperphosphatemia may be caused by intrinsic gene variants or by exogenous causes, such as excessive parenteral administration of phosphate or by the administration of phosphate-containing enemas, acutely increased rate of cellular destruction (crush injuries, rhabdomyolysis, during chemotherapy for malignancy), decreased rate of renal phosphate excretion (renal failure), impaired secretion or action of PTH, and during administration of some bisphosphonates.²⁴¹ Impaired synthesis of FGF23 and/or α -klotho decreases renal tubular excretion of phosphate and impairs renal production of calcitriol leading to hyperphosphatemia, marginal hypocalcemia, and secondary hyperparathyroidism. As a result, there is ectopic calcification of soft tissues—particularly in the periarticular region, a disorder termed *familial hyperphosphatemic tumoral calcinosis* (FHTC). Familial hyperphosphatemic tumoral calcinosis (OMIM 211900) is an autosomal recessive disorder characterized by progressive deposition of calcium phosphate in soft tissues and in periarticular regions of the long bones, manifested clinically by periarticular masses of ectopically deposited calcium and at times by complaints of bone pain.²⁸³ Other sites of ectopic calcification include the brain and blood vessels; radiographically, there is cortical hyperostosis and periosteal reaction involving the long bones. Thus FHTC is the mirror image of ADHR. This disorder is caused by decreased synthesis of FGF23, its rapid degradation, or peripheral resistance to its function related to biallelic inactivating variants of *FGF23*, *GALNT3*, and *KL*, respectively (OMIM 605380, 601756, 604824).^{249,284,285} Thus there may be

decreased synthesis of FGF23 because of loss-of-function mutations in *FGF23* itself, the rapid degradation of FGF23 because it has not been glycosylated because of an inactivating mutation in *GALNT3* encoding UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl transferase 3, or tissue non-responsiveness to FGF23 because of an inactivating mutation in *KL*, encoding its coreceptor α -klotho. *GALNT3* is an enzyme that protectively glycosylates FGF23; its loss enables rapid loss of FGF23. In the epithelial cells of the proximal renal tubule, FGF23 interacts with α -klotho-FGFR1 (IIIc)-heparan sulfate to increase renal phosphate excretion by decreasing expression of phosphate transporters. Management of FHTC is limited to ingestion of a low-phosphate diet, administration of intestinal phosphate binding agents (aluminum hydroxide), and increasing urinary phosphate excretion by use of a diuretic, such as acetazolamide; topical application of sodium thiosulfate has been reported to decrease ectopic calcifications on the skin surface perhaps by chelating and thus solubilizing the calcium in these deposits.²⁵² Surgical excision of tumoral masses that impair function, administration of intestinal phosphate binders, agents that increase renal phosphate excretion (acetazolamide), and diets low in calcium and phosphate have been the major therapeutic strategies used in the treatment of these patients.²⁴¹

Disorders of Alkaline Phosphatase Activity. In its most severe forms, hypophosphatasia (OMIM 241500) is an autosomal recessive disorder due to loss-of-function mutations in *ALPL* (OMIM 171760), the gene encoding tissue nonspecific (bone/liver/kidney) alkaline phosphatase (TNSALP); less damaging forms of this disease are transmitted as autosomal dominant traits (OMIM 241510, 146300).^{112,113} TNSALP is a phosphohydrolase expressed on the surface of bone cells that hydrolyzes phosphorylated compounds, such as pyrophosphate, pyridoxyl-5-phosphate, and phosphoethanolamine. Without TNSALP, the phosphate group of inorganic pyrophosphate cannot be removed; inasmuch as pyrophosphate binds to and hinders conversion of amorphous calcium/phosphate to hydroxyapatite, its persistent presence inhibits enlargement of the hydroxyapatite crystal and hence mineralization of collagenous bone matrix. Alkaline phosphatases are zinc and magnesium-dependent alkaline optimum orthophosphoric monoester phosphohydrolases. Bone, liver, and kidney alkaline phosphatase isoforms differ not by their amino acid structure but by their patterns of posttranslational glycosylation.¹¹³ Homotetrameric bone TNSALP is anchored through its carboxyl terminal to the exterior cell membrane of hypertrophic chondrocytes and osteoblasts (i.e., it is an ectophosphatase) by a phosphatidylinositol-glycan moiety. Pathophysiologically, decreased TNSALP activity leads to accumulation of its endogenous substrates—inorganic pyrophosphate, pyridoxal 5'-phosphate, and phosphoethanolamine. Because pyrophosphate is an inhibitor of osteoid mineralization, failure of degradation leads to its accumulation and then to inadequate formation of hydroxyapatite resulting in rickets or osteomalacia, as well as hypercalcemia, hypercalciuria, suppression of PTH synthesis and secretion, increased renal reabsorption of phosphate, and hyperphosphatemia. (The pathophysiology of bone disease caused by TNSALP deficiency resembles that caused by lack of ENPP1 in which disorder pyrophosphate also accumulates but by a distinctively different mechanism [vide supra].) Failure to metabolize pyridoxal 5'-phosphate to pyridoxine leads to pyridoxine-dependent seizures. The clinical severity of hypophosphatasia varies from early lethality to isolated premature shedding of deciduous teeth and is dependent upon the extent of functional TNSALP deficiency as determined by the site(s) of variations in *ALPL*. The younger the age of onset, the more severe is the disease likely to be. The incidence

of severe forms of hypophosphatasia ranges from 1/2500 newborns in Canadian Mennonite families to 1/300,000 in European populations.¹¹³ Currently, there are seven recognized forms or types of hypophosphatasia—six classical forms (numbered 1–6) and one transitional type (here designated type 1A):

1. The perinatal form of hypophosphatasia is identifiable in the fetus in utero and presents clinically at birth with deformities and fractures of the ribs, vertebrae, and limbs; impaired mineralization of the long bones (metaphyseal fraying) and skull (despite which apparent fusion of the sutures of membranous cranial bones may occur resulting in functional craniosynostosis without radiological evidence thereof); and pyridoxine-dependent seizures. Malformations of the skull are associated with intracranial hemorrhage, whereas fractures of the ribs and deformities of the chest wall result in postnatal respiratory insufficiency and apnea. Radiological findings include absent or extremely poor bone mineralization with rachitic deformities, irregular radiolucent extensions into metaphyses, apparently “missing” vertebrae, and protruding midshaft fibular and ulnar bone spurs. The perinatal form of hypophosphatasia has been almost invariably fatal, although the efficacy of enzyme replacement (vide infra) in these patients has not yet been determined;
 - 1A. In the benign prenatal or transitional form of hypophosphatasia, the fetus and neonate manifest skeletal deformities but not radiographic signs of rickets; shortly after birth, there is spontaneous and substantial improvement; findings that distinguish this less severe type of hypophosphatasia are fetal crowding, normal fetal bone mineralization, and appropriate chest volume; this form of hypophosphatasia may be associated with either monoallelic or biallelic mutations in *ALPL*,^{286,287}
2. The infantile form (OMIM 241500) becomes clinically apparent in the first 6 months of life and is characterized by anorexia, impaired linear growth and weight gain, deformities of the long bones and rib cage, widely “open” fontanelles and sutures (calvarial hypomineralization) with functional craniosynostosis with increased intracranial pressure (manifested by prominence of the anterior fontanelle, proptosis, and papilledema), muscular weakness, developmental delay, pyridoxine-dependent seizures, hypotonia, constipation, hypercalcemia, hypercalciuria, nephrocalcinosis, early shedding of deciduous teeth, and radiographic evidence of rickets with marked skeletal hypomineralization; the neonatal and infantile forms of hypophosphatasia are transmitted as autosomal recessive disorders; until the development of enzyme replacement therapy, the majority of patients with perinatal hypophosphatasia and 50% of those with infantile hypophosphatasia died of respiratory failure before their first birthday (vide infra);
3. Patients with the childhood form of hypophosphatasia (OMIM 241510) are clinically heterogeneous; they are well as neonates, but at and after 6 months of age, abnormalities of varying severity may become apparent including: isolated premature shedding of deciduous teeth, craniotabes, prominence of the costochondral junctions, flaring of the metaphyses, and genu valgus and/or varum. Radiologically, there may be signs of rickets with metaphyseal flaring, intrusion of areas of radiolucency into the metaphyses, deformation of the limbs, osteopenia, and craniosynostosis. These patients may improve spontaneously during puberty and or may evolve into the adult form of this disease. These patients have responded well to enzyme replacement therapy (vide infra);
4. The adult form of hypophosphatasia (OMIM 146300) may be subclinical and identified only during study of a

family with an offspring with a more significant form of hypophosphatasia; the parent may have a history of premature loss of deciduous teeth, osteomalacia with increased susceptibility to recurrent fractures predominantly of the metatarsal bones, pseudofractures of the proximal femur, chondrocalcinosis, or pseudogout because of articular deposition of calcium pyrophosphate dihydrate crystals; subtrochanteric femoral pseudofractures may be demonstrated radiographically;

5. Odontohypophosphatasia (included in OMIM 146300) is manifested only by premature shedding of primary teeth before 5 years of age because of decreased mineralization of cementum without radiological skeletal abnormalities; periodontitis may develop; the childhood and adult forms of hypophosphatasia and odontohypophosphatasia are usually (but not invariably) transmitted as monoallelic, autosomal dominant disorders;
6. Pseudohypophosphatasia is phenotypically and biochemically similar to classic type 2 infantile hypophosphatasia but serum alkaline phosphatase activity *in vitro* is normal, indicating that in this disorder enzyme activity toward artificial substrates in high pH solutions is preserved but alkaline phosphatase activity toward endogenous substrates at pH 7.4 is abnormal.¹¹²

In addition to the site(s) of mutation of *ALPL*, the clinical severity of hypophosphatasia is related inversely to the age at which skeletal disease is evident and to individual biological (possibly epigenetic) factors that affect the expression of the disease with wide individual variability even in family members with the identical *ALPL* mutation(s). In the lethal perinatal form of hypophosphatasia, missense, nonsense, and donor splice site mutations and frameshift deletions in *ALPL* cluster in crucial segments of the protein within (p.Ala94Thr, p.Arg167Trp) or near (p.Gly103Arg, p.Gly317Asp) its catalytically active, enzymatically functional site or at the dimeric interface (p.Ala23Val, p.Arg374Cys) or they may impair movement of TNSALP to its active transmembrane site.^{112,113} Mutations may interrupt binding of TNSALP to the phosphorylated ligand or destabilize attachment of necessary cofactors, such as zinc or magnesium. *ALPL* mutations associated with the childhood form of hypophosphatasia (p.Arg119His, p.Asp361Val) tend to cluster on the three-dimensional surface of the enzyme molecule at sites that relate to its tethering to the cell surface or its formation of tetramers; such variants may retain substantial residual bioactivity. In some patients with moderately severe hypophosphatasia, heterozygotic mutations in *ALPL* (p.Gly46Val, p.Arg167Trp, p.Asn461Ile) exert a dominant-negative effect on the intact TNSALP dimer; these mutations have been clustered at the enzyme active site or at the domain (s) involved with dimerization, tetramerization, or membrane anchoring. Odontohypophosphatasia has been associated with heterozygotic mutations in *ALPL* (p.Pro91Leu, p.Ala99Thr).

After identification of clinical and radiological findings of rickets, the diagnosis of hypophosphatasia is suggested by the presence in the patient of paradoxically low values of circulating alkaline phosphatase (related to age standards) and confirmed by identifying the individual genetic variant of *ALPL* responsible for the disease. In other forms of rickets, alkaline phosphatase values are substantially increased (see Table 20.9). In addition, there are increased serum concentrations of pyrophosphate (controls <200 μmol/g of creatinine) and pyridoxal-5'-phosphate (~5000 nM) and elevated urinary excretion of phosphoethanolamine (controls <15 years of age 83–222 μmol/g of creatinine). Serum concentrations of calcium and phosphate are often elevated in patients with the perinatal and infantile forms of the disease, because intestinal absorption of calcium and renal tubular reabsorption of

phosphate are normal while the bone formation rate is depressed; calcium values are usually normal in the childhood and adult forms of hypophosphatasia.^{112,113} Serum levels of PTH are normal or low; there is no evidence of secondary hyperparathyroidism. Although serum alkaline phosphatase activity in vitro is normal in patients with pseudohypophosphatasia, it is functionally inadequate in vivo—perhaps because the TNSALP protein is sequestered within the cell because of a defect in its transport to the cell surface or because the protein is active with artificial substrate but not with natural substrates at physiological pH. Other disorders associated with hypophosphatasemia include the milk-alkali syndrome, hypothyroidism, hypercortisolemia, vitamin D toxicity, vitamin C deficiency, starvation, magnesium, zinc, or copper deficiency, celiac disease, profound anemia, and massive transfusion.^{112,113} The prenatal diagnosis of hypophosphatasia may be considered in a fetus in whom ultrasonography has demonstrated short ribs and long bones, cupping of the metaphyses, osseous spurs, patchy ossification, and demineralization of the skull and thoracic spine and corroborated by analysis of fetal DNA present in maternal circulation.²⁸⁸ To be included in the differential diagnosis of a fetal sonogram with these findings are achondrogenesis and osteogenesis imperfecta type II.

Treatment of hypophosphatasia depends on the severity of the disease. Supportive measures are appropriate, but administration of bisphosphonate agents are usually contraindicated. Administration of even small doses of vitamin D or its metabolites should be avoided as these patients easily develop hypercalcemia. Hypercalcemia in infants with hypophosphatasia responds to bisphosphonates and transiently to calcitonin. Seizures may be responsive to pyridoxine administration. Milder adult forms of hypophosphatasia may be responsive to teriparatide (rhPTH¹⁻³⁴).²⁸⁹ Recombinant, mineral-targeted human TNSALP (alfotase alfa) has been used successfully to treat the perinatal, infantile, and childhood forms of hypophosphatasia.^{112,290} Alfotase alpha is a biosynthetic human recombinant protein consisting of two identical amino acid chains linked by two disulfide bridges containing the catalytic ectodomain of TNSALP fused to the human IgG1 Fc domain and a terminal deca-aspartate motif that targets bone. It has proven extraordinarily effective in the treatment of patients with the perinatal and infantile forms of hypophosphatasia for up to 7 years.¹¹⁴ Administration of this compound to 11 such patients was followed by radiographic healing of rickets after 24 weeks of treatment; in addition, motor development and pulmonary function improved. There were no serious adverse effects recorded in this study. This agent has proven effective in and has been approved for the treatment of children, adolescents, and adults with hypophosphatasia, as well as infants.^{290,291} A scoring system has been developed for assessment of the changes over time in the skeletal abnormalities of patients with hypophosphatasia treated with alfotase alpha.²⁹²

Hyperphosphatasia may be a normal physiological variant or a biochemical sign of disease. Serum bone-specific alkaline phosphatase activity normally increases during puberty and in response to skeletal injuries and reflects increased osteoblastic activity. Transient increase in serum alkaline phosphatase activity may also be observed in children with viral infections (including human immunodeficiency virus), after liver or kidney transplantation, or during treatment of leukemia/lymphoma.²⁹³ Hyperphosphatasemia is also present in patients with juvenile Paget disease/hyperostosis corticalis deformans juvenilis (OMIM 239000), familial expansile osteolysis (OMIM 174810), and osteosclerotic hyperostosis corticalis generalisata/van Buchem disease (OMIM 239100). Benign transient hyperphosphatasemia of infancy and early childhood is encountered in clinically well infants and children less than 5 years of age (median age 16 months); serum activities of both

bone and liver alkaline phosphatase isoforms are increased (despite absence of liver or bone disease); it is a self-limited process with alkaline phosphatase values returning to normal within several months.^{293,294} Transient hyperphosphatasemia (alkaline phosphatase values >1000 U/L) has been reported to occur in approximately 2% to 3% of children less than 2 years of age.²⁹⁵ It has been suggested that benign and transient hyperphosphatasemia are the consequence of excessive sialylation of alkaline phosphatase that delays its degradation and extends its half-life, but the reason for the excessive sialic acid content of the molecule is uncertain.²⁹³ Rarely, hyperphosphatasemia may be familial and transmitted as a benign autosomal dominant trait. Occasionally, an adolescent with persistent, nonfamilial, benign hyperphosphatasemia may be encountered. Hyperphosphatasia associated with mental retardation is a disorder characterized by developmental delay, seizures, hypotonia, and hyperphosphatasemia of diverse genetic pathogenesis (OMIM 239300). The type 1 (of 6) form of this disorder is caused by biallelic loss-of-function mutations in phosphatidylinositol glycan anchor biosynthesis class V protein (PIGV, OMIM 610274), a glycolipid that attaches proteins including alkaline phosphatase to the surface of a cell.²⁹⁶

Chronic Kidney Disease-Mineral and Bone Disorder

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is the term used to characterize the aberrations in calcium and phosphate metabolism, PTH and vitamin D synthesis and secretion, skeletal homeostasis, and ectopic vascular, extravascular, and cardiac calcifications that are encountered in patients with impaired renal function.²⁹⁷⁻³⁰⁰ CKD-MBD replaces the designation of renal osteodystrophy that pertains solely to the pathological changes present in the bones of patients with chronic renal failure. The major skeletal consequences of chronic renal disease are related to bone resistance to the actions of PTH, subnormal synthesis of calcidiol and calcitriol, and the adverse effects of elevated levels of FGF23, sclerostin, and Dickkopf-related protein 1 (DKK1) on bone formation.^{284,299} The bone disease of chronic renal failure leads to decreased growth, bone mineralization and strength, and increased risk of fracture. It may be characterized as hyperdynamic—associated with high turnover of bone and osteitis fibrosa related to secondary hyperparathyroidism, hypodynamic or low turnover, or adynamic—characterized histologically by little fibrosis, subnormal cellularity, bone turnover rate, and bone volume, but paradoxically normal bone mineralization and PTH values related to aggressive therapy with calcium antireabsorptive agents (e.g., bisphosphonates or antibodies to RANKL, or proanabolic agents [e.g., rhPTH¹⁻³⁴, antibodies to sclerostin]), or most commonly a mixture of the high and low turnover forms, termed *mixed renal osteodystrophy*.^{284,299,300}

As renal function declines, the production of both FGF23 and PTH increases. The secretion of FGF23 increases relatively early in patients with CKD when phosphate retention first occurs—even when the glomerular filtration rate exceeds 80 mL/min/1.73 m².³⁰¹ Thus in patients with CKD-MBD serum concentrations of FGF23 rise before renal function declines and before serum levels of phosphate rise, urinary phosphate excretion increases—initially maintaining normal serum phosphate values. Increasing production of FGF23 as renal failure progresses contributes to declining renal tubular synthesis of calcitriol and lower intestinal calcium absorption. As the glomerular filtration rate progressively declines, urinary phosphate excretion is impaired, leading to its intracellular and extracellular accumulation, hyperphosphatemia (when the glomerular filtration rate falls <20 mL/min/1.73 m²), and modest hypocalcemia.²⁹⁸ The secretion of PTH increases when

the glomerular filtration rate declines to values less than 70 mL/min/1.73 m². Increasing serum concentrations of phosphate resulting in hyperphosphatemia (when the glomerular filtration rate falls <20 mL/min/1.73 m²), falling levels of calcium (because of both decreased intestinal absorption of this cation attributable to deficiency of both calcidiol and calcitriol, and the need to maintain a constant calcium x phosphate product), and rising levels of FGF23 that exert a direct stimulatory effect on *PTH* expression lead to secondary increase in *PTH* generation and when uncontrolled to tertiary hyperparathyroidism. Other factors that contribute to the development of parathyroid chief cell hyperplasia and secondary hyperparathyroidism in CKD-MBD include downregulation of the expression of *CASR* in uremic parathyroid glands (resulting in increase of the set point concentration at which calcium depresses *PTH* expression) and skeletal insensitivity to *PTH*. Phosphate may also slow the rate of degradation of *PTH* mRNA within the parathyroid gland and exert a direct enhancing effect on the growth of parathyroid glands as well. Because calcitriol inhibits parathyroid gland growth and function, decrease in its synthesis also results in increased proliferation of parathyroid chief cells and synthesis of *PTH*. In the presence of elevated *PTH* secretion and various cytokines (IL-1, -6, -11, TNF, macrophage-colony stimulating factor [M-CSF]), osteoclastogenesis and the rates of bone resorption and formation are increased. Acidosis contributes to the dissolution of the mineral phase of bone directly and by impairing osteoprotegerin-mediated inhibition of osteoclast generation. Vascular and soft tissue calcification also occur in subjects with chronic renal failure.²⁹⁹

Chronic renal disease and its associated metabolic bone disease in children may be clinically silent except for failure of linear growth; as the disease progresses, deformities of the extremities, slipped epiphyses, fractures, bone and joint pain, muscular weakness, and lassitude develop. In patients in whom the rate of bone formation is diminished and the volume of unmineralized bone (i.e., osteomalacia) increased, the process has been caused by accumulation of aluminum at the mineralization front; with discontinuation of aluminum-containing phosphate binders development of osteomalacia in renal failure is now unusual.¹⁴³ In the absence of osteomalacia, adynamic bone disease in chronic renal failure is the result of decreased *PTH* generation because of improved control of serum phosphate levels, increased calcium stores, higher levels of *PTH*⁷⁻⁸⁴ and other carboxyl terminal fragments of *PTH* that inhibit bone resorption, and other factors that affect the tissue response to *PTH*. The cardiovascular complications (hypertension, ectopic vascular calcifications, uremic vasculopathy, cardiomyopathy) that develop in children with CKD-MBD begin before the need for and progress during dialysis and are the primary cause of death in children and adolescents with CKD.²⁹⁷ Hyperphosphatemia, secondary hyperparathyroidism, and elevated levels of FGF23 adversely affect the cardiovascular system. Children with autosomal dominant polycystic kidney disease may be hypophosphatemic with decreased renal tubular reabsorption of filtered phosphate perhaps related to resistance to the biological activity of FGF23.³⁰²

Biochemically, CKD-MBD is marked primarily by hyperphosphatemia, low-normal serum calcium levels, and increased serum *PTH* and FGF23 concentrations and TNSALP activity. Serum concentrations of the bone turnover markers—bone alkaline phosphatase, tartrate-resistant acid phosphatase (TRAP) 5b, and carboxyl-terminal FGF23 are increased, whereas sclerostin levels are reduced in children with CKD.³⁰³ Radiographic signs of rickets, low bone mass, and pseudofractures are often present in children with renal osteodystrophy. After clinical assessment, the evaluation of bone disease in children with chronic renal disease includes examination of skeletal roentgenograms and quantitation of

BMC and BMD by DXA and/or peripheral quantitative computed tomography (pQCT) and may require biopsy of the iliac crest followed by histomorphometric analyses of the tissue. Therapeutically, the goals in treating a child with chronic renal failure are to minimize the adverse systemic effects of CKD-MBD; to maintain (near) normal serum calcium, phosphate, and alkaline phosphatase values; and to prevent the development or progression of secondary hyperparathyroidism. Treatment of renal bone disease must be individualized; it includes appropriate intake of dietary calcium, supplemental vitamin D or calcidiol and calcitriol, decreased intake of dietary phosphate and administration of agents that bind intestinal phosphate and prevent its intestinal absorption, phosphaturic drugs, and at times subtotal parathyroidectomy.²⁹⁹ Calcitriol decreases the rate of bone formation in patients with chronic renal insufficiency by inhibiting osteoblast differentiation/function, decreasing *PTH* synthesis, altering degradation of *PTH* within the parathyroid glands, and decreasing expression of *PTH1R*. Calcium-containing oral phosphate binders may also be useful. Dialysis fluids must be prepared with aluminum-free water. When indicated, suppression of *PTH* secretion may be further achieved with the use of calcitriol analogues, such as paricalcitol or of a synthetic ligand of the CaSR—cinacalcet hydrochloride.^{143,298} Experimentally, although immunoneutralization of FGF23 in a rat model of CKD-MBD led to decrease in *PTH* secretion, increased calcitriol and calcium concentrations, and improved bone formation, there was increase in aortic calcification and mortality rate in the anti-FGF23-treated animals.³⁰⁴ Thus suppression of FGF23 secretion in patients with CKD might have paradoxically unwanted consequences. Even after successful renal transplantation, secondary hyperparathyroidism may persist for months or years—its extent and intensity reflecting the severity and duration of chronic renal failure before renal transplantation, development of nodular or monoclonal hyperplasia of the parathyroid glands, and the 20-year life span of the parathyroid chief cell. Five years after renal transplantation during childhood, serum concentrations of PICP, osteocalcin, and ICTP remain significantly increased indicating accelerated bone turnover rate, whereas areal and volumetric BMDs at the distal third of the nondominant radius are normal for height but subnormal for age.³⁰⁵ Hypercalcemia and persistent secondary or tertiary hyperparathyroidism requiring parathyroidectomy may become apparent in children after renal transplantation. The osteopenic effects of glucocorticoids and immune suppressive agents, such as cyclosporin, are observed in postrenal transplantation patients as well.

Disorders of Bone Mineralization

Bone mineralization may be depressed or exaggerated by dysfunction of either osteoblasts or osteoclasts. Abnormalities of osteoblast function result in either impaired (e.g., osteoporosis, osteogenesis imperfecta) or exaggerated bone mineralization (e.g., osteosclerosis, osteopetrosis). Thus in patients with the osteosclerotic disorder, hyperostosis corticalis generalisata/van Buchem disease type 1 (OMIM 239100), decreased expression of *SOST* encoding sclerostin (OMIM 605740, chr. 17q21.31), an inhibitor of WNT signaling by its interaction with the WNT coreceptors low-density lipoprotein receptor-related proteins (LRP) 5 and 6, is associated with enhanced osteoblast function and increased bone mineralization. Although derived from a common hematopoietic stem cell, there are a number of osteoclast subtypes with unique physiological profiles that reflect: the site at which they act (intramembranous or endochondral bone, cartilage); the remodeling process in which they participate (targeted—to sites of bone

replacement, stochastic—hormonally [PTH, calcitonin, calcitriol]-mediated for maintenance of eucalcemia); the type of bone (cortical, trabecular) upon which they act; and the time of day (diurnal variation) at which the osteoclast is most active.³⁰⁶ Decreased osteoclast formation or function results in increased bone mass and various forms of osteopetrosis, whereas augmented osteoclast activity leads to decreased bone mass because of osteolysis. In a number of bone disorders, skeletal areas of both increased and decreased bone mineralization may be present. Juvenile Paget disease/hyperostosis corticalis deformans juvenilis (OMIM 239000) is a hyperphosphatase-mic disorder that often begins in early childhood and is characterized clinically by expanded and bowed extremities, nontraumatic fractures of the long bones, kyphosis, macrocephaly, and muscular weakness; this disorder may progress to wheelchair dependence.³⁰⁷ Serum levels of alkaline phosphatase are markedly increased, a coupled response to increased osteoclastic action. Radiographic skeletal abnormalities include cortical thickening, both osteosclerosis and osteopenia, coarse trabeculations, and progressive skeletal deformities. The disorder is caused by biallelic loss-of-function mutations of *TNFRSF11B* (OMIM 602643) encoding osteoprotegerin, a member of the TNF receptor superfamily that functions as a decoy acceptor for and consequently is an inhibitor of RANK (*TNFRSF11*, OMIM 602642), the stromal cell-osteoblast-derived osteoclastogenic factor. Normally, osteoprotegerin inhibits osteoclastogenesis; hence, inactivating mutations of *TNFRSF11B* are associated with enhanced osteoclastogenesis and osteoclastic activity. The severity of juvenile Paget disease depends on the site of mutation in *TNFRSF11B*—those that result in deletion of the entire gene or those in the ligand binding domain that involve loss of cysteine residues result in marked clinical disease.³⁰⁸ Treatment with recombinant osteoprotegerin has resulted in clinical and radiological improvement as has administration of bisphosphonates, but the latter agents are associated with risk of substantial hypocalcemia.³⁰⁹ Familial expansile osteolysis (OMIM 174810) and early-onset familial Paget disease of bone (OMIM 602080) are autosomal dominant, monoallelic disorders that are pathophysiologically similar to juvenile Paget disease although clinically and etiologically distinct. These two disorders are caused by monoallelic gain-of-function mutations of the gene (*TNFRSF11A*, OMIM 603499) encoding RANK that result in an increase in NF- κ B signaling as a consequence of which there is augmented osteoclastogenesis.³¹⁰ In adolescents with familial expansile osteolysis, focal areas of increased bone turnover in the appendicular skeleton appear in the second decade of life followed by medullary expansion, pathological fractures, and skeletal deformities; deafness and premature loss of dentition may occur in these subjects. Patients with either familial expansile osteolysis or early-onset Paget disease of bone demonstrate focal areas of osteolytic and hyperostotic bone, premature loss of teeth, and deafness. Increased activity of mutant RANK is thought to be caused by heterozygous insertional duplications of 18 or 27 base pairs within the signal peptide region of exon 1 of *TNFRSF11A*.³¹¹ However, because the mutant forms of RANK are trapped in the endoplasmic reticulum and unable to interact with RANKL directly, the mechanism(s) through which these heterozygous mutations increase NF- κ B-mediated osteoclastogenesis are uncertain.^{310,312} It has been hypothesized that mutant RANK may be able to interact with residual intact RANK and either slow its rate of degradation or prolong its interaction with RANKL.³¹⁰ Conversely, loss-of-function mutations of *TNFRSF11A* are associated with autosomal recessive, osteoclast-poor osteopetrosis type 7 and hypogammaglobulinemia (vide infra).^{310,313} Inactivating mutations in *TNFRSF11* encoding RANKL are associated with osteoclast-poor osteopetrosis type 2 (vide infra).

Low Bone Mass

Low bone mass or osteoporosis is often associated with increased fracture risk, although in some disorders with high bone mass fracture rate is increased because abnormal bone microarchitecture leads to decreased bone strength (e.g., osteopetrosis, pyknodysostosis—vide infra).³¹⁴ Some 50% of boys and 40% of girls will sustain a traumatic fracture during childhood or adolescence (peak incidence between 11 and 12 years in girls and 13 and 14 years in boys), most often at the distal radius because of transient decline in cortical strength at this site.^{314,315} It is important to distinguish a high-impact traumatic fracture from one that is the result of increased bone fragility, such as compression vertebral fractures, low-impact fractures, and “spontaneous” femoral fractures caused by a primary or secondary disorder of bone mineralization (Tables 20.10A, 20.10B, 20.10C). In adults, osteopenia and osteoporosis (“porous bone”) are terms that designate states of reduced bone mass and matrix, abnormal bone microarchitecture, and decreased bone strength that increase the risk for fracture; osteomalacia refers to decrease in the mineral phase of bone.^{316,317} Osteoporosis in adults (primarily postmenopausal women) is defined by the World Health Organization (WHO) as an areal BMD at a specific bone site that is -2.5 or more SDs below the mean peak young adult value for gender (T score ≤ -2.5). Postmenopausal osteoporosis is usually the consequence of an increase in the rate of bone resorption relative to that of bone formation because of estrogen deficiency. In adults, osteopenia is present when the areal BMD lies between -1.1 and -2.4 SD below the mean peak young adult value for gender (T score -1.1 to -2.4), and a normal areal BMD is one that is not more than one SD below or above the mean peak young adult value (T score -1.0 to $+1.0$). When the areal BMD is more than $+2$ SD above the mean for age and gender (T score $> +2$), bone mass is considered high. Because in premenopausal women, areal BMD alone is not a good assessor of fracture risk, the International Society for Clinical Densitometry (ISCD) recommends that the term *osteoporosis* be restricted to those patients with both low areal BMD (as defined by a BMD Z score of < -2.0 , i.e., one that is below -2 SDs for age and gender-matched control subjects) and a history of low-impact (fragility) fractures.³¹⁸ Women with areal BMDs that are below -2 SDs but have no history of fracture are designated as having an areal BMD that is “below expected range for age;” the use of the term osteopenia is eschewed in this population. The International Osteoporosis Foundation (IOF) has defined osteoporosis in young adults as a vertebral or hip areal BMD T score that is below -2.5 and is associated with a disorder that adversely affects bone mineralization.³¹⁶ Both groups agree that in young adults, the presence of osteoporosis is not defined by low BMD alone. Many of the diseases that lead to osteoporosis in young adults begin during childhood and adolescence. The WHO and IOF-designated criteria for low or high bone mass do not apply to children and adolescents in whom variability in chronological and bone ages, height, weight, and stage of sexual maturation, as well as gender and ethnicity affect bone mineralization. (The term osteopenia is not used when discussing bone mineralization in infants, children, and adolescents.)

In children and adolescents, osteoporosis or low bone mass is considered present when the child/adolescent has sustained: (1) one or more low-traumatic vertebral fractures in the absence of local disease or high-impact injury or (2) two or more low impact fractures of the long bones (fall from standing height or less) if less than 10 years of age or three or more low-impact fractures before 19 years of age together with whole body minus (–) head and posterior-anterior lumbar spine BMC/BMD as assessed by DXA that are minus (–) 2 SD

TABLE 20.10A Disorders of Bone Mineralization: Low Bone Mass**I Primary/Genetic**

- A Osteogenesis imperfecta (Types I–XIII—see Table 20.10B)
- B Osteoporosis-pseudoglioma syndrome (*LRP5*)
- C Idiopathic juvenile osteoporosis (*LRP5*)
- D Marfan syndrome (*FBN1*)
- E Ehlers-Danlos syndrome (*COL1A1*)
- F Homocystinuria (*CBS*)
- G Fibrous dysplasia (*GNAS*)
- H Glycogen storage disease type I (*G6PC*)
- I Galactosemia (*GALT*)
- J Menkes kinky hair syndrome (*ATP7A*)
- K Hypophosphatasia (*ALPL*)
- L Rickets (genetic forms—see Table 20.8B)

II Secondary

- A Suboptimal nutrition
 - 1 Socioeconomic
 - 2 Cultural
 - 3 Excessive exercise (athletic amenorrhea)
 - 4 Anorexia nervosa
 - 5 Vitamin D deficiency
 - 6 Malabsorption—cystic fibrosis, celiac disease, biliary atresia, “short gut” syndrome, postgastric bypass surgery
 - 7 Idiopathic hypercalciuria
- B Endocrinopathies/metabolic diseases
 - 1 Constitutional delay in growth and sexual development
 - 2 Hypogonadism
 - a Hypergonadotropic—gonadal dysgenesis (Turner, Klinefelter syndromes), aromatase deficiency, estrogen receptor deficiency
 - b Hypogonadotropic—Kallmann syndrome, excessive physical activity, hyperprolactinemia
 - 3 Diabetes mellitus
 - 4 Hyperglucocorticoidism
 - 5 Hyperthyroidism, excessive exogenous thyroxine
 - 6 Hyperparathyroidism
 - 7 Panhypopituitarism
 - 8 Inborn errors of metabolism—homocystinuria, lysinuric protein intolerance, propionic aciduria, methylmalonic aciduria, glycogen storage disease, galactosemia
- C Disuse/immobilization
 - 1 Fracture
 - 2 Cerebral palsy
 - 3 Duchenne muscular dystrophy
 - 4 Quadriplegia/paraplegia
 - 5 Spina bifida
 - 6 Weightlessness
- D Inflammatory diseases
 - 1 Juvenile arthritis
 - 2 Systemic lupus erythematosus
 - 3 Dermatomyositis
 - 4 Inflammatory bowel disease
- E Drugs
 - 1 Glucocorticoids, immune suppressants, anticonvulsant drugs, antiretroviral therapy, warfarin, lithium, methotrexate, cyclosporine
 - 2 Alcohol, tobacco
 - 3 Aromatase inhibitors, medroxyprogesterone
 - 4 Protein pump inhibitors
- F Chronic illness
 - 1 Hemoglobinopathies—thalassemia, sickle cell disease
 - 2 Hemophilia
 - 3 Cranial radiation
 - 4 Renal failure, postrenal transplantation, nephrotic syndrome
 - 5 Malignancy—leukemia, lymphoma, cytotoxic chemotherapy
 - 6 Human immunodeficiency virus infection—highly active antiretroviral therapy
 - 7 Severe burns

Prepared from: Boyce AM, Gafni RI. Approach to the child with fractures. *J Clin Endocrinol Metab.* 2011;96:1943–1952; Ferrari S, Bianchi ML, Eisman JA, et al. Osteoporosis in young adults: pathophysiology, diagnosis, and management. *Osteoporos Int.* 2012;23:2735–2748; Rauch F, Bishop N. Juvenile osteoporosis. In: Rosen CJ (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 7th ed. Washington, DC: The American Society of Bone and Mineral Metabolism; 2008:264–267; Bachrach LK, Ward LM. Clinical review: bisphosphonate use in childhood osteoporosis. *J Clin Endocrinol Metab.* 2009;94:400–409.

TABLE 20.10B Clinical/Phenotypic Classification of Osteogenesis Imperfecta (OI)

Type	Clinical & Skeletal Manifestations	Transmission	Genetic Variants ¹
1	Mild—near normal stature; wormian skull bones, vertebrae are biconcave & flattened, femoral bowing on occasion, increased joint mobility, blue sclerae, brittle teeth (dentinogenesis imperfecta), deafness	Autosomal dominant	<i>COL1A1</i> , <i>COL1A2</i>
2	Lethal perinatally, multiple fractures, wormian skull bones, flattened vertebral bodies throughout spine, bowed long bones, flat iliac wings & acetabulae	Autosomal dominant or recessive	<i>COL1A1</i> , <i>COL1A2</i> , <i>CRTAP</i> , <i>LEPRE1</i> , <i>PPIB</i> , <i>CREB3L1</i>
3	Progressive deformities & fractures of long bones, short stature, soft skull bones, vertebral compression fractures, scoliosis, blue sclerae, deafness	Autosomal dominant or recessive	<i>COL1A1</i> , <i>COL1A2</i> , <i>CRTAP</i> , <i>LEPRE1</i> , <i>PPIB</i> , <i>FKBP10</i> , <i>SERPINH1</i> , <i>SERPINF1</i> , <i>BMP</i> , <i>BMP1</i> , <i>WNT1</i> , <i>SP7</i> , <i>TENT5A</i> , <i>MBTPS2</i>
4	Variable propensity to fracture and deformation of long bones, short stature, light blue sclerae, possible dentinogenesis imperfecta deafness	Autosomal dominant or recessive	<i>COL1A1</i> , <i>COL1A2</i> , <i>CRTAP</i> , <i>PPIB</i> , <i>WNT1</i> , <i>TMEM38B</i> , <i>SP7</i>
5	Similar to OI type IV with exuberant callus formation at sites of healing fractures & calcification of interosseous membranes	Autosomal dominant	<i>IFITM5</i>

Please see Table 20.10C for more details concerning specific gene variants associated with osteogenesis imperfecta.

Prepared from: Bonafe L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. *Am J Med Genet.* 2015;167A:2869–2892; Vierucci F, Saggese G, Cimaz R. Osteoporosis in childhood. *Curr Opin Rheumatol.* 2017;29(5):539–546.

(e.g., Z score -2) below mean for ethnicity, gender, chronological age, and height/height age; when interpreting a DXA study, one should also consider the stage of sexual maturity relative to the chronological age of the subject.^{317,319–323} (Whole-body DXA data that include the head is not considered an optimal measure of BMC/BMD because the skull does not respond directly to environmental factors, such as weight-bearing exercise.) Thus osteoporosis in children and adolescents is suggested by the clinical presentation (bone pain, growth arrest, immobility) because of non/low-traumatic long bone and/or vertebral compression fractures identified by roentgenograms of the long bones and/or findings on lateral radiographs of the thoracolumbar spine.³²² It is essential to differentiate

TABLE 20.10C Gene Variants/Mutations Causally Related to Osteogenesis Imperfecta

Number OMIM Disease—Gene Chromosome/OMIM	Severity/Type	Clinical Features	Growth Impairment	Blue Sclera	Inheritance	Functional Defect
I - 166200 - <i>COL1A1</i> - Collagen type I, alpha-1 17q21.33 1120150 or <i>COL1A2</i> - Collagen type I, alpha-2 7q22.3 120160	Mild - 1	Few (often vertebral) fractures, little deformity, hearing loss in 50%; rarely dentinogenesis imperfecta	Minimal	Present - intense	AD	Nonsense & frameshift mutations result in haploinsufficiency & decreased production of collagen type I
II - 166210 - <i>COL1A1</i> or <i>COL1A2</i>	Perinatal lethal - 2 congenital (see also Types VII, VIII, IX, X)	Many rib & long bone fractures in utero & at birth, severe long bone deformities, "beaded" ribs, unmineralized calvarium	Severe	Present	AD, parental mosaicism	Glycine substitutions in <i>COL1A1</i> or <i>COL1A2</i> result in structurally abnormal collagen type I
III - 259420 - <i>COL1A1</i> or <i>COL1A2</i>	Severe, progressive & deforming - 3	Moderate to severe bowing, multiple long bone & vertebral fractures, dentinogenesis imperfecta, hearing loss	Severe	Present but lighten with age	AD	Glycine substitutions in <i>COL1A1</i> or <i>COL1A2</i> result in structurally abnormal collagen type I
IV - 166220 - <i>COL1A2</i> or <i>COL1A1</i>	Moderately deforming - 4	Mild to moderate bowing, fractures, dentinogenesis imperfecta	Moderate, variable	Grayish or absent	AD	Glycine substitutions in <i>COL1A1</i> or <i>COL1A2</i> result in structurally abnormal collagen type I
V - 610967 - <i>IFITM5</i> - Interferon-induced transmembrane protein-5 11p15.5 614757	Moderately deforming - 5	Mild to moderate bone fragility, ossification of interosseous membranes of forearm, hyperplastic callus formation at fracture sites; "mesh-like" bone lamellation pattern	Mild to moderate	Absent	AD	Osteoblast specific membrane protein essential for osteoblast differentiation; functional consequence of the constant mutation (c.-14C>T) in <i>IFITM5</i> is uncertain
VI - 613982 - <i>SERPINF1</i> - Serpin peptidase inhibitor, clade F, member 1 17p13.3 172860	Moderately to severely deforming - 3	Onset of fractures of long bones & vertebrae in infancy, increased alkaline phosphatase activity & osteoid, "fish-scale" pattern of lamellation	Moderate to severe	Grayish	AR	Loss-of-function mutations in pigment epithelium-derived factor (PEDF)—regulator of bone mineralization & osteoclast differentiation
VII - 610682 ^a <i>CRTAP</i> - Cartilage associated protein 3p22.3 605497	Moderately deforming to severely deforming or lethal - 2, 3, 4	Fractures may be present at birth, rhizomelia, limb deformities	Moderate	Absent or faint	AR	Inactivating mutations (duplication) of <i>CRTAP</i> impair 3-hydroxylation of prolyl-986 of procollagen $\alpha 1(I)$
VIII - 610915 ^a <i>P3HI (LEPRE1)</i> - Leucine- and proline-enriched proteoglycan 1 (Prolyl hydroxylase 1) 1p34 610339	Severely deforming to lethal - 2, 3	Markedly decreased bone mineralization, scoliosis, platyspondyly, prominent metaphyses, long phalanges	Severe	Absent	AR	Inactivating mutations of <i>LEPRE</i> encoding prolyl-3-hydroxylase 1 (leprecan) impair 3-hydroxylation of prolyl-986 of procollagen $\alpha 1(I)$
IX - 259440 - ^a <i>PP1B</i> - Cyclophilin B - 15q22.31 123841	Lethal to severely deforming, see - 2, 3, 4	Shortened, bowed, & fractured long bones in midgestation	Severe	Gray	AR	Inactivating mutations of <i>PP1B</i> encoding peptidyl-prolyl isomerase B impair 3-hydroxylation of prolyl-986 of procollagen $\alpha 1(I)$
X - 613848 - <i>SERPINH1</i> - Serpin peptidase inhibitor, clade H, member 1 11q13.5 600943	Severe - 3	Short bowed femora in utero, multiple fractures in first month of life, dentinogenesis imperfecta	Severe	Present	AR	Loss-of-function mutations in CBP2 (HSP47)—chaperone, essential for maintenance of integrity of the triple helical structure of collagen type 1 & its resistance to proteolytic degradation
XI - 610968 <i>FKBP10</i> - FK506-binding protein 10 17q21.2 607063	Severe & progressive deformities - 3	Limbs are short & bowed, joint contractures; dentinogenesis imperfecta; fish-scale pattern of bone lamellation (see Type VI)	Moderate	Gray	AR	Loss-of-function mutations in a chaperone protein—FKBP65—essential for posttranslational processing of procollagen type I; also mutated in Bruck syndrome type 1

TABLE 20.10C Gene Variants/Mutations Causally Related to Osteogenesis Imperfecta—cont'd

Number OMIM Disease—Gene Chromosome/OMIM	Severity/Type	Clinical Features	Growth Impairment	Blue Sclera	Inheritance	Functional Defect
XII - 613849 - <i>SP7</i> - Transcription factor specificity factor (Sp)7 12q13.13 606633	Moderate to severe - 4	Multiple fractures in early infancy; short, bowed long bones, highly arched palate	Severe	Absent	AR	Inactivating mutations in Osterix, a transcription factor essential for osteoblast differentiation & bone mineralization
XIII - 614856 - <i>BMP1</i> - Bone morphogenetic protein 1 8p21.3 112264	Moderate to severe - 3	Generalized deformities of all bones, multiple fractures despite increased bone mineral density	Severe	Faint blue	AR	Inactivating mutations of procollagen type I C-terminal propeptide endoproteinase impair collagen formation
XIV - 615066 - <i>TMEM38B</i> - Transmembrane protein 38B 9q31.1 611236	Moderate - 3	Variable severity; fractures occur in utero or during early childhood; moderate bowing of the lower limbs	Moderate	Absent	AR	Inactivating mutations of a component of an intracellular monovalent cation channel necessary for Ca ²⁺ release from storage sites
XV - 615220 - <i>WNT1</i> - Wingless integration site family, member 1 12q13.12 164820	Progressively deforming - 3	Multiple fractures & bone deformities in childhood; cerebellar hypoplasia, profound developmental delay	Present	Present	AR	Mutated WNT1 fails to activate LRP5-beta catenin signaling resulting in impaired formation & function of osteoblasts
XVI - 616229 - <i>CREB3L1</i> - Cyclic AMP response element-binding protein 3-like 1 11p11.2 616215	Lethal prenatally - 2	Fractures present in utero -		Present	AR	Loss of transcriptional activation of <i>COL1A1</i>
XVII - 616597 - <i>SPARC</i> - Secreted protein, acidic cysteine-rich 5q33.1 182120	Moderate severity - 4	Limb & vertebral fractures in early childhood, joint laxity, muscular weakness	Present	Not stated	AR	Encodes osteonectin—phosphoprotein secreted by osteoblasts that enables calcification of bone collagen
XVIII - 617592 - <i>TENT5A</i> - Terminal nucleotidyltransferase 5A 6q14.1 611357	Moderately severe - 3	Congenital bowing, multiple fractures by age 1 year, vertebral collapse, progressive scoliosis, dentinogenesis imperfecta	Present	Present	AR	Nucleotidyltransferase catalyzes transfer of nucleoside monophosphate to hydroxyl group of acceptor molecule; expressed in osteoblast
XIX - 301014 - <i>MBTPS2</i> - Membrane-bound transcription factor protease site 2 Xp22.12 300294 Undesignated	Severe - 3	Fractures occur prenatally & postnatally, bowing of long bones, pectus carinatum	Moderate	Present	XLR	Zinc metalloproteinase; regulates proteolysis of transcription & other factors required for secretion & cross-linking of collagen type 1
<i>PLOD2</i> - Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 3q24 601865	Severe - 3; osseous fragility with congenital joint contractures	Fractures in infancy, pterygia, scoliosis	Severe	Absent	AR	Encodes a procollagen lysyl hydroxylase necessary for formation of collagen type 1 telopeptide; Bruck syndrome 2
300131 - <i>PLS3</i> - Plastin 3 Xq23 300131	Mild - 1	Osteopenia - incidental finding	Minimal	Absent	XLR	Actin & calcium binding protein required for integrity of osteocyte cytoskeleton
176790 - <i>P4HB</i> - Procollagen-proline, 2-oxoglutarate-4-dioxygenase, beta subunit 17q25.3	Severe - 3	Cole-Carpenter syndrome 1 - Bone fragility, craniosynostosis, hydrocephalus, ocular proptosis	Modest	Present	AD	Beta subunit of prolyl 4-hydroxylase - catalyzes synthesis of 4-hydroxyproline in preprocollagen

Continued

TABLE 20.10C Gene Variants/Mutations Causally Related to Osteogenesis Imperfecta—cont'd

Number OMIM Disease—Gene Chromosome/OMIM	Severity/Type	Clinical Features	Growth Impairment	Blue Sclera	Inheritance	Functional Defect
607186 <i>SEC24D</i> SEC24-related gene family, member D 4q26	Severe - 3	Cole-Carpenter syndrome 2		Present	AR	Component of vesicles that facilitate transport of protein from endoplasmic reticulum
603506 <i>LRP5</i> Low-density lipoprotein receptor-related protein 5 11q13.2	Severe - 3	Osteoporosis-pseudoglioma syndrome (259770) - bone fragility, intraocular pseudoglioma, corneal opacity, glaucoma	Severe	Corneal opacities	AR	Coreceptor with LRP6 & Frizzled for WNT1; inactivating variants impair osteoblastogenesis

AD, Autosomal dominant; AR, autosomal recessive.

^aComplex of enzymes regulating 3-hydroxylation of prolyl 986 of procollagen $\alpha 1(I)$. (From Marini JC. Osteogenesis imperfecta. In: Rosen CJ (ed). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 7th ed. Washington, DC: The American Society of Bone and Mineral Metabolism; 2008:446–450; Barnes AM, Chang W, Morello R, et al. Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta. *N Engl J Med*. 2006;355, 2757–2764; Bishop N. Characterising and treating osteogenesis imperfecta. *Early Hum Dev*. 2010;86:743–746; Rauch F, Lalic L, Roughley P, Glorieux FH. Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. *J Bone Miner Res*. 2010;25:1367–1374.)

vertebral compression fractures from variants of normal vertebral morphology using appropriate semi-quantitative methods. In children and adolescents, bone mass may be quantitated by DXA determination of areal BMD of the “whole body minus head” and of body regions (lumbar spine, hip, distal one-third radius). (DXA analyses of hip bone mass are variable in children and adolescents and not usually used for interpretation of BMC or BMD data.) A portable DXA unit that measures areal BMD in the ultradistal, middistal, and one-third distal regions of the nondominant forearm may also be used to determine areal BMD; it provides data comparable to a fixed DXA instrument. Despite its limitations (provision of areal rather than volumetric BMD data and failure to distinguish between trabecular and cortical bone), DXA is the most widely used bone densitometric method in children at this time. Other methods used for determination of bone mineralization include QCT, pQCT of the tibia and radius, high-resolution pQCT (HRpQCT), and QUS. Estimation of bone mass by QCT provides quantitative three-dimensional information about cortical and trabecular bone volume and mass. Relative to bone strength determined by calculation of the stress-strain index of a long bone with data garnered by pQCT, DXA whole body areal BMC (minus the head) for height also affords a reliable measurement for determining cortical bone strength and hence fracture risk. The report of the BMD/content should include not only the methodology used but also the specific instrument used, the software version used for data analysis, and the ethnic mix of the reference population. Interestingly, in otherwise healthy prepubertal and pubertal boys who sustain a high-impact traumatic fracture, regional BMDs tend to be within the lower half of the normal range; additionally, bone microstructure tends to be aberrant, and bone strength decreased—perhaps reflecting an intrinsic bone defect.³¹⁵

In general, impaired bone strength may develop as a consequence of congenital or acquired disorders that adversely affect bone formation or mineralization.^{322,324} These disorders may be related to suboptimal nutritional intake or nutrient absorption (anorexia nervosa, cystic fibrosis, celiac disease), chronic inflammatory disease (bowel, joint), immobilization (paresis, muscular dystrophy, cerebral palsy), hematological abnormalities (leukemia and its chemotherapy, sickle cell anemia, thalassemia), medications (glucocorticoids, immunosuppressant drugs, anticonvulsant agents), or endocrinopathies (growth,

thyroid, or sex hormone deficiencies or excessive secretion of glucocorticoids). In patients with endogenous or therapeutic excess of cortisol and related glucocorticoids, bone mass and mineralization are decreased whereas fracture risk is increased because of the suppressive effects of these agents on bone formation and acceleration of bone resorption with consequent increase in skeletal fragility.^{325–327} GH deficiency is associated with decreased bone turnover rate and subnormal mineralization of the skeleton, as well as increase in fracture risk with improvement during treatment with recombinant human GH.

Although heritable factors account for 60% to 80% of optimal bone mineralization, modifiable factors that contribute to the development of osteopenia and osteoporosis in adulthood (weight-bearing exercise, nutrition, body mass, hormonal milieu) have their genesis in utero, infancy, childhood, and adolescence.^{328,329} In children as in adults, bone mass, composition, microarchitecture, and size determine bone strength. Because many youths consume excessive amounts of carbonated beverages and diluted fruit juices thus limiting their intake of milk, children and adolescents ingest only 55% to 70% of the recommended daily calcium allowance (1300 mg/day), although late pubertal males tend to consume more than do pubertal females. In adolescent and adult females, excessive intake of cola drinks with high phosphoric acid levels lower body calcium content by sequestering dietary calcium in the intestinal tract and increasing dissolution of bone mineral to neutralize acid with the possible consequent development of mild secondary hyperparathyroidism. Sedentary, non-weight-bearing activities encouraged by television, video, and computer games also impair bone mineralization.³³⁰ In a study of 300 male and female adolescents and young adults (13–21 years of age) using both DXA assessment of body composition and QCT measurement of the axial and appendicular skeletons, a positive correlation between lean mass and all bone measurements has been reported in both genders.³³¹ In children and adolescents, fat mass, waist circumference, and insulin resistance are negatively related to DXA-BMC of the lumbar spine and total body BMD.³³² These observations suggest that bone mass and strength are determined by dynamic muscular forces—not solely by static load. The odds ratios for fractures of the foot, ankle, leg, and knee increase as body mass index rises particularly in 6- to 11-year-old children.³³³

Nutritional deprivation depresses the rate of bone accrual; extreme weight loss, such as in patients with anorexia nervosa has many adverse effects upon bone mass, including decreased areal BMD as assessed by DXA, and low distal radial total and trabecular volumetric BMD, decreased cortical bone thickness and area, and higher cortical porosity as determined by HRpQCT leading to reduced bone strength and increased fracture risk.³²² Anorexia nervosa is a psychiatric disorder in which chronic undernutrition is self-induced because of an underlying emotional disturbance.³³⁴ It occurs more often in females than males and often begins in adolescence (peak age of onset of anorexia nervosa is between 13 and 18 years of age) and may persist well into adulthood. There is a substantially increased fracture risk in these patients that is caused by failure to accrue sufficient bone mass as thickness and area of bone cortex, trabecular number and thickness, turnover rate, and bone strength are reduced relative to normal subjects. Pathophysiologically, low bone mass and decreased bone strength are the consequences not only of a paucity of the protein and mineral components of bone and chronic acidemia, but also of the hormonal adaptation to starvation. Thus resistance to the biological effects of GH manifested by decreased synthesis of IGF-1 and resistance to the appetite-stimulating effects of the GH secretagogue ghrelin, stress-related increase in glucocorticoid production resulting in hypercortisolemia, and depressed synthesis of estrogens and androgens (both testosterone and dehydroepiandrosterone) caused by hypogonadotropism synergize to impede osteogenesis and bone strength, thereby increasing fracture risk. Patients with anorexia nervosa are also hypoleptinemic because of scarcity of adipose tissue. Leptin is an adipokine that decreases appetite, enhances gonadotropin secretion, and may affect bone mass as it directly increases chondrogenesis and osteoblastogenesis and inhibits osteoclastogenesis.³³⁵ Administration of estrogen/progestin to females with anorexia nervosa does not increase bone mass or prevent its loss; bisphosphonates, IGF-1, and dehydroepiandrosterone may maintain or increase BMD in such patients but their use should be limited. Even in adolescents with anorexia nervosa whose disorder is managed successfully, increased fracture risk persists well into adulthood. The “athletic triad” of suboptimal body fat mass, amenorrhea in women, and decreased bone mass attributed to low sex hormone production is encountered in the highly trained female athlete and the elite long-distance male runner. There is a population of well children, adolescents, and young adults with “constitutional thinness” that is characterized by long-standing suboptimal weight for height but normal growth, sexual maturation, and fertility in whom the fracture risk is not increased despite the areal BMD being low.

Although the longer and more intense is weekly sporting activity in children and adolescents, the greater is the vertebral areal BMD independently of calcium intake, excessively prolonged engagement in high-impact activities, such as basketball, gymnastics, and cheerleading the greater is the likelihood of sustaining a fracture in females.^{225,336} In pre- and peripubertal children, simple school physical education programs using jumping, hopping, and skipping exercises 2 to 3 times weekly significantly increases areal BMD at the femoral trochanter in as little as 8 months relative to children engaged in a standard physical education curriculum. Suboptimal nutrition and sedentary activities during childhood and adolescence, as well as consumption of colas and alcohol and smoking of cigarettes, prevent optimal bone mineralization and increase the likelihood of later development of osteoporosis and its complications. Because the risk of developing an osteoporotic fracture as an adult declines by 40% for every 5% increase in peak bone mineral mass, the foundation for the prevention of osteoporosis in the adult must be constructed in the child and adolescent by maintaining

adequate calcium intake, vitamin D stores (serum concentrations of calcidiol >20 ng/mL), and complementary weight-bearing activity during these formative years. Other nutrients, for example, magnesium, protein, vitamins C and K, and copper must also be consumed for optimal bone matrix synthesis.²¹²

Acute immobilization of the healthy, active child and adolescent and the consequent reduction in weight-bearing and decrease in the mechanical load on bones results in a lowered rate of bone formation; in the presence of continued bone resorption, hypercalciuria and later hypercalcemia and lowered bone mass develop.³³⁷ In the chronically immobilized child or adolescent (cerebral palsy, spastic quadriplegia, muscular dystrophy), fracture rate (primarily of the femur) is high during simple maneuvers, such as turning, dressing, or feeding. In this group of subjects, not only lack of weight-bearing but also the severity of the primary illness, body size and pubertal status, state of general nutrition, vitamin D and calcium intake, coexisting inflammatory states, and medications (e.g., anticonvulsants, glucocorticoids), as well as indoor confinement adversely impact bone mass and fracture risk.²²⁵ Distal femoral and lumbar vertebral areal BMDs increase at slower than normal rates in the child with spastic cerebral palsy, resulting in diminution of areal BMD Z scores as an older subject.³³⁸ The intravenous administration of pamidronate and zoledronic acid and subcutaneous injections of recombinant human GH have increased areal BMD in small selected groups of children with quadriplegic and spastic cerebral palsy.³³⁹ Areal BMD increased substantially in 26 immobile patients with quadriplegic cerebral palsy (3–17 years of age) during administration of the bisphosphonate alendronate (1 mg/kg/week orally), calcium (600 mg/day), and vitamin D (400 IU/day) over 1 year of treatment.³⁴⁰ Assisted standing with weight-bearing by itself may also increase BMD in children with severe cerebral palsy.

The importance of normal gonadal sex steroid secretion during age-appropriate sexual maturation is emphasized by the observation that in adult males with delayed sexual development, radial, vertebral, and femoral areal BMDs are lower than in males with normal timing of pubertal maturation.³¹⁶ Histomorphometrically, in adult sex-steroid-deprived bone, there is decrease in cortical width, trabecular number, osteoid, and mineralization activity; reduced bone mass and abnormal microarchitecture result in decreased skeletal strength and increased risk of fracture.³⁴¹ As a consequence of estrogen (and androgen) deficiency, there is increase in the production but decline in the life span of osteoblasts and osteocytes, while osteoclastogenesis is stimulated and the life span of osteoclasts prolonged; these events reflect the sum of the activities of multiple proosteoclastogenic cytokines whose synthesis is regulated by estrogen, including M-CSF, IL-1 and -6, TNF α , RANKL, and osteoprotegerin. Although osteopenia responsive to estrogen has been recorded in adult males with aromatase deficiency, mature women with complete androgen insensitivity are also osteopenic despite normal to increased estrogen production, clearly indicating that androgens are also important for normal bone mineralization.³⁴¹

The increased risk of fractures in prepubertal and adolescent girls and adult women with Turner syndrome (gonadal dysgenesis) has been attributed to both intrinsic abnormalities of bone itself as manifested by the characteristic malformations present in this disorder, including Madelung deformity of the wrist, mesomelia, scoliosis, cubitus valgus, and shortened metacarpal and metatarsal bones—the consequence of haploinsufficiency of Short stature Homeobox-containing gene (*SHOX*, OMIM 312865) and of decreased bone mineralization as a result of chronic estrogen deprivation.³⁴² In patients with Turner syndrome, there is selective deficiency of cortical bone, whereas trabecular bone density

may be normal as may areal and volumetric BMDs when adjusted for height. Inasmuch as FSH stimulates osteoclastogenesis both directly and by enhancing TNF α -mediated increased expression of RANKL and subsequent osteoclast differentiation, the elevated levels of this gonadotropin present in the female with ovarian failure may contribute to the suboptimal bone mineralization of patients with Turner syndrome.³⁴² In addition, disorders that may exert an adverse impact on bone mineralization (e.g., inflammatory bowel disease, celiac disease, hypothyroidism) are more common in patients with Turner syndrome. The frequency of wrist fractures is increased during childhood as is the general risk for fractures in adults with Turner syndrome. In subjects with gonadal dysgenesis, estrogen replacement therapy during early adolescence increases bone calcium deposition and areal BMD and cortical and trabecular bone mass, whereas delayed estrogen replacement is associated with persistent decrease in cortical bone mass albeit normalization of trabecular bone mass.³⁴³ Recombinant human GH administered during childhood increases growth rate, adult stature, and bone size in many patients with Turner syndrome, and perhaps increases bone strength and reduces fracture risk.³⁴⁴ In girls with Turner syndrome, appropriate intake of calcium and vitamin D and weight-bearing exercises are also necessary. Bisphosphonates are not recommended for treatment of Turner syndrome adolescents because these agents act primarily on trabecular bone, the primary target of estrogen. In children with central precocious puberty, administration of a gonadotropin-suppressing agent may decrease the rate of bone mineral accrual, a process that is apparently reversible after completion of therapy.³⁴⁵ Appropriate intake of calcium and vitamin D and weight-bearing exercises should be prescribed for subjects receiving a gonadotropin-suppressing agent.

Pubertal subjects with primary (e.g., Klinefelter syndrome, galactosemia, postradiation or chemotherapy) or secondary hypogonadism (anorexia nervosa, excessive physical training, hypogonadotropism) also have decreased bone mineralization. By decreasing estrogen production, even short-term (6 months) use of the intramuscular contraceptive depot medroxyprogesterone acetate (MPA) results in significant loss in bone mass in adolescent females and young women (18–21 years of age) when BMD would ordinarily be increasing; however, bone mass increases over time after this agent is discontinued.³⁴⁶ The adverse impact of depot MPA on BMD may be prevented by concomitant administration of estrogen.

The low bone mass of glucocorticoid excess is the result of inhibition of osteoblastogenesis and increase in the rate of apoptosis of the osteoblast and osteocyte—leading to decrease in the rate of bone matrix formation and microfracture repair; they enhance osteoclastogenesis and increase osteolysis—the result of increase in expression of RANKL in association with decrease in the rate of apoptosis of the osteoclast—permitting prolonged and excessive bone resorption.^{322,327,347,348} Because glucocorticoids decrease osteocyte-mediated bone formation, during each remodeling cycle the amount of bone replaced is far less than the amount removed and skeletal microarchitecture degraded resulting in declining bone mass and strength over time. Indirectly, glucocorticoids impair bone accretion by suppressing secretion of IGF-1 and by decreasing intestinal calcium absorption.^{327,348} By decreasing muscle strength, glucocorticoids lower the forces exerted on bone further impairing its formation. Children receiving glucocorticoids for treatment of asthma or rheumatic disorders should also receive 2000 IU of vitamin D daily.³²⁴

At the molecular level, glucocorticoids suppress expression and synthesis of *RUNX2* (OMIM 600211, chr. 6p21.1) and *BMP2* (OMIM 112261, chr. 20p12.3), factors essential for prenatal and postnatal osteoblast differentiation, respectively, and

increase osteoblast expression of *TNFSF11* encoding RANKL and decrease expression of *TNFRSF11B* encoding osteoprotegerin—the decoy receptor for RANKL, changes that favor osteoclastogenesis.^{347,349} Glucocorticoids impair WNT/ β -catenin signaling and consequently inhibit synthesis of collagen type I, as well as increase its rate of degradation; they impair IGF-1 formation and function. They also direct the mesenchymal osteoblast precursor into the pathway of adipocyte differentiation. To a limited extent, glucocorticoids inhibit normal vitamin D metabolism and thereby vitamin D-dependent intestinal absorption of calcium. They increase renal loss of calcium by a direct effect on the renal tubule, leading to secondary hyperparathyroidism.^{347,349} Glucocorticoids also reduce production of sex hormones in the adolescent and adult. The muscle weakness of chronic glucocorticoid exposure reduces the impact of mechanical forces on bone formation. Glucocorticoid-mediated inhibition of linear growth and widening of bones contributes to impairment of bone mineralization in children.²³² Glucocorticoids adversely affect trabecular bone formation initially and later impair cortical bone accumulation.³⁵⁰ The disease (e.g., asthma, nephrotic syndrome, acute lymphoblastic leukemia, chronic inflammatory disorders, such as juvenile arthritis) for which pharmacological doses of glucocorticoids have been prescribed contributes to decreased bone mass by impairing mobility and by elaboration of osteoclastogenic cytokines. Although glucocorticoid-reduced bone mass accumulation is greater with frequent intermittent bursts of oral glucocorticoids than with inhaled glucocorticoids in boys with asthma, in high doses over prolonged periods, inhaled glucocorticoids can also lead to decreased bone mass.^{351–353} In young adults with asthma, there is an inverse relationship between vertebral and femoral areal BMDs and cumulative dose of inhaled glucocorticoid with increasing fracture risk as the dose and duration of glucocorticoid administration increases; a cumulative dose of 5000 mg of hydrocortisone leads to a 1-SD decline in vertebral BMD.³⁵⁴ In adult women with 21-hydroxylase deficient congenital adrenal hyperplasia treated with glucocorticoids, bone mineralization is modestly reduced, in part related to the extent of suppression of adrenal androgen production. It is suggested that when children begin to receive either oral or inhaled glucocorticoids that they also be instructed to ingest age-appropriate amounts of calcium and vitamin D and to engage in weight-bearing exercises. In children experiencing adverse effects of glucocorticoids on growth and bone mineralization, it is important to lower their steroid dose to the greatest extent possible and to withdraw them if at all feasible. Although often recommended for adults being treated with glucocorticoids, routine administration of a bisphosphonate to children receiving these agents is not recommended unless there is steady decline in bone mass or the patient has had a low impact or fragility fracture attributable to low bone mass.^{348,350} Nevertheless, early use of a bisphosphonate (e.g., alendronate) might prevent bone loss in glucocorticoid-treated patients with childhood-onset rheumatic disease.

GH exerts direct and indirect actions upon bone formation.³⁵⁵ In growth plate cartilage, GH stimulates local synthesis of IGF-1 that in turn enhances proliferation of resting chondrocytes and endochondral ossification; in bone, GH enhances osteoblast differentiation, proliferation, and function resulting in increased osteoblast synthesis of IGF-1, IGFBP-3, osteocalcin, bone-specific alkaline phosphatase, and procollagen type I; GH stimulates osteoblast synthesis of osteoprotegerin, thereby impairing osteoclastogenesis but it also stimulates osteoclastogenesis and bone resorption indirectly acting through IGF-1-mediated synthesis of RANKL.^{355–357} GH primarily stimulates formation of cortical bone. IGF-1 regulates differentiation of chondrocytes and maintains their rate of

proliferation.³⁵⁷ Osteoblast and osteocyte-synthesized IGF-1 affects osteoblast maturation and the rates of matrix mineralization and bone turnover and mediates the response of bone to a mechanical load and to PTH.³⁵⁷ PTH, thyroid hormone, and estrogen stimulate and glucocorticoids inhibit osteoblast secretion of IGF-1.³⁵⁵ Administration of GH to GH-deficient children and adults increases the rates of both bone formation and destruction, the latter predominating initially; over long periods of treatment (12–18 months) GH increases areal BMD in these patients; to achieve peak bone mass, however, GH therapy must be continued into adulthood. Nevertheless, in many untreated adults with GH deficiency, volumetric BMD is often normal despite low areal BMD values reflecting the smaller size of their bones. In part, low bone mass in the GH-deficient child may also be attributed to the small bone size of the short child compared with age peers when examined as areal BMD by DXA. Measurement of bone mass by either size-corrected areal BMD or volumetric methods (e.g., pQCT) suggests that volumetric BMD is normal or slightly low in the majority of GH-deficient subjects; nevertheless fracture risk is increased in adults with GH deficiency of childhood onset.³⁵⁵ In patients with hypopituitarism of adult onset associated with multiple pituitary hormone deficiencies, fracture risk is increased, but this may be attributable to loss of gonadotropin stimulated sex hormone secretion and to the decreased mechanostat effects of reduced muscle mass on bone accretion in these fully grown subjects. GH primarily enhances cortical bone growth; thus in a state of excessive GH secretion (e.g., acromegaly), increased membrane bone growth results in the characteristic facial features and related bony overgrowth of those affected; however, GH excess may also decrease vertebral areal BMD and increase vertebral fracture risk in these patients.³⁵⁵

Triiodothyronine, the biologically active thyroid hormone, downregulates proliferation of chondrocytes and increases their rate of differentiation to prehypertrophic and hypertrophic chondrocytes.³⁵⁸ Triiodothyronine stimulates osteoblast differentiation and synthesis of osteocalcin, osteopontin, type I collagen, alkaline phosphatase, and IGF-1; it also enhances osteoclastogenesis and, thus the rate of bone resorption, but likely does so indirectly by increasing osteoblast synthesis of RANKL. In children with hyperthyroidism, the rate of cartilage maturation is accelerated, fusion of cranial sutures is enhanced, and osteoclastogenic effects are prominent. Thus in thyrotoxic patients, there is increase in the rate of bone turnover and decrease in the length of the bone remodeling cycle (primarily because of shortening of the bone formation phase) resulting in a net loss of mineralized bone.³⁵⁸ As in adults with thyrotoxicosis, whole body, vertebral, and femoral BMDs are low in children and adolescents with hyperthyroidism, but they substantially increase within the first 12 to 24 months after restoration of the euthyroid state. In hypothyroid children, endochondral maturation is delayed and calcification abnormal; administration of physiological replacement doses of thyroxine to children with acquired or congenital hypothyroidism advances cartilage maturation and endochondral bone formation; treatment does not adversely affect bone mineralization during childhood, although adults with congenital hypothyroidism may have a 10% reduction in radial bone mass.

Hyperglycemia is associated with accumulation of highly glycated proteins (protein bound glucose/fructose) that impair differentiation of mesenchymal stem cells to chondrocytes and osteoblasts with consequent decreased synthesis of osteocalcin and other products of the osteoblast; osteoclastogenesis is also impaired.^{359,360} In patients with type 1 diabetes mellitus, areal BMD is decreased, bone size is reduced, and fracture risk is increased, reflecting the adverse effects of chronic hyperglycemia and insulin deficiency upon bone formation. In adolescents with type 1 diabetes mellitus, whole body, axial, and

appendicular bone mass assessed by DXA is decreased relative to control subjects and inversely related to Hb A1c values. Using pQCT in young, prepubertal subjects with type 1 diabetes mellitus, cortical bone cross-sectional area and BMD were found to be decreased implying an increased risk for fracture.³⁶¹ Although patients with type 2 diabetes mellitus tend to have increased areal BMD (related to decline in the rate of bone turnover perhaps related to hyperinsulinemia), their fracture risk is nevertheless increased.

Bone mass is decreased in the majority of children with acute lymphoblastic leukemia and 26% of these patients sustain a fracture often of a vertebra before or within the first four years of treatment.³²² Pathogenetic factors involved in the development of low bone mass in these subjects include: adverse effects of the disease itself directly on bone—mediated by cytokine-induced increase in osteoclastogenesis and activity and suppression of bone remodeling, radiation injury of bone; inhibitory effects of glucocorticoid, chemotherapeutic, antibiotic, and immunosuppressive agents on bone formation; diminished physical activity; decreased caloric, protein, and vitamin D intake; sex hormone deficiency caused by delayed or arrested adolescent development; and GH deficiency in children who have received cranial radiation.³⁶² After initial treatment of the neoplasm and achievement of a remission, administration of a bisphosphonate, such as zoledronic acid, may substantially increase bone mineralization in these children and adolescents.³⁶³ In solid organ transplant recipients, cyclosporine A induces bone loss by increasing osteoblast expression of RANKL and decreasing production of osteoprotegerin, thereby augmenting osteoclastogenesis. Methotrexate and intrathecal chemotherapy exert significant inhibitory effects on bone mineralization in children with malignancies. These patients should receive appropriate calcium and vitamin D supplements and weight-bearing exercises to the extent possible.³⁶² Those who develop GH deficiency following cranial radiation may be treated with recombinant hGH if they remain GH deficient after the primary illness has been in prolonged remission. Decreased bone mineralization is common in the postbone marrow or solid organ transplant subject; its diverse pathogenesis includes the primary disease itself and the chronic illness that may accompany it, the use of high-dose glucocorticoids and antirejection medications, as well as altered intestinal, renal, hepatic, and gonadal function.^{322,364} In addition to provision of adequate nutrition, calcium, and vitamin D, in adults the effects of transplantation on bone accrual may be partially ameliorated by administration of bisphosphonates.

In severely burned patients and children with hemophilia, sickle cell disease, central diabetes insipidus, Marfan syndrome, homocystinuria, lysinuric protein intolerance, and propionic and methylmalonic aciduria, areal BMD is also decreased. Children with cystic fibrosis may have low vertebral and femoral neck areal BMD as a consequence of suboptimal nutrition, vitamin D deficiency, chronic inflammation, concomitant diabetes mellitus, pubertal delay, and drug therapy. However, approximately one-third of optimally managed cystic fibrosis patients with good clinical control may nevertheless have a subnormal BMD, although this is not necessarily translated into increased fracture risk.³⁶⁵ Low bone mass and vertebral collapse may be early manifestations of chronic inflammatory bowel disease.³²⁴ Vitamin D deficiency and secondary hyperparathyroidism, as well as the chronic inflammatory state and therapeutic agents, such as glucocorticoids, likely contribute to decreased bone formation and increased bone resorption in this illness. That whole body areal BMC in children, adolescents, and young adults with chronic inflammatory bowel disease may be normal relative to lean body mass (although reduced relative to ethnic, age, and height norms) does not necessarily imply that bone strength in these patients is normal as evidenced by the

increased fracture risk of adults with this disorder. Low bone mass is common in children and adults with celiac disease.³⁶⁶ Pathogenetic factors associated with low bone mass in children with celiac disease include malabsorption of vitamin D, calcium, protein, and other nutrients, synthesis of osteoclast activating and proinflammatory cytokines, such as IFN- γ and IL-15, -18, and -21, and secondary hyperparathyroidism. In children and adolescents infected with the human immunodeficiency virus, whole body areal BMD may be decreased as a consequence of the infective agent itself, the chronic inflammatory state it induces, suboptimal nutrition including subtle vitamin D deficiency, and the administration of highly active antiretroviral therapy that may have direct effects upon osteoblast and osteoclast generation and function.³⁶⁷ Despite clinical well being and normal linear growth, the rate of accrual of bone mass is decreased in these subjects while the rate of bone resorption is increased. Bone mass is reduced in children with a variety of connective tissue diseases (juvenile idiopathic arthritis, systemic lupus erythematosus, juvenile dermatomyositis) caused by the chronic inflammatory state and production of proosteoclastic cytokines and to therapy with glucocorticoids.³²⁴

Idiopathic juvenile osteoporosis (OMIM 259750) is a disease of unknown pathogenesis manifested by bone pain and bone fractures with minimal trauma that occurs in the late prepubertal-early pubertal girl or boy (8–12 years of age) and spontaneously abates over time with variable residual manifestations.^{368,369} In affected subjects, roentgenograms obtained for evaluation of joint, muscle, and/or back pain, difficulty walking, foreshortening of the trunk, and/or the presence of kyphosis reveal biconcave vertebrae and/or vertebral compression fractures, radiolucent areas in long bones, and metaphyseal fractures. Analyses by DXA and QCT demonstrate decreased bone mineralization. Chemical studies are normal. Dynamic histomorphometric studies reveal low rates of bone formation and turnover with reduction in cancellous bone volume and trabecular thickness primarily because of decreased osteoblast activity on the endosteal but not the periosteal bone surface; there is no evidence of increased bone resorption. Idiopathic juvenile osteoporosis is quite likely to be genetically heterogeneous in origin. Mutation analyses of *COL1A1* and *COL1A2* have been normal in these subjects. In 15% of patients with juvenile osteoporosis, a familial heterozygous loss-of-function mutation in the gene encoding LDL receptor-related protein 5 (*LRP5*, OMIM 603506) has been detected.³⁷⁰ Biallelic loss-of-function mutations of *LRP5* result in the osteoporosis-pseudoglioma syndrome, whereas heterozygous gain-of-function variants lead to forms of osteopetrosis and osteosclerosis (vide infra). One of the most difficult diagnostic challenges is the clinical distinction between idiopathic juvenile osteoporosis and osteogenesis imperfecta type I (vide infra). The latter disease is characterized clinically by a positive family history, onset in early infancy, lifelong persistence, diaphyseal fractures, wormian bones, lax ligaments and decreased muscular strength, blue sclerae, abnormal dentition, hearing loss, and a high rate of bone turnover.³⁶⁹ In addition, there are some children with low bone mass and recurrent fractures whose clinical picture is not as severe as those with idiopathic juvenile osteoporosis, suggesting that there is a wide spectrum of clinical findings in children and adolescents with marginal bone mineralization. In children with classical idiopathic juvenile osteoporosis, symptomatic treatment is offered; in some patients, calcitriol or supplemental sodium fluoride has been of benefit. Although the disorder ameliorates and even disappears at puberty, treatment of the prepubertal patient with sex steroids does not seem to accelerate the healing process. Administration of the bisphosphonate pamidronate has been helpful in reducing

bone pain and increasing vertebral BMD in small numbers of children with idiopathic juvenile osteoporosis.^{368,369}

LRP5 (vide supra) is a cell membrane protein expressed by osteoblasts that serves as coreceptor for signal transduction through the WNT-Frizzled- β -Catenin pathway leading to differentiation and function of osteoblasts. WNT signaling enhances differentiation of pluripotential mesenchymal precursor cells into the pathway of chondrogenesis and osteogenesis and impedes its differentiation into adipocytes.³⁷¹ By stimulating *RUNX2*, β -catenin further directs the osteochondroprogenitor cell into the osteoblastic track, and in the mature osteoblast, β -catenin enhances expression of osteoprotegerin and thus depresses osteoclastogenesis.³⁷² Sclerostin (encoded by *SOST*, OMIM 605740) binds to the extracellular domain of LRP5 and thereby inhibits WNT signaling.³⁷³ Intrinsic abnormalities of *LRP5* expression have been associated clinically with disorders of both impaired and excessive bone mineralization. The osteoporosis-pseudoglioma syndrome (OMIM 259770) is characterized clinically by congenital or early infantile onset of severe visual impairment because of microphthalmia and hyperplasia of the vitreous (pseudoglioma that may be erroneously identified as retinoblastoma), leading to retinal detachment, glaucoma, and blindness, marked osseous fragility with craniotables and fractures during late infancy, childhood or adolescence, and variable cognitive impairment, ligamentous laxity, and hypotonia.³⁷⁴ The disorder is transmitted as an autosomal recessive trait and is caused by biallelic (homozygous or compound heterozygous) inactivating (missense [p.Val336Met, p.Arg494Gln], nonsense [p.Arg428Ter, p.Arg1002Ter], frameshift, splice-site) mutations in *LRP5*—primarily located in the extracellular domain of LRP5.^{375,376} Missense mutations likely prevent normal binding of LRP5 to the product of the mesoderm development gene (*MESDC2*, OMIM 607783, chr. 15q25.1), a chaperone protein that directs LRP5 to the cell membrane. Although often asymptomatic, heterozygous carriers may be osteopenic; however, vision is not impaired. Intravenous administration of pamidronate over several years can increase bone mass in children with this disorder.³⁷⁶ Loss-of-function mutations in *LRP5* have also been associated with familial exudative vitreoretinopathy (OMIM 133780), a developmental disorder of retinal vasculature that may be transmitted as either an autosomal dominant (p.Leu145Phe) or autosomal recessive (p.Arg570Gly, p.Arg752Gly) trait; these patients also have reduced bone mass. LRP5 transduces not only the WNT signal but also that of *NDP* (OMIM 300658) encoding Norrin whose signal modulates ocular vitreoretinal formation. Gain-of-function mutations in *LRP5* are associated with increased bone mass (vide infra).

Osteogenesis Imperfecta

Osteogenesis imperfecta or “brittle bone disease” is a disorder of increased bone fragility usually associated with low bone mass that varies in clinical severity from lethality in utero or perinatally to mildly increased susceptibility to fractures in later life and that may be transmitted as either autosomal dominant or autosomal recessive traits.^{190,193,377} The original Silence classification of four types of osteogenesis imperfecta based upon underlying pathogenesis, clinical characteristics, and disease course has been modified by establishment of five clinical forms of this disease (designated clinical types 1–5) (see Table 20.10B) whose clinical and radiological manifestations may be attributed to variants of several of the 19 mutated gene currently associated with osteogenesis imperfecta (designated gene types I–XIX) (see Table 20.10C). Thus osteogenesis imperfecta is a primary form of osteoporosis that is caused by heritable errors of bone formation and mineralization of variable severity ranging from mild to lethal that is caused by genetic

variants critical for osteoblast differentiation (osteoblastogenesis) and function, collagen synthesis and processing, and formation of hydroxyapatite. Types (forms) of osteogenesis imperfecta may be classified according to the phenotype, that is, the extent of bone fragility and resulting clinical severity (see Table 20.10B) or by the site of the genetic error(s) in osteoblastogenesis or collagen formation and its modifications (see Table 20.10C).^{190,193,377–380} (The currently designated 19 genetic types [i.e., variant genes] of osteogenesis imperfecta are numbered in the order of their initial clinical description. In addition, there are several genes whose variants are associated with increased osseous fragility that may later be designated as other genetic forms of osteogenesis imperfecta [see Table 20.10C].) The hallmark of each of the five clinical types of osteogenesis imperfecta is increased bone fragility, but severity varies with clinical type 1 being least severe as those so designated having straight limbs and heights within the low normal range and clinical type 2 being lethal in the perinatal period because of respiratory insufficiency as a consequence of multiple rib fractures that reduce chest volume.³⁷⁷ Osteogenesis imperfecta clinical type 3 is associated with substantial deformities of the limbs, but affected patients live through infancy and childhood albeit with marked growth restriction and limited mobility. The severity of osteogenesis imperfecta clinical type 4 is intermediate between clinical types 1 and 3; adult stature is substantially restricted with heights between –3.6 and –4.6 SDs below the mean for gender. The phenotype of patients with osteogenesis imperfecta clinical type 5 (OMIM 610967) is similar to that of clinical type 4 but unique in that this form is associated with exuberant callus formation at sites of healing fractures and calcifications of interosseous membranes that occur only in patients with mutations in IFN-induced transmembrane protein-5 or bone-restricted IFITM5-like protein (*IFITM5*, OMIM 614757) (vide infra). The following classification of osteogenesis imperfecta is based on the functional site of the genetic error in osteoblastogenesis, osteoblast activity, or collagen synthesis, processing, and/or secretion.

Errors in Osteoblast Differentiation, Maturation, Function. Loss-of-function variants of *WNT1* (Wingless-type mouse mammary tumor virus [MMTV] integration site, member 1, OMIM 164820) and *LRP5* (OMIM 603506) have adverse impacts upon differentiation of osteoblasts and consequently inactivating mutations in these genes impair bone formation leading to osteogenesis imperfecta (*WNT1*—gene type XV, clinical types 3 and 4) and the osteoporosis-pseudoglioma syndrome (OMIM 259770) because of variants of *LRP5*. *WNT1* is a key ligand for the canonical WNT signaling pathway of osteoblast differentiation from mesenchymal stem cells. *WNT1* stimulates osteoblastogenesis by binding to the LRP5-Frizzled receptor complex that then activates an intracellular signal transduction pathway that inhibits proteasomal degradation of cytoplasmic β -catenin (*CTNNB1*, OMIM 116806). Accumulated β -catenin translocates to the nucleus of the mesenchymal stem cell where it inhibits differentiation into chondrocytes or adipocytes and stimulates osteoblastogenesis, resulting in increased bone formation and diminished bone resorption.³⁷² *WNT1* indirectly stimulates osteoclastogenesis as well. Inactivating mutations in *WNT1* may be monoallelic or biallelic; the clinical severity of deleterious *WNT1* mutations may vary and may include extraskelatal malformations, such as hypoplasia of a cerebellar hemisphere.

Osteogenesis imperfecta gene type XII (OMIM 613849) is a moderately severe form of this illness (clinical type 4) characterized by multiple fractures in early infancy with short, bowed long bones and highly arched palate but normal teeth, hearing, and sclerae.³⁸¹ It is transmitted as an autosomal recessive

disorder and is caused by inactivating mutations in transcription factor specificity factor 7 or osterix (*SP7*, OMIM 606633). Osterix is a 431 aa essential WNT1-dependent zinc-finger transcription factor requisite for maturation of preosteoblasts into osteoblasts and for functional expression of *COL1A1*; it stimulates both endochondral and intramembranous bone development; its expression is regulated by *RUNX2* (OMIM 600211).³⁸² Although its relevant function in the osteoblast is unknown, biallelic inactivating mutations in *TENTA5* (OMIM 611357) encoding a nucleotidyltransferase termed *FAM46A* that catalyzes transfer of nucleoside triphosphate to a hydroxyl group of an acceptor molecule is associated with osteogenesis imperfecta gene type XVIII.³⁸³ After nuclear transcription and initiation of translation of *COL1A1* and *COL1A2*, their nascent peptide products leave the nucleus and enter the endoplasmic reticulum where they undergo further modifications requiring chaperones for movement through this system. Thus biallelic loss-of-function mutations in serpin peptidase inhibitor, clade F, member 1 (*SERPINF1*, OMIM 172860) encoding the collagen chaperone heat shock protein 47 result in severe osteogenesis imperfecta (gene type VI, clinical type 3).³⁸⁴ Variants of *IFITM5* encoding IFN-induced transmembrane protein-5 (OMIM 614757), a protein synthesized by osteoblasts whose primary function may be to stabilize *SERPINF1*, are associated with a distinct form of osteogenesis imperfecta characterized by ossification of interosseous membranes of the forearm, hyperplastic callus formation at fracture sites, dense metaphyseal bands, and a “mesh-like” bone lamellation pattern (gene type V, clinical type 5).³⁷⁹ Deletion of or mutations in *PLS3* (OMIM 300131) sited on chromosome Xq23, encoding plastin 3—an actin and calcium binding protein essential for the integrity and remodeling of the actin filaments that form the cytoskeleton of the osteocyte, result in an X-linked form of osteogenesis imperfecta of modest severity (clinical type 1).³⁸⁵ Males with monoallelic deleterious variants of *PLS3* have decreased bone mineralization and experience vertebral compression and long bone fractures in early childhood, but do not exhibit blue sclerae, dentinogenesis imperfecta, or joint hyperflexibility. Low BMD may be present in heterozygous female carriers of *PL3* variants in whom clinical manifestations may vary from childhood-onset bone fragility fractures as in males to postmenopausal osteoporosis. Plastin 3 may also regulate osteoclastogenesis and the process of mechanosensing.

Errors in Synthesis and Processing of Collagen Type 1.

Collagen type 1 is a trimer composed of two cross-linked molecules of collagen type 1A1 (*COL1A1*, Collagen type I, alpha-1, OMIM 120150) and one molecule of collagen type 1A2 (*COL1A2*, Collagen type I, alpha-2, OMIM 120160) that intertwine to form collagen type I in bone, skin, ligaments, tendons, sclerae, and teeth.¹⁹¹ The fibers of both collagen type 1A1 and 1A2 are composed of triple repeats of glycine and two additional amino acids—often proline, hydroxyproline, or lysine. Mutations in *COL1A1* (gene type I) or *COL1A2* (gene type II) that lead to a stop codon result in a truncated procollagen product that is rapidly degraded; thus only normal collagen type I is produced but in reduced amounts. Patients with mutations in *COL1A1* or *COL1A2* that change (e.g., substitution of cysteine for glycine at critical positions) its three-dimensional configuration result in the formation of abnormal collagen 1 α 1 fibers and thus more severe bone disease. Usually, variants of *COL1A1* compromise collagen synthesis more severely than do variants of *COL1A2*. Thus osteogenesis imperfecta gene type I (OMIM 166200) is an autosomal dominant disorder (new mutation in 33% of patients) resulting primarily from “functional null alleles”—the result of splicing defects or point mutations leading to insertion errors or truncation of collagen type

1A1 (*COL1A1* - p.Gly178Cys, p.Arg963Ter), mutations that result in decreased transport of procollagen- α 1(I) into the cytoplasm or its release into matrix—thereby modestly decreasing production of intact procollagen type I. Its clinical manifestations are relatively benign: intensely blue sclerae are present at birth and persist throughout adulthood, modestly low bone mass, infrequent fractures with little deformity, low normal adult stature, hearing loss in 50%, mitral valve prolapse in 18%, and rarely dentinogenesis imperfecta (osteogenesis imperfecta gene type IB). (Paradoxically, patients with mild osteogenesis imperfecta gene type I caused by mutations at selected procollagen type I carboxyl terminal propeptide cleavage sites [*COL1A1*—p.Asp1219Asn; *COL1A2*—p.Ala1119Thr] may have normal or increased lumbar spine BMD by both DXA and QCT examinations despite increased bone fragility).³⁸⁶ Lethal mutations in *COL1A1* are those that alter an amino acid with a branched or charged side chain and those within the binding sites of the collagen monomer for integrins, matrix metalloproteins, fibronectin, and cartilage oligomeric matrix protein and those that result in binding to and degradation of intact procollagen subunits.¹⁹¹ Lethal mutations in *COL1A2* are those that interfere with its binding to proteoglycans. Mutations (p.Arg134Cys) in *COL1A1* may also be found in patients with classical Ehlers-Danlos syndrome (OMIM 130000) of hyperextensible skin and laxity of ligaments of the spine and large and small joints. Children with clinical features of both osteogenesis imperfecta (osseous fragility) and Ehlers-Danlos syndrome have been described.³⁸⁷ In these patients, the mutations have been concentrated within the first 90 aa of the helical region of collagen 1 α 1 and prevent normal posttranslational removal of the procollagen aminopropeptide; although the mutant protein can be incorporated into collagen, the structural integrity of the product is impaired as its fibrils are thin and weak.³⁸⁸ Osteogenesis imperfecta gene types I-IV describe forms of variable severity of this disease associated with loss-of-function mutations in *COL1A1* or *COL1A2* (see Table 20.10B).

Mutations of *COL1A1* and *COL1A2* are usually heterozygous (monoallelic) and transmitted as autosomal dominant diseases; the mutant collagen type 1 subunit may exert a dominant-negative effect as it interacts with the intact subunit (gene types I-IV; clinical types 1-4). After synthesis, COL1 α 1 and Col1 α 2 undergo posttranslational modifications, including hydroxylation of selected lysine and proline residues, cross-linking of collagen strands, release from the osteoblast into surrounding matrix, removal of amino and carboxyl-terminal propeptides, and assembly into collagen fibers. Among the most important posttranslational modifications of type I procollagen is hydroxylation of proline and lysine residues by prolyl and lysyl hydroxylases. Hydroxylation of proline residues at carbon 4 affords thermal stability, whereas hydroxylation of lysyl residues permits binding of carbohydrates—galactose or glucosyl-galactose—and the formation of crosslinks within or between procollagen chains.¹⁹³ Hydroxylations of proline at carbon 3 in position 986 of *COL1A1* and at position 707 of *COL1A2* are especially critical for stability of the three-dimensional configuration of type I procollagen and its subsequent maturation to collagen type I.¹⁹³ After synthesis, COL1 α 1 and COL1 α 2 are further modified by hydroxylation on selected proline and lysine residues. Failure to form 3-hydroxyproline at aa 986 of collagen 1 α 1 and aa 707 of collagen 1 α 2 may be caused by loss-of-function variants of one of the three genes involved in this process—prolyl 3-hydroxylase 1 (*P3H1*, OMIM 610339)—also termed *leprecan*, cartilage associated protein (*CRTAP*, OMIM 605497), and cyclophilin B (*CypB*, Peptidyl-prolyl isomerase B, *PPIB*, OMIM 123841). In the absence of any one of these factors, the helical domain of collagen 1 α 1 is modified by other enzymes leading to

alterations in its strength.³⁷⁹ Biallelic loss-of-function variants in any one of these three genes leads to severe to lethal forms of osteogenesis imperfecta (clinical types 2, 3, 4; osteogenesis imperfecta gene types VII, VIII, IX; see Tables 20.10B, 20.10C). Osteogenesis imperfecta gene type VIII (OMIM 610915) caused by mutant *P3H1* occurs primarily in African-American and Middle Eastern subjects.³⁸⁹⁻³⁹¹ Prolyl 3-hydroxylase 1 specifically hydroxylates carbon 3 of the proline residue at codon 986 in collagen 1A1 and at codon 707 in collagen 1A2. The phenotype of osteogenesis imperfecta gene type VIII overlaps with those of clinical types 2 and 3; in addition to osseous fragility, it is associated with substantial growth retraction, white sclerae, and bulbous metaphyses. *CRTAP* and *CypB* stabilize the collagen prolyl 3-hydroxylation complex and also chaperone the movement of collagen from the endoplasmic reticulum.^{193,392} Loss-of-function mutations in *PPIB* have been designated osteogenesis imperfecta type gene type IX, an often lethal disorder in which osseous fragility is evident in utero (see Table 20.10B).³⁹³⁻³⁹⁵ *CRTAP* is expressed in the proliferative zone of developing cartilage and at the chondroosseous junction. *Crtap* “knock-out” mice develop an osteochondrodysplasia (kyphoscoliosis, rhizomelic shortening of the proximal segment of the limbs) and severe osteopenia, the latter caused by reduced production and alteration in the quality of osteoid and consequent decreased rate of mineral deposition.³⁹⁶ Mice deficient in *Crtap* are unable to 3-hydroxylate the proline residue near the carboxyl terminus of bone collagen 1A1, leading to increased hydroxylation of lysine residues and resultant abnormal structure of the collagen fibril—changes that result in defective mineralization of bone collagen type I. Nonhydroxylated proline at codon 986 in bone collagen 1A1 was demonstrated in 3/11 patients with a recessively transmitted lethal or severe osteogenesis imperfecta characterized by multiple fractures of the long bones resulting in rhizomelic shortening of the limbs with externally rotated and abducted legs, poorly mineralized calvaria and ribs, proptotic eyes, and white or light blue sclerae.³⁹⁷ This proved caused by homozygous or compound heterozygous loss-of-function mutations in *CRTAP* (frameshift [c.879delT], 16-bp duplication in exon 1, nonsense [p.Gly276Ter], missense [p.Met1Ile], splice donor site of exon 1 at the first intronic nucleotide [IVS1+1G \Rightarrow C]) that interfered with effective hydroxylation of the proline residue at codon 986 of bone procollagen 1A1. This disorder has been designated osteogenesis imperfecta gene type VII (OMIM 610682). In other patients with osteogenesis imperfecta gene type VII, a homozygous mutation in *CRTAP* has been identified in which alteration of one nucleotide (c.472-1021C \Rightarrow G) generates a cryptic splice donor site that leads to inclusion of 73 bp of intron 1 into the genome of *CRTAP* thus extending exon 2.³⁹⁶ This mutation results in more rapid degradation of *CRTAP* and consequently leads to decreased 3-hydroxylation of proline 986 in bone collagen 1A1. Osteogenesis imperfecta gene type VII has been identified in a Native American population in northern Quebec. Clinically, it is an autosomal recessive disorder in which fractures are present at birth; often the frequency of fractures declines with advancing age—particularly after adolescence; the sclerae are slightly bluish; there is progressive skeletal deformation leading to rhizomelic shortening of the limbs and restricted ambulation.³⁹⁸

Loss-of-function variants in *PLOD1* (OMIM 153454) impair hydroxylation of lysine in the helical regions of collagen type I and have been associated with Ehlers-Danlos syndrome, kyphoscoliosis type 1 (OMIM 225400). Inactivation of *PLOD2* (OMIM 601865) results in the association of osteogenesis imperfecta, congenital joint contractures and pterygia (Bruck syndrome 2, OMIM 609222). (Bruck syndrome 1 [OMIM 259450] has been attributed to inactive variants of *FKBP10* [OMIM 607063], a chaperone protein required for movement

of collagen type I propeptides through the osteoblast.) Deleterious biallelic mutations (p.Phe249Leu; c.34G>C) in *BMP1* (OMIM 112264) prevent removal of the carboxyl terminal propeptides of collagen 1 α 1 and collagen 1 α 2 and hence formation of the collagen type I fibril thus resulting in a moderately severe form of osteogenesis imperfecta (gene type XIII; clinical type 3). Gene type XIII osteogenesis imperfecta is associated with multiple fractures annually, either borderline low or elevated bone mass by DXA, substantial growth retardation, faintly blue sclerae, and normal teeth.^{399,400} *BMP1* is a multifunctional protein; one of its most important activities is as procollagen type I C-terminal propeptide endoproteinase. Inactivation of *BMP1* impairs proteolytic removal of the carboxyl terminal propeptide from procollagen type I and the normal assembly of mature collagen type I fibrils. Interestingly, this form of osteogenesis imperfecta may be associated with either borderline low or even increased lumbar spine areal BMD by DXA despite which treatment with bisphosphonates has been clinically and radiographically useful.³⁹⁹ In this regard, it should be noted that patients with mild osteogenesis imperfecta gene type I/clinical type 1 caused by mutations in the procollagen type I carboxyl terminal propeptide cleavage sites, also have normal or increased lumbar spine BMD by both DXA and pQCT examinations.³⁸⁶ Heterozygous mutations in *IFITM5* (OMIM 614757) result in osteogenesis imperfecta of moderate severity (gene type V; clinical type 5) characterized by formation of painful hyperplastic callus at fracture sites, calcification of the membrane between adjacent radius and ulna bones, and a dense metaphyseal band below the growth plates of the long bones.

Osteogenesis imperfecta gene type II/clinical type 2 is a disorder that is lethal in the perinatal period or in early infancy. It is usually the result of de novo heterozygous mutations in *COL1A1* or *COL1A2* with substitution of an alternative amino acid for glycine in the triple helical domains of the procollagen α 1(I)/ α 2(I) chains (*COL1A1*—p.Gly94Cys, p.Gly391Arg, p.Gly1003Ser; *COL1A2*—p.Gly547Asp, p.Gly865Ser, p.Gly976Asp); these mutations lead to the synthesis of abnormal procollagen chains that bind to and thereby inactivate intact procollagen peptides in a dominant-negative manner severely curtailing the synthesis of intact collagen type I. Osteogenesis imperfecta clinical type 2 is manifested by in utero fractures, long bone deformities, very little calvarial mineralization, and death because of respiratory insufficiency. Lethal phenotypes of osteogenesis imperfecta are also associated with homozygous loss-of-function mutations in *LEPRE*, *CRTAP*, *PPIB*, and *CREB3L1*, the latter encoding cAMP response element-binding protein 3-like 1 (OMIM 616215) (see Table 20.10C).¹⁹⁴ Osteogenesis imperfecta gene type III (OMIM 259420) is an autosomal dominant trait resulting from point or frameshift mutations in *COL1A1* (p.Gly154Arg, p.Gly844Ser) or *COL1A2* (p.Gly526Cys). Osteogenesis imperfecta clinical type 3 is characterized by recurrent fractures leading to progressive bone deformities that are often apparent at birth, kyphoscoliosis, extreme short stature, blue sclerae that lighten with age, abnormal dentition (in 80% of children <10 years of age), and hearing loss; it may be caused by variants of many genes (see Table 20.10B). Osteogenesis imperfecta clinical type 4 (gene type IV, OMIM 166220) is an autosomal dominant disease most often associated with point mutations or small deletions in *COL1A2* (Gly586Val, Gly646Cys, Gly1012Arg) and occasionally in *COL1A1* (p.Gly175Cys, p.Gly832Ser). It is of variable severity with prolonged survival, mild to moderate bone deformities, short stature, normal sclerae, dentinogenesis imperfecta, and hearing loss. Osteogenesis imperfecta clinical type 4 may also be caused by variations in *CRTAP*, *PPIB*, *WNT1*, *TMEM38B*, or *SP7*. Osteogenesis imperfecta clinical type 5 (gene type V, OMIM 610967) is manifested not only by osseous fragility but also by dislocation of the radial head, exuberant callus formation at

sites of healing fractures and calcification of interosseous membranes between adjacent radius/ulna and tibia/fibula and is presently associated only with variants of *IFITM5* encoding IFN-induced transmembrane protein-5 (OMIM 614757, chr. 11p15.5).^{401–403} Other clinical characteristics of gene type V osteogenesis imperfecta are autosomal dominant transmission, moderate to severe bone fragility (lumbar spine areal BMD Z scores range between -7.7 and -0.7), severe to mild growth retardation (adult height Z scores vary from -8.7 to -0.1), dislocation of the radial head, white sclerae, and normal dental development. Histologically, there is irregular arrangement of lamellae. The most frequent mutation in *IFITM5* by whole exome sequencing is a heterozygous c. $-14C>T$ transition within its 5' untranslated region.^{401–404} This mutation is 14 bp upstream of the reference initiation codon and creates a new initiation signal that adds five aa (Met-Ala-Leu-Glu-Pro) to the amino terminus of IFITM5 increasing its length from 132 to 137 aa. The *IFITM5* variant p. Ser40Leu has also been identified in patients with osteogenesis imperfecta gene type V, albeit without its classical features of interosseous calcification and excessive callus formation.⁴⁰⁵ IFITM5 is a protein that is abundantly expressed within osteoblast membranes during embryonic and postnatal development of the skeleton; it has amino and carboxyl extracellular termini, two transmembrane domains, and an intracellular helical domain. IFITM5 encodes an osteoblast differentiation factor that is involved in protein trafficking and folding; experimentally, loss of *Ifitm5* results in decreased bone formation, particularly in utero and in decreased expression of *SERPINF1* (OMIM 172860).^{405,406} Decreased expression of *SERPINF1* results in autosomal recessive osteogenesis imperfecta gene type VI (OMIM 610968) (clinical type 3) and diminished synthesis of its product pigment epithelium-derived factor (PEDF).⁴⁰⁵ PEDF is a chaperone protein, whose concentration may be measured in serum, that regulates osteoblast differentiation and bone mineralization; it is synthesized by chondrocytes, osteoblasts, and osteoclasts; PEDF interferes with function of vascular endothelial growth factor (VEGF), a protein that enhances migration of osteoblasts and osteoclasts into cartilage; PEDF may regulate bone mineralization by increasing synthesis of osteoprotegerin, thereby impeding osteoclastogenesis. Osteogenesis imperfecta gene type VI (OMIM 610968) is phenotypically similar to gene type IV and is characterized clinically by severe bone fragility that first manifests after 6 months of age and microscopically by excess unmineralized osteoid, and a "fish-scale" pattern of lamellation of bone matrix consistent with a defect in bone mineralization.^{193,407–409} Loss-of-function mutations in *SERPINF1* may interfere with bone formation while increasing osteoclast generation and bone dissolution. Serum concentrations of PEDF are undetectable in patients with osteogenesis imperfecta gene type VI providing a diagnostic tool for this illness.⁴¹⁰ Interestingly, patients with osteogenesis imperfecta gene type VI do not respond to treatment with bisphosphonates as well as do patients with other forms of this heterogeneous disease.

Osteogenesis imperfecta gene type XI (clinical type 3) is the result of inactivating mutations in *FKBP10* (FK506-binding protein 10, OMIM 607063) encoding an endoplasmic reticular protein (FKBP65) that is also a chaperone for procollagen type I—one that is essential for hydroxylation of lysine residues in its telopeptide, a modification necessary for cross-linking, as well as its transcellular movement and secretion.⁴¹¹ In the absence of functional FKBP65, procollagen type I accumulates in the endoplasmic reticulum of the osteoblast. This disorder is transmitted as an autosomal recessive trait that begins in infancy; affected patients have repeated long bone fractures and progress to wheel chair dependency in early childhood; they may or may not develop joint contractures but do not

display dentinogenesis imperfecta.^{412–414} Mutations in *FKBP10* have also been recorded in patients with Bruck syndrome 1 (OMIM 259450—pterygia, congenital contractures, fractures in infancy resulting in limb deformities, growth retardation, scoliosis) suggesting that osteogenesis imperfecta gene type XI and Bruck syndrome 1 are likely allelic disorders whose clinical manifestations depend on the mutation site and other modifying factors.⁴¹²

Collagen Secretion and Stress of the Endoplasmic Reticulum. Osteogenesis imperfecta gene type X is an autosomal recessive disorder caused by biallelic inactivating mutations in *SERPINH1* (OMIM 600943) encoding a multifunctional 418 aa peptide designated collagen type 1 binding protein (CBP) 2 that is also termed heat shock protein (HSP) 47. Gene type X osteogenesis imperfecta (clinical type 3) is a severe disorder with fractures occurring throughout the skeleton that begins in utero.^{193,414a} CBP2/HSP47 is essential for cartilage organization, the formation of endochondral bone, and the fabrication and maintenance of the integrity of the triple helical structure of procollagen type I, its transcellular movement and secretion, and its resistance to proteolytic degradation.^{414a,415,416} Biallelic variants of *CREB3L1* (OMIM 616215), a component of the endoplasmic reticular stress response in osteoblasts, have been identified in fetal siblings with severe osteogenesis imperfecta (gene type XVI, clinical type 2).⁴¹⁷ Mutations in membrane-bound transcription factor protease site 2 (*MBTPS2*, OMIM 300294) (encoding a zinc metalloproteinase that regulates intramembrane proteolysis of several transcription factors that regulate secretion of type 1 collagen and is also involved in the stress response of the endoplasmic reticulum) are transmitted as an X-linked trait and result in osteogenesis imperfecta of moderate severity (gene type XIX, clinical type 3).⁴¹⁸ Biallelic variants of transmembrane protein 38B (*TMEM38B*, OMIM 611236) encoding an intracellular potassium channel that alters intracellular Ca^{2+} levels that adversely affect the release of procollagen type I from osteoblasts (and thus increases accumulation of procollagen type I in the osteoblast's endoplasmic reticulum, thereby impairing its further function and leading to endoplasmic reticular stress) has been designated osteogenesis imperfecta gene type XIV.⁴¹⁹ *TMEM38B* encodes the 291 aa trimeric intracellular cation channel type B (TRICB), one component of TRIC, a monovalent cation channel that is critical for the release of Ca^{2+} from intracellular storage sites, such as the sarcolemma and endoplasmic reticulum and thus maintenance of cytosolic Ca^{2+} concentrations, a process necessary for normal cell differentiation, division, and function (including that of the osteoblast). Gene type XIV osteogenesis imperfecta is an autosomal recessive disorder of variable severity (clinical types 3, 4) with fractures occurring in utero or during early childhood but with normal dentition, hearing, and sclerae that has been identified in Middle Eastern families.^{420,421}

Although undesigned as causes of osteogenesis imperfecta, a number of gene variants result in osseous fragility other than those so defined (see Table 20.10C), including those that result in Cole-Carpenter syndromes 1 (OMIM 112240) and 2 (OMIM 616294) characterized clinically by pre- and postnatal fractures of long bones, frontal and coronal craniosynostosis, proptosis, hydrocephalus, and macrocranium. Cole-Carpenter syndrome 1 is the result of inactivating variants of procollagen-proline, 2-oxoglutarate-4-dioxygenase, beta subunit (*P4HB*, OMIM 176790) resulting in decreased hydroxylation of proline residues in preprocollagen type 1. Cole-Carpenter syndrome 2 is the consequence of inactivating variants of *SEC24D* (SEC24-related gene family, member D, OMIM 607186) encoding a component of osteoblastic vesicles that transports/exports protein from the endoplasmic

reticulum within the osteoblast facilitating movement of procollagen type 1 molecules.

Evaluation of Children and Adolescents With Low Bone Mass

Identification of the pathogenesis of decreased bone mineralization in childhood is initiated with careful review of the patient's general medical history, as well as that related to the skeletal system beginning prenatally together with those of immediate family members through several generations, followed by documentation of physical findings (stature, skeletal deformities, scleral color, and dental status), biochemical status (measurement of serum calcium, magnesium, phosphate, creatinine, alkaline phosphatase, calcidiol, calcitriol, and other clinically relevant studies), pertinent roentgenograms, assessment of skeletal mineralization, and ultimately examination of the integrity of genes related to formation and maintenance of the skeleton. Various forms of rickets should be excluded as should disorders known to be associated with suboptimal bone mineralization, such as anorexia nervosa, asthma treated with glucocorticoids, rheumatic joint and bone diseases, chronic inflammatory disorders (e.g., inflammatory bowel disease), and neoplastic disorders and their treatment. Decreased bone mineralization may also occur in patients with disorders of mobility (e.g., cerebral palsy, muscular dystrophy, spinal muscular atrophy) and those with endocrinopathies (GH deficiency, excessive exposure to or overproduction of glucocorticoids, sex hormone deficiencies, type 1 diabetes mellitus), hematological diseases (thalassemia, sickle cell anemia, leukemia), systemic abnormalities (cystic fibrosis), and chronic renal failure before and/or after kidney transplantation.^{321,322,422}

Osteoporosis caused by osteogenesis imperfecta is most often made clinically apparent by the occurrence of nontraumatic fractures occurring in utero, postnatally, during childhood, or in adolescence and confirmed by radiological evaluation of the skeleton and determinations of BMC and concentration.^{323,324,422,423}

Thus evaluation of the child/adolescent with a history of low-impact fractures (defined as two or more long bone fractures sustained by a fall from a distance that is less than the standing height of the patient before chronological age 10 years, or three or more before age 19 years, or a vertebral fracture without local disease or substantial trauma) begins with historical review and physical examination directed to the identification of factors that might adversely affect bone formation, mineralization, and strength.^{314,317} Fractures of the tibia and femoral diaphyses occur often in children with osteogenesis imperfecta.³⁷⁷ In addition to genetic and hormonal influences, the most important elements necessary for the accrual and maintenance of normal bone mass that need to be evaluated are those that relate to diet (sufficient intake of calcium and protein; absence of anorexia nervosa), sustained normal vitamin D stores either by exposure to sunlight or ingestion of supplements, normal mobility with consistent weight-bearing exercise, and the presence of chronic diseases, particularly those that may be treated with glucocorticoids (e.g., asthma, chronic inflammatory states) that might impair bone development. Examination of the patient's growth pattern to determine whether statural growth has been normal and normal weight for stature and gender has been attained and maintained and to assess by physical examination stages of primary and secondary sexual maturation are essential. Determination of skeletal maturation (bone age) is useful to determine if the child is growing in accord with her/his genetic potential. If pertinent, systemic illnesses, such as chronic renal disease, celiac disease, inflammatory bowel disease, and endocrinopathies should be eliminated. Assessment of areal bone mineralization and bone

mineral apparent density is most often accomplished by DXA in children with referral to specific reference data for chronological age, gender, and ethnicity/ancestry, while also taking into consideration such factors as height, pubertal stage, and skeletal maturation.^{317,320,321,424} In a series of 304 children and adolescents undergoing DXA bone mineralization studies, 36% were doing so because of a history of fractures, 27% because of hypogonadism, and 22% because of gastrointestinal (celiac, inflammatory bowel) disease.⁴²⁵ Low body mass index and low vitamin D stores were the most significant predictors of subnormal BMD (<-2 SDs for age and gender). Depending on the individual patient, measurements of serum calcium, phosphate, alkaline phosphatase, creatinine, PTH, calcidiol (25OHD), and reproductive hormones, as well as disease-specific analytes (e.g., for inflammatory bowel or celiac diseases) may be indicated. Bone turnover markers are of marginal diagnostic utility in children, although useful as indices of therapeutic response.^{426,427}

Clinical manifestations of osteogenesis imperfecta vary from mild to moderate, severe, or lethal.^{193,377} Radiographic findings in subjects with osteogenesis imperfecta include, in addition to diffusely low bone mass, thin cortices, metaphyseal flaring, fractures and bone deformities resulting therefrom, wormian skull bones (frequent but not pathognomonic of osteogenesis imperfecta), platybasia that may compress overlying hindbrain, vertebral compression, and a triradiate pelvis.¹⁹¹ In children with osteogenesis imperfecta, bone densitometry, usually but not invariably, reveals decreased mineralization, the extent of which correlates to a degree with clinical manifestations. The diagnosis of osteogenesis imperfecta is established by clinical criteria and confirmed by genotyping of *COL1A1* and/or *COL1A2* or other pertinent gene(s), although failure to detect a genetic mutation does not necessarily rule out this disorder but suggests that more extensive genetic inquiry is warranted (whole exome or whole genome sequencing). Undetectable serum levels of PEDF are indicative of osteogenesis imperfecta caused by inactivation of *SERPINF1*.⁴¹⁰ Occasionally, biopsy of the iliac crest and histological examination of the bone may be necessary for subclassification of the disorder. Osteogenesis imperfecta clinical type 2 can be identified prenatally by fetal ultrasonography; other types might be determined prenatally by analysis of collagen synthesized by cells cultured from chorionic villus biopsies and by genetic analyses. Included in the differential diagnosis of recurrent fractures in children are child abuse, various forms of rickets including hypophosphatasia, the McCune-Albright syndrome of fibrous dysplasia (OMIM 174800), juvenile Paget disease, and juvenile osteoporosis.

Other clinical findings of note in patients with osteogenesis imperfecta include: progressive hearing impairment—present in 40% to 60% of subjects, joint hypermobility in perhaps 60% to 70% of patients that may lead to joint dislocation and/or tendon rupture, and craniocervical junction insults. Cranial-cervical deformities occur in approximately 30% of patients with osteogenesis imperfecta and may be categorized as: basilar invagination, basilar impression, and platybasia—the most common of these complications. The presence of dentinogenesis imperfecta, substantial short stature, and very low lumbar axial BMD are strongly associated with skull-based anomalies. Symptoms/signs of cranial involvement are headaches on movement, coughing or sneezing; trigeminal neuralgia; weakness of arms/legs; and difficulties with balance. Screening of subjects with osteogenesis imperfecta for these complications is essential. It is emphasized that in the absence of a vertebral fracture, the diagnosis of osteoporosis is not established by DXA data alone but requires the corresponding and relevant clinical history of associated disease and documented increased fracture risk. Serial areal BMD measurements at 6- to 12-month intervals

are useful in determining the trend in bone mineralization in children with diseases that place them at risk for development of osteoporosis.⁴²²

Management of Children and Adolescents With Low Bone Mass/Osteogenesis Imperfecta. Keys to the prevention of children and adolescents from developing low bone mass is the provision of a diet adequate in calcium and vitamin D, encouragement of weight-bearing activities and exercise, and the avoidance of exposure to agents that may impede normal accrual of bone mineral. After assessment and when necessary and appropriate, interventions may include treatment of an accompanying underlying systemic disease or endocrinopathy that may be of pathogenetic significance in the development of low bone mass, such as elimination, such as elimination or reduction in glucocorticoid dose in an asthmatic child. Therapeutic agents used in the treatment of patients with low bone mass increase skeletal mass either by inhibiting bone resorption (antiresorptive or antiremodeling drugs) or by stimulating bone formation (anabolic agents).⁴²⁸⁻⁴³⁰ In adults, the most widely used antiresorptive medications are sex hormones, selective estrogen receptor modulators, denosumab (a monoclonal antibody that binds to and inhibits RANKL-mediated osteoclastogenesis—thus mimicking the effect of osteoprotegerin), and bisphosphonates. Selective estrogen receptor modulators (SERMs), such as triphenylethylene, benzothioephene, or naphthalene-related compounds (e.g., raloxifene) bind with high affinity to estrogen receptor α in specific tissues where they alter the three-dimensional configuration of the receptor and recruit tissue-selective cohorts of various cofactors, thus either reducing (breast, brain) or inducing (bone) receptor function in targeted sites.⁴³¹ SERMs decrease osteoclast formation primarily at trabecular bone sites, but their efficacy in increasing BMD is less than that of estrogens themselves.⁴³² Although nasal salmon calcitonin inhibits osteoclast function directly and has modest bone restorative effects, it is seldom used for the treatment of osteoporosis in adults or children.⁴³³ Bone anabolic medications include teriparatide (PTH¹⁻³⁴), abaloparatide (PTH¹⁻³⁴), and romosozumab (a humanized monoclonal antibody against sclerostin, an inhibitor of LRP5/6-WNT- β -catenin-mediated osteoblast differentiation). Although approved for treatment of osteoporosis in adults, denosumab and romosozumab await further development and evaluation in children with osteoporosis of varied pathogenesis.⁴³⁴ Despite having been proven useful in the treatment of adults with osteoporosis, both teriparatide (PTH¹⁻³⁴) and abaloparatide (PTH¹⁻³⁴) have the potential risk of inducing osteosarcoma and are not used in children.

Infants/children/adolescents with osteogenesis imperfecta require care by a team of experienced endocrinologists, orthopedic surgeons, and physiatrists and their corresponding healthcare associates. Rehabilitative services and physical therapy to improve muscle strength and mobility with the restraints of bone fragility are encouraged as is protected exercise—such as walking and swimming. Patients with primary osteogenesis imperfecta (and secondary forms of osteoporosis) with documented vertebral and/or long bone fractures have been treated most often with bisphosphonates. Bisphosphonates are compounds related to pyrophosphates in which the linking oxygen molecular bridge between two phosphate moieties is replaced by a carbon link to which two side chains are attached; one side chain is often a hydroxyl group or chloride atom that together with phosphate residues binds tightly to hydroxyapatite and coats bone surface; the second side chain may be “simple” and contain chloride or sulfur atoms or more complex and heavier with nitrogen atoms and ring structures composed of carbon and oxygen (Fig. 20.14).⁴³⁵ When one side chain attached to the central carbon atom is a hydroxyl group

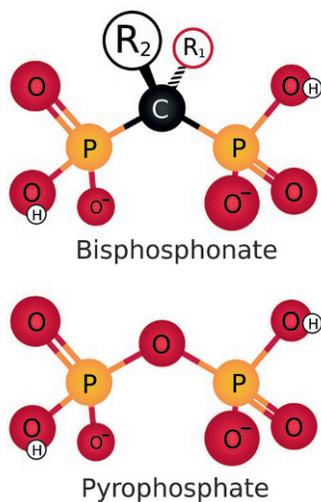


Fig. 20.14 Bisphosphonates are analogues of pyrophosphate in which carbon has been substituted for the oxygen bridge between two phosphate groups; two side chains (R1, R2) are attached to the carbon atom: R1 may be a hydroxyl group or chloride atom that together with phosphate residues binds tightly to hydroxyapatite and coats bone surface; R2 may contain chloride or sulfur atoms or it may be composed of ring structures containing carbon, oxygen, and nitrogen. (Courtesy Wikimedia Commons User Adenosine.)

(OH⁻), binding of the bisphosphonate to hydroxyapatite is increased and the solubility of the mineral phase of bone is reduced, thus decreasing resorption of hydroxyapatite. The second side chain determines the potency and longevity of the bisphosphonate. Bisphosphonates that contain nitrogen as a constituent in one of the side chains are substantially more potent than are the “simple” bisphosphonates.⁴³⁶ In zoledronic acid, a bisphosphonate commonly used in childhood because of its safety profile and prolonged duration of action, the second side chain is CH₂-imidazole.

Bisphosphonates chelate (bind) the calcium ions of hydroxyapatite and thus are targeted to bone; within the resorption lacuna beneath an osteoclast, bisphosphonates dissociate from hydroxyapatite as the pH is lowered by osteoclast secretion of H⁺ and are then endocytosed into the interior of the osteoclast. After bisphosphonates are ingested by osteoclasts, they inhibit the cell’s energy producing capacity, leading to their death and hence decrease in bone resorption and remodeling without impairment of osteoblast-mediated synthesis of new bone with consequent increase in bone mass and strength.⁴²³ Within the osteoclast, bisphosphonates impede osteoclast function by hastening their death by two mechanisms: (1) “simple” bisphosphonates, such as medronate, etidronate, and clodronate are metabolized into analogues of ATP that interfere with release of phosphate and hence with energy generation; additionally, simple bisphosphonates prevent movement of ATP into the osteoclast’s mitochondria as a consequence of which mitochondria disintegrate and osteoclast apoptosis is initiated—thereby further interfering with bone resorption. (2) Large, nitrogen containing bisphosphonates (pamidronate, zoledronic acid) act by impairing cholesterol synthesis by inhibiting farnesyl pyrophosphate synthase activity in osteoclasts. This enzyme is essential for synthesis of cholesterol through the mevalonate pathway; its inhibition prevents prenylation of proteins, a posttranslational modification that enables prenylated proteins to interact with proteins and to bind to cell membranes.³⁹³ Inhibition of this pathway in osteoclasts impairs formation of the ruffled border and actin rings, movement

of osteoclast products into the resorption lacuna, and resorption of degraded bone products—all metabolic functions of osteoclasts essential for bone resorption. The biological activity of a bisphosphonate on osteoclast function is observed immediately after its administration as serum calcium concentrations decline rapidly; indeed, this rapid effect has been used in the treatment of hypercalcemic patients. The effects of bisphosphonates on bone last long after the agent has been discontinued (the “residence time”) enabling some compounds to be given as infrequently as once yearly or half-yearly (e.g., zoledronic acid).³⁸⁰ Because the bisphosphonates remain in bone for extremely long intervals, their long-term effects are cumulative. Histomorphometric analysis has revealed that bisphosphonate-mediated inhibition of osteoclast stimulated bone resorption increase bone mineralization by decreasing the number of resorption cavities and thus the remodeling space, preserving cancellous (trabecular) bone architecture, and decreasing porosity of cortical bone.

Bisphosphonates have been useful in improving mineralization in children with osteogenesis imperfecta and related syndromes, as well as in those with glucocorticoid-induced osteoporosis and those in whom mobility is limited, such as muscular dystrophies and cerebral palsy.⁴³⁵ In many infants and children, intravenous pamidronate (0.5–1 mg/kg/dose on three consecutive days up to 2–15 mg/kg/y administered once every 3 to 6 months for an annual dose of 9 mg/kg/y) has been used. Intravenous infusion of zoledronic acid (first dose 0.025 mg/kg over 30 minutes; 0.05 mg/kg dose at +6, 12, 18, 24 months; continuing dose if required 0.0125 mg/kg every 6 months) has proven effective in increasing BMD, reducing fracture incidence, and improving mobility and wellbeing in patients with osteogenesis imperfecta.^{314,423,429,435,437} Histomorphometric analysis of an iliac bone biopsy after administration of bisphosphonate to children with osteogenesis imperfecta demonstrates increase in thickness of cortical bone and in trabecular number but not trabecular thickness. Discontinuation of bisphosphonate administration may lead to increase in fracture risk between weaker new bone and stronger treated bone. Therefore if there has been no adverse response and positive clinical benefit (absence of new fractures, decreased bone pain, enhanced mobility) and increase in BMD/BMC by serial DXA measurements, treatment with bisphosphonate at half-dose may be continued until adult height is achieved at which point bisphosphonate administration is discontinued and thorough reevaluation undertaken; this plan may be modified depending on the individual’s response to the therapeutic drug.^{324,423} Protocols for the treatment of children/adolescents with secondary forms of osteoporosis have also been presented.^{322,423} Bisphosphonates have also been used in the treatment of children with hypercalcemia recalcitrant to other management (e.g., hydration, diuretics).⁴²³ An expected side effects of bisphosphonate administration is hypocalcemia—anticipated by the provision of supplemental calcium and vitamin D before and for several days after therapeutic administration of the bisphosphonate. To avoid medication-related systemic effects, such as fever, malaise, and myalgia, administration of an analgesic and antipyretic agent for 48 to 72 hours after administration of the bisphosphonate is often used. Serious side effects of bisphosphonates are unusual but include iritis, atypical femoral fracture caused by decreased bone remodeling, and osteonecrosis of the jaw, the latter complication being unusual in children.³⁸⁰ Limited data indicate that oral bisphosphonates (alendronate, olpadronate) administered daily also increase areal BMD in children with osteogenesis imperfecta and connective tissue disease but with lower efficacy than intravenous administration. However, currently although bisphosphonates are not approved for use in children by the FDA, they are widely used.⁴³⁷

Side effects of bisphosphonates have been both acute (fever, myalgia, abdominal pain, vomiting, hypocalcemia) and chronic (inflammatory disorders of the eye, osteonecrosis of the jaw in the elderly, and induced “osteopetrosis”) but have not generally occurred in the pediatric population.⁴³⁷ Experimentally and in adults receiving long-term therapy, bisphosphonates can suppress bone turnover and contribute to hypermineralization, the latter leading paradoxically to reduced mechanical strength and increased fracture risk.⁴³⁸ Therefore when considering a child for treatment with bisphosphonates one must carefully evaluate the primary diagnosis and whether the patient’s low bone mass and fracture frequency merit therapy in view of the potential, albeit rare, side effects of bisphosphonates. Treatment with bisphosphonates several years before conception does not appear to have an adverse effect on fetal outcome, but treatment during pregnancy is contraindicated because of possible toxicity.⁴³⁹

Although not appropriate for use in children and adolescents because of concern about its potential to induce osteosarcoma, PTH¹⁻³⁴ (teriparatide) is a widely used bone anabolic agent in adults. When administered continuously, PTH¹⁻³⁴ increases osteoclast-mediated dissolution of bone, but when administered intermittently it exerts anabolic effects on osteoblast function and bone formation. This property is used clinically through the use of teriparatide (PTH¹⁻³⁴) and abaloparatide (PTHrP¹⁻³⁴) in the treatment of postmenopausal women with osteoporosis. When administered intermittently in small amounts, both agents preferentially accelerate the rates of bone remodeling and of bone formation relative to that of bone resorption by direct effects upon osteoblast differentiation, maturation, and longevity.⁴³⁰ PTH¹⁻³⁴ also acts upon the osteocyte to decrease the production of sclerostin, an inhibitor of bone synthesis that acts by repressing LRP5/6-WNT- and BMP-mediated bone formation.⁴⁴⁰ The quantity of bone formed in each remodeling unit is increased with consequent augmentation of trabecular thickness and interconnectivity and periosteal new bone formation and cortical thickness resulting in increased bone size, mass, and strength. Side effects of PTH¹⁻³⁴ administration include transient hypercalcemia, hypercalciuria, and development of antibodies to the peptide. Although osteosarcoma has been observed in mice receiving very high doses of these agents, no malignant disorders have been recorded in adults receiving either agent. The pediatric use of PTH¹⁻³⁴ has been primarily limited to children with hypocalcemia because of a gain-of-function mutation in *CASR* and consequent hypercalciuric hypocalcemia—one form of familial isolated hypoparathyroidism.⁵⁸

The basic management of patients with osteogenesis imperfecta is directed to prevention of fractures to the extent possible and to the treatment of fractures that do occur by sound orthopedic procedures and by orthopedists familiar with this disorder.³⁷⁷ Expert orthopedic management is essential for the child with osteogenesis imperfecta, as the use of intramedullary rods for correction of long bone deformities and for their linear growth and the proper correction of scoliosis require substantial experience.⁴⁴¹ Dental and rehabilitative services and physical therapy to improve dentition, muscle strength, and mobility within the constraints of bone fragility are to be encouraged as are protected ambulation and exercises, such as swimming.^{191,377} The intermittent administration of bisphosphonates to infants, children, and adolescents with moderately severe forms of osteogenesis imperfecta (clinical type I, III, IV) has been of substantial benefit.⁴³⁵ Patients with these types of osteogenesis imperfecta have responded symptomatically (decreased musculoskeletal pain, increased mobility) to the intermittent intravenous administration of a bisphosphonate—for example, pamidronate, zoledronic acid; bone mass has increased, fracture risk has declined, and

vertebral dimensions may be normalized although the prevalence of scoliosis has not declined.^{377,435,441}

It has been recommended that bisphosphonates be used in infants with osteogenesis imperfecta with congenital and recurrent fractures, deformities of the long bones, and decreased bone mass. Infants as young as 2 months of age have safely tolerated 4-hour intravenous infusions of pamidronate (0.5 mg/kg/d for 3 consecutive days every 6–8 weeks) realizing clinical improvement, such as apparent decline in bone pain, increase in lumbar vertebral BMD of 86% to 227%, and decrease in fracture rate after 1 year of therapy.¹⁹¹ Bisphosphonate administration is also recommended for children with osteogenesis imperfecta and recurrent fractures of the extremities or vertebral collapse that is symptomatic in concert with demonstrated decreased bone mineralization.⁴²⁹ In children (3–16 years of age), pamidronate administered as a 4-hour infusion (1.5–3.0 mg/kg/d) for 3 consecutive days every 4 months, resulted in increases in lumbar spine BMD of 42% per year, metacarpal cortical width of 27% per year, and vertebral size, as well as decline in fracture rate, and symptomatic improvement. During 2 to 4 years of intravenous pamidronate administration, increase in vertebral (trabecular) bone mass and size are accompanied by decline in the extent of vertebral compression and fewer compressed vertebrae than in untreated patients.⁴⁴² In the iliac crest, bisphosphonates increase cortical bone thickness and trabecular number but not trabecular thickness; in metacarpals bisphosphonates enhance cortical thickness. Administration of bisphosphonates is also accompanied by transient hypocalcemia (managed symptomatically), increased levels of PTH and calcitriol, and decreased values of bone turnover markers. Near maximal benefits of bisphosphonates on lumbar vertebral areal BMD by DXA and on mean cortical width, cancellous bone volume, and trabecular bone formation rate by histomorphometric analysis of iliac crest bone biopsies are achieved within the first 2 to 4 years of treatment with little further change with more prolonged therapy. The use of monoclonal antibodies to sclerostin (romosumab) or RANKL (denosumab) may also prove useful in the treatment of children with osteogenesis imperfecta.^{443–445} Transplantation of mesenchymal stem cells, human fetal mesenchymal stem cells or chorionic stem cells, or bone marrow stromal cells have been undertaken in patients with osteogenesis imperfecta but remains experimental therapy as do specific gene replacement and mutation silencing strategies.^{446,447}

Fibrous Dysplasia

Fibrous dysplasia is a usually nonmalignant fibro-osseous disorder that involves long bones, ribs, and skull in which normal bone and bone marrow are replaced by fibrous tissue; it may be monostotic, polyostotic, or panostotic.^{448–450} This disorder may be monostotic or polyostotic and presents as the cause of a pathological fracture with minor injury. Although fibrous dysplasia may be an isolated abnormality, it occurs in patients with the McCune-Albright syndrome (OMIM 174800) in association with very large, irregularly edged café-au-lait pigmentations and various endocrinopathies, including isosexual precocious puberty, hypersomatotropism, thyrotoxicosis, and hyperadrenocorticism, as well as dysfunction in many other tissues (heart, liver, pancreas). Bone and skin lesions are often on the same side of the body.⁴⁴⁹ Fibrous dysplasia is caused by mosaicism for postzygotic, early embryonic, somatic gain-of-function missense mutations (p.Arg201Cys/His/Ser/Gly; p.Gln227Leu) in *GNAS* (OMIM 139320), the gene encoding the α subunit of the Gs-protein that render a G_{s α} constitutively active by prolonging its biological life.⁴⁵¹ The extent and severity of disease is determined by the point in fetal development at which the mutation occurs and its tissue distribution. The

mutations result in loss of intrinsic guanosine triphosphatase activity within the $G_{\alpha s}$ subunit; thus the stimulatory effect of $G_{\alpha s}$ on adenylyl cyclase is extended thereby increasing generation of cyclic AMP. Among the targeted signaling pathways of cyclic AMP is that involving WNT/ β -catenin in osteoblast progenitor cells.⁴⁴⁹ In response to excess cyclic AMP, clones of mutated mesenchymal preosteoblasts proliferate but their differentiation to mature osteoblasts is incomplete and their secreted fibrocellular matrix is abnormal; continued expansion of osteoprogenitor cells in bone marrow leads to local fibrosis. As the osteogenic cells increase in number, they steadily erode contiguous bone. These lesions can also synthesize FGF23 and thus lead to hyperphosphaturia, hypophosphatemia, and excess unmineralized osteoid and a rickets-like clinical state. Fibrodysplastic lesions are initially silent whereas osteoclasts at the periphery of the lesions actively compress and thin bone cortices, ultimately resulting in bone pain and pathological fractures of the long bones, particularly the proximal femoral metaphyses. Children between 6 and 10 years of age have the highest fracture rate (0.4 fractures per year). Within the skull base and facial bones, expansion of fibrous dysplastic lesions leads to disfigurement and compression of cranial nerves. Radiographically, the fibrodysplastic lesion is viewed as a “cyst-like” medullary structure with a “ground-glass” consistency without a trabecular pattern.⁴⁴⁹ Histologically, fibrous dysplasia is characterized by abundant immature bone marrow stromal cells, incompletely differentiated osteoblasts, irregularly formed bone trabeculae that may resemble Chinese characters, many undermineralized osteoid seams characteristic of osteomalacia, and islands of cartilage. The clinical manifestations of fibrous dysplasia depend on the sites and extent of bone involvement and associated endocrinopathies. Diagnosis of fibrous dysplasia is based on clinical characteristics, imaging of the bone lesions (“ground glass” appearance), and confirmation of the genetic mutation in *GNAS*. In addition to managing the multiple endocrinopathies and organ defects, attention must be paid to the osseous lesions. Fractures are repaired by standard techniques, including intramedullary nailing when indicated; occasionally it may be feasible to evacuate a fibrodysplastic lesion surgically and to fill the cavity with bone grafts. In patients with polyostotic fibrous dysplasia without or associated with the McCune-Albright syndrome, administration of the oral bisphosphonate alendronate did not alter the radiographic appearance of the skeletal lesions, ameliorate bone pain, or improve function.⁴⁵² However, in patients with either isolated polyostotic fibrous dysplasia or that associated with the McCune-Albright syndrome, treatment with parenteral pamidronate or zoledronic acid has ameliorated bone pain and decreased serum levels of alkaline phosphatase reflecting decline in bone turnover rate but has not repaired the skeletal lesions.^{453,454} In a 9-year-old boy with a rapidly growing femoral mass because of fibrous dysplasia (and a somatic mutation in *GNAS1*), treatment with denosumab led to rapid decline in the rate of tumor growth and in lowering of the levels of bone turnover markers.⁴⁵⁵ However, the child developed hypocalcemia and secondary hyperparathyroidism requiring supplemental calcium, phosphate, and calcitriol. Cessation of denosumab was marked by rebound increase in values of bone turnover markers and hypercalcemia.

High Bone Mass

Abnormally increased bone mass is the consequence of disruption of the normal equilibrium between the coupled processes of bone formation and resorption. Osteosclerosis is the designation applied to a heritable or acquired disorder characterized by or associated with increase in bone mass and BMD. Specifically, increase in cortical bone width is termed *hyperostosis*;

thickening of trabecular bone is termed *osteosclerosis*.⁸ Osteopetrosis or “marble bone disease” is a group of diseases associated with increased calcified bone but paradoxically complicated by osseous fragility, leading to fractures with minor or no trauma that is primarily caused either by decreased osteoclastogenesis or impaired osteoclast function or rarely by an increase in the rate of bone formation because of excessive osteoblast activity. Failure of osteoclast-mediated bone resorption leads to osteopetrosis that may be associated with a large number of poorly functioning osteoclasts (“osteoclast-rich” osteopetrosis) or with a normal number or paucity of osteoclasts (“osteoclast-poor” osteopetrosis).^{306,456,457}

The 2019 designated genetic causes of osteopetrosis and related forms of osteosclerosis are listed in Tables 20.11A, 20.11B and schematically illustrated in Fig. 20.15. These clinical disorders are transmitted as either autosomal recessive or dominant or X-linked disorders; osteopetrosis caused by mutations of *CLCN7* and *PLEKHM1* may be transmitted either as biallelic or a monoallelic abnormalities.⁴⁵⁶ Osteopetroses that are caused by errors in osteoclast differentiation and associated with “osteoclast poor” osteopetrosis are related to mutations in *TNFRSF11A*, *TNFSF11*, and *IKBKG*. Those associated with “osteoclast-rich” osteopetroses are caused by variants of *TCIRG1*, *CLCN7*, *OSTM1*, *SNX10*, *PLEKHM1*, and *CAII*. Microscopically, the bones of patients with autosomal recessive osteopetrosis types 1, 3, 4, 5, 6, and 8 have abundant but functionally impaired osteoclasts (osteoclast rich); in autosomal recessive osteopetrosis types 2 and 7 and the X-linked form, there are few osteoclasts caused by a paucity of RANKL or RANK (“osteoclast poor”). Autosomal dominant type 1 osteopetrosis is not related to an error in osteoclast differentiation or function and defective bone resorption but rather is associated with increased osteoblastogenesis resulting in enhanced formation and mineralization of bone. This form of osteopetrosis is caused by a gain-of-function variant (p.Gly171Val) of *LRP5* (OMIM 603506) that leads to increased bone mass by impairing the binding of *LRP5* to sclerostin (*SOST*, OMIM 605740) and to *DKK1* (*Dickkopf*, *xenopus*, *homolog of*, OMIM 605189), cofactors that physiologically bind to and inhibit linking of *LRP5* to its ligand. Thus in the presence of mutated *LRP5* the WNT1/Frizzled receptor/*LRP5* coreceptor/ β -catenin signaling pathway within the mesenchymal stem cell is programmed toward osteoblastogenesis. This activating variant of *LRP5* expressed by the osteoblast is associated with increased bone formation (autosomal dominant osteopetrosis type I)—primarily of the cranium; this disorder may be asymptomatic or associated with cranial nerve insults resulting in trigeminal neuralgia, facial nerve paresis, or auditory nerve dysfunction, albeit without the more severe deleterious consequences of the osteoclastic forms of osteopetrosis.

The recessive forms of osteopetrosis (see Table 20.11A) are often clinically more severe than those transmitted in a dominant manner. Manifestations of increased bone mineralization may be present in utero (fractures) or develop in the first several months of life (fractures, trapping of cranial nerves in narrowing foramina with consequent compression atrophy of these nerves and loss-of-function—particularly sight and hearing; compression of the growing brain may lead to developmental delay). Autosomal recessive osteopetrosis type 1 is caused by variants of T cell immune regulator 1 (*TCIRG1*, OMIM 604592) and is associated with increased osteoblastogenesis, leading to enhanced formation and mineralization of bone. Variants in *CLCN7* result in a wide range of clinical manifestations from benign carrier status to rather severe manifestations of osteopetrosis. Inactivating biallelic mutations of either *OSTM1* or *CLCN7* are often associated with a lysosomal storage disease, neurodegeneration, and impaired cognition. Because of loss of bone marrow, hematopoiesis is compromised in

TABLE 20.11A Gene Variants/Mutations Pathogenetically Related to Osteopetrosis

Type OMIM Inheritance: Autosomal recessive (AR) Autosomal dominant (AD)	Gene Protein OMIM Chromosome	Functional Defect	Clinical Abnormality
AR1 259700	<i>TCIRG1</i> T cell immune regulator 1 604592 11q13.2	Encodes the V-H+ATPase $\alpha 3$ subunit, the pump responsible for movement of H^+ from osteoclast cytoplasm to the subosteocytic lacuna where H^+ dissolves the mineral phase of bone; also expressed in gastric mucosa where it is required for intestinal absorption of Ca^{2+}	Osteoclast rich Severe "malignant" osteopetrosis with early lethality; paradoxical rachitic-like osseous phenotype
AR2 259710	<i>TNFSF11</i> Tumor necrosis factor ligand superfamily, Member 11 602642 13q14.11	Osteoblast factor (also designated receptor activated nuclear factor- κB -ligand [RANKL]) that stimulates osteoclastogenesis	Osteoclast poor Severe osteopetrosis
AR3 259730	<i>CAII</i> Carbonic anhydrase II 611492 8q22	Enzyme that catalyzes H^+ production by osteoclastic synthesis & ionization of carbonic acid (H_2CO_3); excess HCO_3^- removed through Cl^-/HCO_3^- ion exchanger	Osteoclast rich Osteopetrosis of intermediate severity; associated with renal tubular acidosis, cerebral calcifications, cognitive defects
AR4 611490 AD2 166600	<i>CLCN7</i> Chloride channel 7 602727 16p13	Chloride channel expressed in osteoclasts that facilitates acidification of subosteocytic lacuna & dissolution of mineral phase of bone	Osteoclast rich AR4—Biallelic variants result in severe osteopetrosis, decreased cognition associated with neurodegeneration because of a lysosomal storage disease; AD2—Monoallelic variants lead to sclerosis of the base of the skull, pelvis, and vertebral endplates resulting in a "sandwich" x-ray appearance of the spine; not associated with increase in rate of fracture but entrap & impair cranial nerve function (hearing, sight)
AR5 259720	<i>OSTM1</i> Osteopetrosis-associated transmembrane protein 1 607649 6q21	Chaperone-like factor that associates with <i>CLCN7</i> during its maturational & translocational processes	Osteoclast rich Associated with severe osteopetrosis, neurodegeneration caused by a lysosomal storage disease
AR6 611497 AD3 618017	<i>PLEKHM1</i> Pleckstrin homology domain containing protein, Family M, Member 1 611466 17q21.31	Factor necessary for vesicular trafficking of proteins in the osteoclast	Osteoclast rich Osteopetrosis of moderate severity; osteoclasts do not form ruffled borders
AR7 612301	<i>TNFRSF11A</i> Tumor necrosis factor receptor superfamily, Member 11A 603499 18q21.33	Transmembrane receptor for RANKL—expressed by osteoclast precursor cell	Osteoclast poor Also designated receptor activated nuclear factor- κB (RANK); severe, associated with hypogammaglobulinemia
AR8 615085	<i>SNX10</i> Sorting nexin 10 614780 7p15.2	Regulator of vacuole formation & vesicle associated protein movement in osteoclasts & stomach	Osteoclast poor (\pm) Severe osteopetrosis, anemia & leukopenia because of obliteration of medullary cavity of bone; paradoxical rachitic-like osseous phenotype
AR osteopetrosis	<i>FERMT3</i> Fermitin family, member 3 607901 11q13.1	Integrin signaling, platelet aggregation	Osteoclast rich. Encodes kindlin-3 protein; mutations associated with osteopetrosis, infections, hemorrhage
AD1	<i>LRP5</i> Low-density lipoprotein receptor-related protein 5 603506 11q13.2	Coreceptor for WNT1 with Frizzled that is essential for osteoblastogenesis	Gain-of-function variants of this WNT1-Frizzled receptor associated protein result in sclerosis of the cranial vault but not with increased frequency of fractures

Continued

TABLE 20.11A Gene Variants/Mutations Pathogenetically Related to Osteopetrosis—cont'd

Type OMIM Inheritance: Autosomal recessive (AR) Autosomal dominant (AD)	Gene Protein OMIM Chromosome	Functional Defect	Clinical Abnormality
AD2	<i>CLCN7</i>		
AD3	<i>PLEKHM1</i>		
X-linked	<i>IKBKG</i> Inhibitor of kappa light polypeptide gene enhancer in B cells, Kinase of, Gamma 300248 Xq28	Serine/threonine kinase that enhances function of NF-κB thereby stimulating osteoclastogenesis	Osteoclast poor (±) Also designated NF-κB essential modulator (NEMO); associated with moderate osteopetrosis, ectodermal dysplasia, immunodeficiency

(From Teti, A., Econs, M.J. (2017). Osteopetroses, emphasizing potential approach to treatment. *Bone*, 102:50–59; Penna, S., Capo, V., Palagano, E., et al. (2019). One disease, many genes: Implications for the treatment of osteopetroses. *Front Endocrinol*, 10:85.)

TABLE 20.11B Gene Mutations Causally Related to Increased Bone Mass/Osteosclerosis

Disorder OMIM	Gene Gene Protein OMIM Chromosome	Function	Functional Defect
Pycknodysostosis 265800	<i>CTSK</i> Cathepsin K 601105 1q21.2	Cysteine proteinase	Failure of enzymatic dissolution of organic matrix of bone during bone remodeling, AR
Van Buchem disease 1 239100	<i>SOST</i> Sclerostin 605740 17q21.31	Factor that binds to extracellular domain of LRP5 and LRP6 inhibiting their binding to WNT1 & its signal transduction	Hyperostosis corticalis generalisata—increased bone mineralization d/t enhanced osteoblastogenesis involving cortex of cranium & long bones resulting in cranial nerve entrapment & loss-of-function, AR
Sclerosteosis 1 269500	<i>SOST</i> Sclerostin 605740 17q21.31		Generalized skeletal osteosclerosis & hyperostosis— increased bone mineralization with enhanced osteoblastogenesis associated with gigantism, syndactyly, increased intracranial pressure, early demise, AR
Van Buchem disease 2 - 607636	<i>LRP5</i> Low-density lipoprotein receptor-related protein 5 603506 11q13.2	Factor that binds to the WNT1-β-catenin complex & enhances osteoblastogenesis	Hyperostosis corticalis generalisata, AD
Sclerosteosis 2 614305	<i>LRP4</i> 604270 11p11.2	Factor that binds to sclerostin & facilitates its inhibitory effect on osteoblastogenesis	Generalized skeletal osteosclerosis & hyperostosis, AR/AD
Raine syndrome 259775	<i>FAM20C</i> Family with sequence similarity 20, member C 611061 7p22.3	Phosphorylase of SIBLING proteins that impede biomineralization	Prenatal generalized osteosclerosis with periosteal bone formation often resulting in perinatal death, AR

AD, Autosomal dominant; AR, autosomal recessive, *SIBLING*, small integrin-binding ligand N-linked secreted glycoproteins.

most subjects with osteopetrosis. Impaired development of osteoclasts results in osteoclast-poor osteopetrosis and is attributable to mutations in genes encoding RANKL (*TNFRSF11*) and RANK (*TNFSF11A*). *IKBKG* is an X-linked gene that encodes a subunit of the inhibitor of kappa B (IκB) kinase complex that is also essential for activation of NF-κB and osteoclastogenesis. Despite being present in normal number or in abundance in patients with osteoclast-rich

osteopetrosis, osteoclast activity may be reduced because of decreased synthesis of acid (because of loss-of-function mutations in *CAII*, the gene encoding carbonic anhydrase), decreased number or activity of ion channels that transport Cl⁻ and H⁺ into the subosteocytic lacunae (channel proteins encoded by *CLCN7* and *TCIRG1* or its cofactor *OSTM1*, respectively), or subnormal generation of proteinases that degrade bone matrix, such as cathepsin K (because of

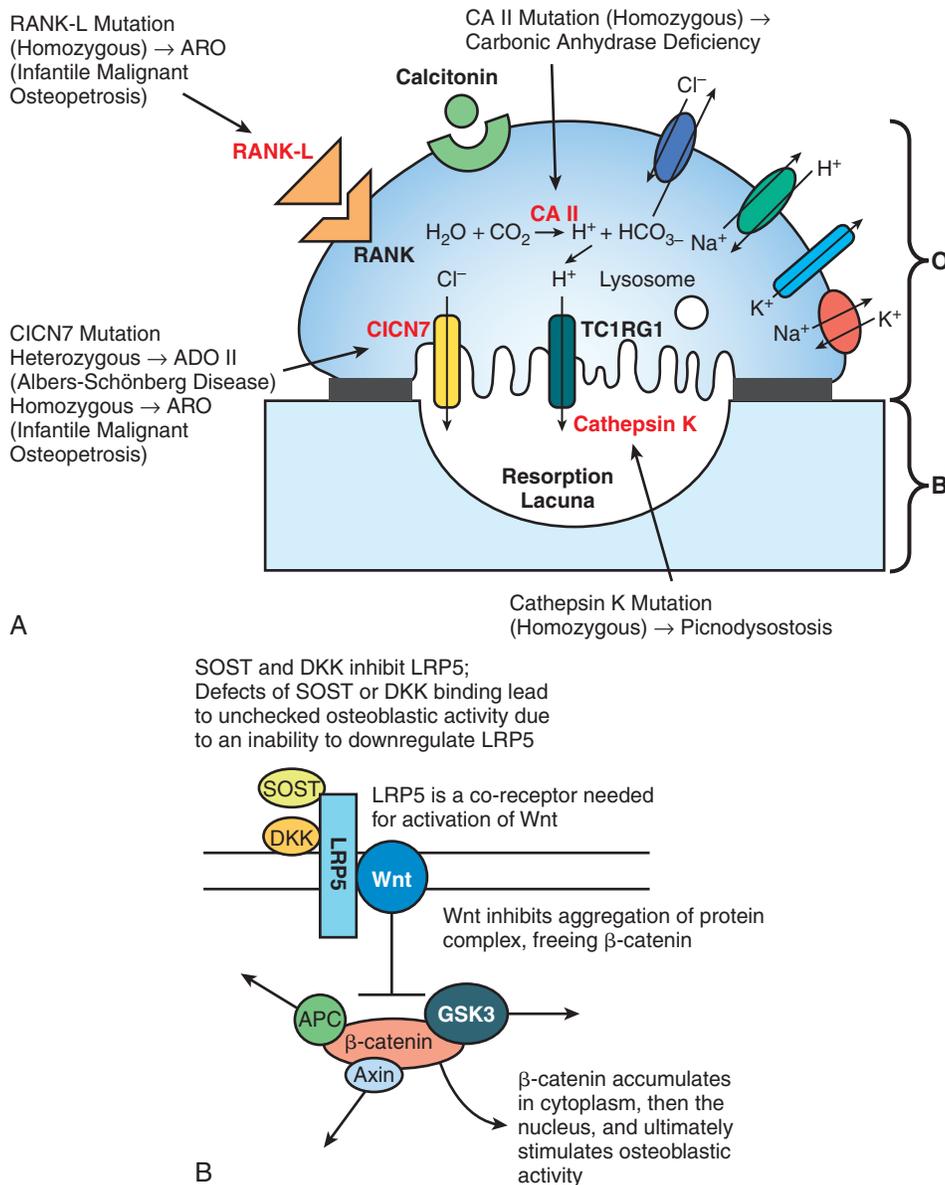


Fig. 20.15 Biochemical defects in osteoclasts (A) and osteoblasts (B) associated with osteopetrosis (see text for details). (Adapted with permission, from de Vernejoul MC. Sclerosing bone disorders. *Best Pract Res Clin Rheumatol* 2008;22(1):71–83.)

inactivating mutations in *CTSK*). Rarely, excessive deposition of bone mineral because of increased osteoblast function may be a consequence of enhancement of the WNT/ β -catenin signal transduction pathway because of gain-of-function mutations in *LRP5* as discussed or to decreased inhibition of this process because of inactivating variants of *SOST* (encoding sclerostin).⁴⁵⁸ Interestingly, in patients with mutations in *LRP5* and autosomal dominant osteopetrosis type I (see Table 20.11A), there is also a paucity of osteoclasts.³⁰⁶ Inactivation of *SOST* (OMIM 605740) leads to sclerosteosis type 1 (OMIM 269500), characterized by bony overgrowth and entrapment of cranial nerves in association with syndactyly attributable to sclerostin-unregulated activity of the LRP5-Frizzled receptor- β -catenin osteoblast stimulating pathway.⁴⁵⁸ Inactivating variants of *LRP4* (OMIM 604270) encoding a protein that binds to sclerostin are also associated with high bone mass (sclerosteosis type 2, OMIM 614305)—the possible consequence of impaired LRP4 assisted sclerostin activity within bone or of the extraskeletal sequestration of sclerostin by mutated LRP4.⁴⁵⁹

Histopathologically, bone in the majority of forms of osteopetrosis is characterized by the presence of quiescent osteoclasts—either increased, normal, or few in number—and retained “islands” of calcified cartilage formed during endochondral ossification (primary spongiosa) because of failure of reabsorption of immature bone.⁸ Although densely packed with minerals, osteopetrotic bone is quite fragile because the abnormality in bone remodeling resulting from decreased osteoclastic bone resorption leads to incorporation of weak calcified growth plate cartilage into bone and to delay in repair of microfractures. Radiographically, osteopetrosis is characterized by diffuse increase in bone mass involving both cortical and trabecular bone, diaphyseal/metaphyseal widening with an “Ehrlenmeyer flask” appearance, alternating bands of sclerotic and lucent bone at the ends of the long bones (“bone-within-bone”), iliac crest, and vertebrae (“rugger jersey” or “sandwich” vertebrae), sclerotic changes at the base of the skull, narrow medullary cavities, and pathological fractures.^{8,457} Cranial CT often reveals narrowing of the bony canals through which pass cranial nerves II, III, IV, VII, and VIII. Although classically, three

clinical forms of osteopetrosis of variable severity were identified—infantile malignant (autosomal recessive), intermediate (autosomal recessive), and adult (autosomal dominant)—as information about the genetic mutations responsible for the disease have been delineated this classification has been replaced by one that identifies its mode of transmission (autosomal dominant or recessive or X-linked recessive) and in accord with the responsible mutated gene (see Tables 20.11A, 20.11B). Thus the infantile/malignant form of osteopetrosis (OMIM 259700) may be caused by biallelic mutations in *TCIRG1*, *CLCN7*, or *OSTM1*—genes encoding osteoclast membrane hydrogen and chloride transport channel proteins or a comodulator through which the osteoclast secretes acid into the subosteoclastic lacuna to dissolve hydroxyapatite, the mineral phase of bone. A severe form of osteopetrosis associated with hypogammaglobulinemia is attributable to deleterious variations in *TNFRSF11A* encoding RANKL. Forms of osteopetrosis of intermediate severity are the result of mutations in *TNFSF11*, *CAII*, *PLEKHM1*—genes encoding RANK, carbonic anhydrase, and a protein that is involved with intraosteoclastic trafficking of lysosomes, respectively. X-linked osteopetrosis is a disorder of moderate severity related to variants of *IKBKG*, a serine/threonine kinase that enhances function of NF- κ B, thereby stimulating osteoblastogenesis (also designated NF- κ B essential modulator [NEMO]; this is an osteoclast-poor (\pm) form of moderately severe osteopetrosis in association with ectodermal dysplasia and immunodeficiency. Activating mutations in *LRP5* and heterozygous inactivating variations in *CLCN7* are associated with osteopetrosis of mild to intermediate severity that are transmitted as autosomal dominant disorders.

The infantile/malignant form of osteopetrosis is an autosomal recessive (AR type 4) disorder with attenuated growth particularly of the limbs, delayed development, increased fracture rate, and failure of tooth eruption because of variants of *CLCN7* (see Table 20.11A). Bony overgrowth leads to macrocephaly, occasionally hydrocephalus, maldevelopment of the paranasal sinuses, and to symptomatic nasal “stiffness.” Narrowing of cranial foramina compromises cranial nerve function (II, III, VII, VIII) with consequent blindness, deafness, or cranial nerve paralysis. Decrease in bone marrow volume results in depressed intramedullary hematopoiesis, anemia, and leukopenia partially compensated by extramedullary hematopoiesis and ensuing hepatosplenomegaly with consequent increased susceptibility to infection and hemorrhage. Retention of teeth within the sclerotic jaw leads to recurrent and persistent mandibular and maxillary osteomyelitis. Physical examination reveals short stature, macrocephaly, frontal bossing, small facial features, and hypertelorism; vision may be compromised by exophthalmos and retinal atrophy. Osteopetrotic neonates and infants are often hypocalcemic (because of inability to reabsorb calcium deposited in bone) with secondary hyperparathyroidism and elevated calcitriol values; levels of serum acid phosphatase and the brain isoenzyme of creatine kinase are also increased.^{7,8} Death usually occurs often within the first decade of life because of sepsis, anemia, or hemorrhage. Autosomal recessive osteopetrosis may be caused not only by biallelic mutations in the osteoclast’s chloride channel—*CLCN7*, but also in the $\alpha 3$ subunit of the osteoclast’s vacuolar proton pump—*TCIRG1*, the osteopetrosis-associated transmembrane protein-1 (*OSTM1*), or a protein that guides *TCIRG1* to the osteoclast’s ruffled membrane (*SNX10*) (vide infra). Biallelic mutations in *TNFRSF11A* encoding RANK give rise to a severe form of osteopetrosis often in association with hypogammaglobulinemia.⁴⁶⁰

The intermediate clinical forms of osteopetrosis are also transmitted as autosomal recessive traits associated with short stature, macrocephaly, variable compromise of cranial nerve function, recurrent fractures, abnormal dental development

predisposing to osteomyelitis of the mandible or maxilla, and anemia. Pathogenetically, they represent the variable penetrance of one of the genetic mutations that may be associated with the infantile form of osteopetrosis, predominantly of *CLCN7*, but also of *CAII*, *TNFSF11*, and *PLEKHM1*. There are three designated clinical and radiographic forms of monoallelic, autosomal dominant osteopetrosis: type 1 (OMIM 607634) is characterized by an enlarged and dense cranial vault and diffuse vertebral sclerosis and is related to activating mutations of *LRP5*; it is not associated with an increase in fracture rate as bone strength is actually increased—hence it has also been termed *high bone mass disease*. Autosomal dominant osteopetrosis type 2 (OMIM 166600) is typified by thickening of the vertebral endplates resembling “bone-within-bone” and resulting in a “rugger jersey spine” and sclerotic bands of bone in the pelvis and base of the skull. It is a variant of Albers-Schonberg disease that results from heterozygous loss-of-function mutations in *CLCN7*.⁸ Affected subjects manifest cranial nerve compromise (16%), mandibular and nonmandibular osteomyelitis (19%), osteoarthritis of the hip (27%), and fractures (78%). Clinical evidence of the disease tends to worsen over time. However, the expression of the trait is variable. Thus one-third of carriers of an inactivating mutation in *CLCN7* have no radiographic or clinical manifestations, although they do have significantly higher BMD than do subjects with the wild-type gene.⁴⁶¹ In one-quarter of clinically apparent patients with a heterozygous loss-of-function mutation in *CLCN7*, the expression of illness (fractures, osteomyelitis, compromised vision) is identifiable at birth or early in infancy or childhood. Patients with radiologic/clinical manifestations of this disorder have elevated serum concentrations of TRAP and the BB isoform of creatine kinase elaborated by osteoclasts; these values are normal in unaffected carriers.⁸ Autosomal dominant osteopetrosis type 3 is the result of monoallelic variants in *PLEKHM1* (OMIM 611466) encoding a factor necessary for trafficking of intraosteoclast organelles. X-linked osteopetrosis has been related to inactivating variants of *IKBKG* (OMIM 300248) encoding a serine/threonine kinase that enhances function of NF- κ B, thereby stimulating osteoclastogenesis; its loss is associated with moderate osteopetrosis, ectodermal dysplasia, and immunodeficiency.⁴⁵⁷

Most often genetic variants that impede resorption of bone in osteopetrotic subjects are related to abnormalities in osteoclastogenesis or osteoclast function—particularly the efficiency of acidification of the resorption lacuna beneath the osteoclast’s ruffled border enabling mineral dissolution or enzymatic degradation of organic bone matrix. *TNFSF11* encodes RANKL, a 317 aa transmembrane protein expressed on the surface of stromal cells and osteoblasts that interacts as a trimer with its receptor—RANK (encoded by *TNFRSF11A*), a plasma membrane protein expressed by osteoclast precursor cells to form a heterohexameric complex that stimulates NF- κ B and thereby induces osteoclastogenesis.⁴⁶² Inactivating mutations in *TNFSF11* and *TNFRSF11A* result in impaired osteoclast formation and thus osteoclast-poor osteopetrosis. Loss-of-function variations in the structure of RANKL associated with osteopetrosis include a missense mutation (p.Met199Lys) and 2-bp (828delCG) and 5-bp (intron 7 – 532 + 4_532 + 8) deletions of *TNFSF11*.⁴⁶³ Patients with osteopetrosis caused by inactivating mutations in *TNFSF11* do not respond to hematopoietic stem cell transplantation because the abnormality in osteoclastogenesis is extrinsic to the osteoclast cell line; because recombinant RANKL protein can transform the monocytes of affected patients into functioning osteoclasts in vitro, a potentially alternative therapeutic avenue might be possible.⁴⁶³ Biallelic missense (p.Gly53Arg, p.Arg 170Cys), nonsense (p.Trp244Stop, p.Gly280Stop), and insertion loss-of-function mutations in

the extracellular domain of RANK also impair binding to RANKL and consequent stimulation of NF- κ B and osteoclastogenesis.^{310,460} Many patients with inactivating mutations in *TNFRSF11A* also develop impaired B lymphocyte function and hypogammaglobulinemia.³¹³ Monocytes from these subjects are functionally unresponsive to recombinant RANKL and to M-CSF *in vitro*; hematopoietic stem cell transplantation is curative in osteopetrotic subjects with deleterious mutations in *TNFRSF11A*.⁴⁶⁰ Inactivating mutations in *IKBKKG*, encoding the inhibitor of the kinase of kappa light polypeptide gene enhancer in B cells (gamma subunit), alternatively termed *NEMO*, also impair NF- κ B generation and osteoclastogenesis resulting in the syndrome of osteopetrosis, lymphedema, anhidrotic ectodermal dysplasia, and immunodeficiency (OL-EDA-ID—OMIM 300301).⁴⁶⁴ *IKBKKG* is a 419 aa component of the I κ B kinase complex that activates NF- κ B. *IKBKKG* is located on the long arm of the X chromosome, and thus its loss is transmitted as an X-linked trait.

CAII (one of several zinc metalloenzymes) is a protein that is expressed in osteoclasts, erythrocytes, brain, and kidney; it regulates the formation of carbonic acid from water and carbon dioxide ($\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$) that then dissociates to form proton/hydrogen (H^+) and bicarbonate (HCO_3^-) ions. Loss-of-function homozygous or compound heterozygous (p.Lys17Glu, p.Tyr40Ter, p.His107Tyr, p.Asn252Asp) mutations in *CAII* lead to autosomal recessive osteopetrosis (type 3) that presents in childhood with failure to thrive, short stature, visual impairment, and developmental delay in association with mild proximal and severe distal renal tubular acidosis, cerebral calcifications within the cortex and basal ganglia, and osteopetrosis with increased fracture risk.⁸ Osteopetrosis is of modest severity and usually nonprogressive; it may even improve at puberty. Bicarbonate may be used to normalize acid-base balance. After generation by CAII, H^+ is extruded from the osteoclast into the subosteoclastic resorption lacuna through transporters and proton pumps. *TCIRG1* (OMIM 604592) encodes an 822 aa, 116 kDa protein that is a subunit of the osteoclast's vacuolar proton pump (H^+ -ATPase). (By alternative splicing, this gene also encodes a 614 aa protein—TIRC7—that is essential for activation of T lymphocytes.) Biallelic inactivating (missense, nonsense, deletion, insertion, splice site) mutations in *TCIRG1* whose loss impairs transport of H^+ and thus decreases bone mineral resorption have been found in 50% of subjects with the neonatal/infantile form of lethal osteopetrosis (AR type 1) (OMIM 259700).^{460,465} *TCIRG1* may be guided to the osteoclast's ruffled membrane above the subosteoclastic lacuna by the product of *SNX10*; biallelic mutations (p.Arg51Gln; c.212+1G>T) in *SNX10* also result in infantile malignant osteopetrosis (autosomal recessive type 8) associated also with stenosis of the foramen magnum, herniation of the cerebellar tonsils, and mandibular osteomyelitis.^{466,467} Osteoclasts in this disorder are distinguished by the increased number of cytoplasmic vesicles they contain, their large size, and their inability to form ruffled borders attributable to defects in the trafficking patterns of endosomes and lysosomes needed to form these structures. Loss-of-function mutations in *CLCN7*, a chloride channel expressed in the ruffled membrane of the activated osteoclast and its lysosomes, also impair acidification of the subosteoclastic resorption space and hence mineral dissolution.⁴⁶⁸ Heterozygotic inactivating mutations (p.Arg767Trp; 2-bp deletion—1423AG) of *CLCN7* lead to an autosomal dominant form of osteopetrosis (type 2), whereas biallelic loss-of-function mutations (p.Ile261Phe, p.Arg762Gln, p.Leu766Pro) are found in infants with the lethal, autosomal recessive, and intermediate forms of this disease (type 4).^{469,470} Mutations in *CLCN7* account for 15% of subjects with severe and 40% of patients with intermediate autosomal recessive osteopetrosis and 75% of adults with autosomal

dominant osteopetrosis type 2. *CLCN7* is coexpressed with and complexed to Osteopetrosis-associated TransMembrane protein 1 (*OSTM1*, OMIM 607649) in endosomes and lysosomes and in the ruffled membrane of activated osteoclasts.⁴⁷¹ By decreasing posttranslational stability of *CLCN7*, homozygous loss-of-function (nonsense, deletion) mutations in *OSTM1* have been pathogenetically related to autosomal recessive lethal osteopetrosis (type 5); variants of *OSTM1* are associated with a severe neurodegenerative process characterized by cerebral atrophy and loss of myelination.^{472,473} In addition, a secreted sequence of *OSTM1* binds to the cell membrane of precursors of osteoclasts, thereby inhibiting fusion and hence formation of multinucleated osteoclasts.⁴⁷⁴ The product of *PLEKHM1* is a protein that is essential for lysosomal movement and distribution within the osteoclast, linking lysosomes to microtubules, fusion with the osteoclast plasma membrane and secretion from the osteoclast and for formation of their supralacunar ruffled borders; biallelic inactivating mutations of *PLEKHM1* result in osteopetrosis of intermediate or substantial severity (autosomal recessive type 6).^{475,476}

If not identified prenatally, clinical findings in infants with osteopetrosis may include failure to thrive, ocular prominence and abnormal extraocular movements, loss of visual and auditory function, facial nerve paresis/paralysis, and signs of increased intracranial pressure. In older children with the intermediate form of osteopetrosis, a nontraumatic fracture may be a presenting complaint of osteopetrosis; genu valgum may be present, as well as abnormalities of cranial nerve function, impacted primary teeth, and hepatosplenomegaly. Other manifestations of this disease include anemia, hypocalcemia, delayed eruption of primary dentition, dental caries, infections of the mandible, hepatosplenomegaly, and growth retardation. Radiographic findings in patients with osteopetrosis may be present *in utero* or early infancy and include: widened calvarium, uniformly increased density of long bones, widening of cortical and medullary bone resulting in narrowing of the marrow cavity, metaphyseal bands of dense bone that alternate with lucent bone areas resulting in a "bone-in-bone" appearance, "club-shaped" metaphyseal modeling (Ehrlemeyer flask deformity), increased rib width, notch in anterior portion of uniformly dense vertebrae or vertebrae with dense superior and inferior margins (rigger-jersey or sandwich vertebrae), new or healed fractures.^{456,457} Symptoms and signs in patients with autosomal dominant osteopetrosis include: fractures associated with minimal trauma, dental abscesses, and osteomyelitis of the mandible. Thus in most patients, the diagnosis of osteogenesis imperfecta is suspected and established on the basis of clinical and radiographic findings. Foremost among diagnostic laboratory investigations in a patient with osteopetrosis is analysis of the genes known to be associated with this disease (see Table 20.11A) or as is rapidly becoming more common—whole genome sequencing. The Osteopetrosis Working Group also recommends in the evaluation and observation of children with suspected/diagnosed osteopetrosis: determination of a complete blood count and measurement of serum concentrations of calcium, phosphate, creatinine, calcidiol, PTH, BB isozyme of creatine kinase, lactate dehydrogenase; urine calcium/creatinine; renal ultrasonography; and magnetic resonance imaging of the cranial nerve foramina/canals.⁴⁵⁷

A multidisciplinary team skilled in the management of patients with osteopetrosis is essential for the optimal care of infants and children with this disorder. Symptomatic therapy of these patients includes avoidance of fracture-inducing activities, transfusion of needed blood components, and administration of supplemental calcium and vitamin D/calcitriol to manage hypocalcemia and rickets ("osteopetrorickets").^{8,477} Osteopetrorickets most often develops in patients with variants of *TCIRG1* because of decreased gastric acidity and consequent

decline in intestinal calcium absorption. Administration of IFN- γ (an indirect stimulus of osteoclastogenesis) may be of limited benefit in patients with autosomal recessive forms of osteopetrosis awaiting stem cell therapy. Administration of high doses of calcitriol to patients with osteopetrosis is not recommended.⁴⁵⁷ When performed in early infancy, hematopoietic stem cell transfusions from HLA-identical donors with replacement of defective osteoclasts by normal osteoclast progenitor cells, although hazardous, have been of value in patients with osteoclast-rich forms of osteopetrosis, although unlikely to reverse already sustained consequences of the disease.^{8,456,457,478,479} Hematopoietic stem cell transplants allow the engraftment of donor-derived osteoclast precursors that differentiate into mature functional osteoclasts permitting bone remodeling and hematopoiesis. Hematopoietic stem cell transfusions are not generally recommended in patients with osteopetrosis who also have primary neurodegeneration (*CLCN7*, *OSTM1*) or in osteoclast poor forms of osteopetrosis related to inactivating mutations in *TNFSF11*, *TNFRSF11A*, and *IKBKG*. The possibility of in utero hematopoietic stem cell transplantation may also be considered. Hypercalcemia often complicates the posttransplantation period as osteoclast function resumes, particularly in patients with inactivating mutations in *TNFRSF11A*.⁴⁶⁰ Hypercalcemia has been managed with dietary restriction, calcitonin, and bisphosphonate administration and/or by use of an inhibitor of RANKL–denosumab, a monoclonal antibody raised against this protein.⁴⁸⁰ Replacement of the defective gene by genome editing or other advancing genetic techniques with restoration of normal gene function may be of benefit to patients with osteopetrosis in the future. In addition to general clinical care by the child's primary care physician, the endocrinologist, hematologist, ophthalmologist, orthopedist, neurologist, and geneticist have fundamental roles to play in the management and coordination of care of patients with osteopetrosis.⁴⁵⁷ With improved treatment, there has been increased life span and improved developmental progress. Osteopetrosis caused by deficiency of CAII is not corrected by restoration of normal systemic acid-base balance, but bone marrow transplantation may be useful.⁸

In addition to the OMIM designated forms of osteoclast-rich osteopetrosis previously discussed, inactivating mutations in *FERMT3* (OMIM 607901) also result in a severe, infantile form of osteopetrosis associated with increased susceptibility to hemorrhage and infection.⁴⁷⁹ Variants of *MITF* encoding microphthalmia-associated growth factor (OMIM 15684), *LRKK1* encoding leucine-rich repeat kinase 1 (OMIM 610986), and *USB1* encoding U6 small nuclear RNA biogenesis phosphodiesterase 1 (OMIM 613276) have also been associated with osteopetrosis/osteosclerosis-like clinical syndromes.⁴⁷⁹ In a rare patient, genetic variants of *SLC29A3* encoding solute carrier family 29 member 3 (OMIM 612373) and *CSF* encoding macrophage colony-stimulating factor (OMIM 120420) have been associated with osteoclast poor forms of osteopetrosis.⁴⁷⁹ Related to the long-acting inhibitory effects of bisphosphonate on bone modeling and remodeling, administration of high doses of intravenous pamidronate (2800 mg) over a 3-year period resulted in an acquired "osteopetrosis-like" disorder in a 12-year-old boy with unexplained hyperalkaline phosphatasemia—an abnormality that persisted for several years after this agent was discontinued.⁴⁸¹ The metaphyses of this lad were extremely dense and club-shaped, the base of the skull sclerotic, and the vertebral endplates thickened. Histologically, iliac crest biopsy revealed bars of calcified cartilage and quiescent osteoclasts. Despite the severity of the radiographic and microscopic findings, the patient was clinically well with normal growth and without evidence of bone marrow suppression or extramedullary hematopoiesis, although the risk for future fractures may have been increased.

In addition to osteopetrosis, there are other clinical forms and genetic variants that cause increased bone mineralization in children (see Table 20.11B). Pycnodysostosis (OMIM 265800) is clinically manifested by disproportionate short stature during infancy and childhood with macrocranium and open cranial sutures, high forehead, small facial features, proptosis, bluish sclerae, beaked and pointed nose, micrognathia, highly arched palate, retained primary teeth, short fingers with hypoplastic nails, narrow thorax, pectus excavatum, and kyphoscoliosis with lumbar lordosis.^{8,482} Radiologically, there is increased bone density that becomes progressively worse with age despite which susceptibility to fractures is increased, open fontanelles, impaction of permanent and supernumerary teeth, clavicles that are slim and hypoplastic bilaterally, partial or total absence of the ribs and hyoid bone, and acroosteolysis of the distal phalanges—a pathognomic characteristic of pycnodysostosis.^{482–484} Laboratory data and bone histology are usually normal, although there may be biochemical evidence of decreased osteoblastic and osteoclastic activity. The microscopic observation that an abundance of osteoclasts with ruffled borders surrounded by enlarged clear zones suggested that dissolution of bone mineral was normal but that degradation of matrix was abnormal in these patients. Indeed, there are large amounts of decalcified matrix both within the subosteoclastic lacunae and the osteoclasts.³⁰⁶ Pycnodysostosis is caused by biallelic loss-of-function mutations in *CTSK* encoding cathepsin K, the osteoclast's lysosomal cysteine protease that degrades organic matrix after the mineral phase of bone has been reabsorbed. Among the variations in *CTSK* identified in patients with pycnodysostosis have been: unipaternal isodisomy for chromosome 1 with paternal *CTSK* harboring an inactivating p.Ala277Val mutation, p.Leu9Pro substitution in the signal peptide of the precursor form of the enzyme protein preventing completion of its posttranslational processing, and p.Ter330Trp substitution permitting the addition of 19 aa to the carboxyl terminus of this enzymatic protein.

In contrast with diseases that increase bone mass by decreasing bone resorption are those disorders that primarily increase bone formation. An example of the latter is the familial, relatively benign form of autosomal dominant high bone mass (OMIM 601884) that is associated with a heterozygous gain-of-function mutation (p.Gly171Val) in *LRP5* already discussed. Dickkopf and sclerostin are proteins that bind to the extracellular domain of *LRP5* and internalize the receptor complex thereby blocking WNT signaling.³⁷³ Although generally benign, activating mutations of *LRP5* may also be associated with neurological complications, such as hearing loss, headaches, and pain in the extremities. In some families, heterozygous activating mutations (p.Ala242Thr) of *LRP5* and exuberant bone formation have been associated with autosomal dominant generalized endosteal osteosclerosis (van Buchem disease, type 2, OMIM 607636). Variants of *SOST* (encoding sclerostin) are associated with van Buchem disease type 1 (OMIM 239100) that has been attributed to a 52-kb deletion three prime (3') of *SOST* with resultant loss of a regulatory element of the gene; this disorder is characterized by markedly thickened cranium—resulting in loss of cranial nerve function, mandible, and long bones, and autosomal recessive sclerosteosis type 1 (OMIM 269500), a generalized, progressive disorder of skeletal overgrowth in association with syndactyly; involvement of the skull results in entrapment and functional impairment of cranial nerves, as well as increased intracranial pressure. Sclerosteosis type 2 (OMIM 614305) is associated with biallelic inactivating variants of *LRP4* (OMIM 604270) encoding low-density lipoprotein-related coreceptor 4 that prevent its interaction with sclerostin thereby disinhibiting sclerostin.⁴⁸⁵ Sclerosteosis type 1 is first manifested in childhood and is characterized by very thick peripheral and cranial

bones with calvarial overgrowth, leading to facial disfigurement, entrapment of cranial nerves II, VII, and VIII, increased intracranial pressure, and brainstem compression.^{8,373,458} Affected patients also have variable asymmetric cutaneous or bony syndactyly of the index and middle fingers and excessive somatic growth; they are extraordinarily resistant to fractures. Serum levels of alkaline phosphatase and procollagen type 1 N-terminal propeptide (P1NP) are elevated in these subjects while concentrations of sclerostin are low or unmeasurable.⁴⁵⁸ Sclerosteosis type 1 is caused by biallelic loss-of-function mutations in *SOST* encoding sclerostin, a 213 aa peptide secreted primarily by osteocytes embedded within bone. Physiologically, sclerostin inhibits WNT-mediated bone formation by binding to and internalizing LRP5, the coreceptor for WNT. When sclerostin activity is decreased, increased bone formation ensues. Sclerostin also increases osteoclastogenesis by increasing osteocytic synthesis of RANKL.⁴⁵⁸ In heterozygous carriers of inactivating mutations in *SOST*, bone mass is increased but not to pathological levels. Hyperostosis corticalis generalisata/van Buchem disease type 1 is an autosomal recessive disorder characterized by thickening and enlargement of the bones of the skull, mandible, ribs, and diaphyses of the long bones that result in increased bone density and encroachment upon cranial nerve passageways resulting in abnormalities of vision, hearing, and facial movement.^{8,458} However, patients with van Buchem disease type 1 do not have syndactyly or tall stature thus distinguishing it from sclerosteosis type 1. This disorder is also caused by biallelic loss of expression of *SOST* but as a result of 52-kb deletion of a regulatory element that is 35 kb downstream (3') of *SOST* rather than by a mutation within the exonic/intronic structure of *SOST* itself.⁴⁸⁶ Thus this disease is allelic to sclerosteosis; it differs clinically in that gigantism and hand abnormalities are present in patients with sclerosteosis type 1 but not in those with van Buchem disease type 1. The clinical manifestations of sclerosteosis type 2 are similar to those of type 1 but are pathogenetically related to biallelic loss-of-function mutations in *LRP4*.⁴⁸⁷ The inhibitory effect upon bone formation of the interaction of sclerostin and LRP5/LRP6 is facilitated by binding of sclerostin to LRP4. Thus inactivating mutations (p.Arg1170Trp, p.Trp1186Ser) in *LRP4* impede the interaction of sclerostin with LRP5/6 and thus the inhibitory effect of sclerostin upon osteoblastogenesis. Treatment of these disorders is primarily symptomatic.

Progressive diaphyseal dysplasia (Camurati-Englemann disease; OMIM 131300) is an autosomal dominant, cranial-peripheral hyperostotic disorder with variable expression that presents in children with problems, such as limping, waddling gait, and/or leg pain, fatigue, and nonprogressive muscular weakness. Radiographically, there is symmetrical cortical thickening (hyperostosis) because of increased periosteal and endosteal bone formation in the diaphyses of the long bones, axial skeletons, and skull, genu valgum, and scoliosis.^{488,489} Pathogenetically, this disorder is caused by missense activating mutations within the "latency associated peptide" domain of the precursor propeptide of *TGFβ1* (OMIM 190180). Normally, after posttranslational processing, two latency associated peptides are noncovalently linked to two mature TGFβ1 peptides to form a "latency complex." Gain-of-function mutations within the latency associated peptide domain of *TGFβ1* (particularly at codon 218—p.Arg218His, p.Arg218Cys—a mutational hotspot) impair this association resulting in premature activation of TGF-β1 and consequent stimulation of intramembranous bone formation and repression of bone resorption. TGF-β1 also inhibits myogenesis and adipogenesis. Because of the inhibitory effects of glucocorticoids on bone formation and their stimulatory effects on bone resorption, these agents have been useful in alleviating many of the clinical symptoms and radiological abnormalities in patients with progressive

diaphyseal dysplasia as have inhibitors of angiotensin receptor II (e.g., losartan).⁴⁸⁹ Other hereditary sclerosing bone dysplasias include osteopoikilosis (OMIM 166700)—nodules of bone overgrowth within pelvic and long bones, cranium, and/or ribs because of heterozygous inactivating mutations in *LEMD3* (OMIM 607844) encoding a nuclear membrane protein that modulates signal transduction by BMPs and TGFβ; osteopoikilosis is associated with hyperdense deformations of cortical bone—melorheostosis; and osteopathia striata (OMIM 300373) is an X-linked disorder caused by loss-of-function mutations in *AMER1* (OMIM 300647) encoding a protein that promotes the proteasomal degradation of β-catenin—its loss thus promotes transfer of β-catenin to the nucleus and extends the biological activity of the WNT/LRP5,6/β-catenin signal transduction system that promotes osteoblast maturation and function leading to endosteal hyperostosis.⁴⁸⁸ The hereditary and nonhereditary (e.g., intramedullary osteosclerosis) sclerosing bone dysplasias must be differentiated from acquired sclerosing diseases, such as sickle cell anemia and myelofibrosis.⁴⁸⁸

Heterotopic Bone Formation/Ectopic Calcification

Disorders of heterotopic ossification are those in which bone develops outside of the skeleton and within soft tissues (Table 20.12).⁴⁸ Dysregulation of the processes of differentiation and maturation enable precursor cells to develop as osteoblasts that then produce normal endochondral or membrane bone but in abnormal, extraskeletal sites. Sporadic heterotopic bone formation occurs in sites of severe wounds and burns, after spinal cord injuries, and in areas of pressure ulcers. Fibrodysplasia ossificans progressiva (OMIM 135100) is a disabling disorder of ectopic bone formation that may develop spontaneously or at sites of injury and lead to ankylosis of all major joints that severely limits mobility.⁴⁸ It is characterized by progressive ectopic ossification of skeletal muscle and connective tissue (fascia, tendons, ligaments) leading to immobility and fusion of the mandible, neck, spine, hips, and other joints and the development of a "second skeleton" that encases and imprisons the body. The disorder may be present at birth and is often manifest by 5 years of age. Fibrodysplasia ossificans progressiva is also associated with congenital abnormalities of the great toes (hallux valgus, malformed first metatarsal bones, monophalangism), characteristic facial features (long narrow face, small mandible, low set ears), deafness, scalp baldness, and mild developmental delay.⁴⁹⁰ Microscopically, there is normal but ectopic endochondral osteogenesis that occurs after a preceding inflammatory phase that develops either in the absence of trauma or following minor injury, such as an immunization; the pathological process proceeds through the phases of monocytic infiltration, muscle fiber degeneration, fibrous proliferation, angiogenesis, chondrogenesis, and osteogenesis.⁴⁸ Although usually sporadic because affected subjects rarely reproduce, fibrodysplasia ossificans progressiva can be transmitted as an autosomal dominant trait. This disease is primarily caused by a highly specific variant (c.617G>A transition leading to p.Arg206His) of *ACVR1* (encoding activin A Receptor, type-1, OMIM 102576); another described *ACVR1* mutation in patients with classical fibrodysplasia ossificans progressiva is c.744G>C transversion leading to p.Arg258-Ser.⁴⁸ Activins are members of the TGFβ superfamily that includes the BMPs together with the inhibitors and Mullerian duct inhibiting factor. *ACVR1* encodes a type I BMP receptor (activin receptor type Ia) that is expressed in chondrocytes and osteoblasts.⁴⁸ The p.Arg206His mutation in *ACVR1* resides at the junction of the receptor's cytoplasmic glycine-serine activation and tyrosine kinase domains and results in a constitutively active BMP type I receptor. Overactivation of the

TABLE 20.12 Genetic Causes of Heterotopic Bone Formation and Ectopic Calcification

Disease Type OMIM	Gene Chromosome OMIM	Clinical Manifestations	Pathophysiology
HETEROTOPIC BONE FORMATION			
Fibrodysplasia ossificans progressiva 135100	<i>ACVR1</i> : Activin A receptor, type I 2q24.1 102576	Progressive ossification of skeletal muscles, fascia, tendons, and ligaments	Specific monoallelic activating mutation (Arg206His) in the cytoplasmic domain of a type 1 BMP receptor increases extraskelatal chondrogenesis and osteogenesis
Progressive osseous heteroplasia 166350	<i>GNAS</i> : GNAS complex locus 20q13.32 139320	Ossification of dermis beginning in infancy followed by membranous bone formation within deep muscle and fascia in childhood	Monoallelic inactivating mutations in the paternally encoded G _{sα} subunit of G-protein enable subcutaneous bone formation; related to pseudopseudohypoparathyroidism
ECTOPIC CALCIFICATION			
Tumoral calcinosis, hyperphosphatemic, familial 211900	<i>GALNT3</i> : UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyl-transferase 3 2q24.3 601756	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic mutations in a cofactor required for synthesis of bioactive FGF23 and renal tubular excretion of phosphate
Tumoral calcinosis, hyperphosphatemic, familial 211900	<i>FGF23</i> : Fibroblast growth factor 23 12p13.32 605380	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic inactivating mutations in a factor that inhibits renal tubular resorption of phosphate
Tumoral calcinosis, hyperphosphatemic, familial 211900	<i>KL</i> : Klotho 13q13.1 604824	Progressive deposition of calcium phosphate crystals in periarticular region, soft tissue, and bone	Biallelic inactivating mutation in a cofactor enabling FGF23 to inhibit renal tubular resorption of phosphate
Tumoral calcinosis, normophosphatemic, familial 610455	<i>SAMD9</i> : Sterile alpha motif domain-containing protein 9 7q21.2 610456	Postinflammatory periarticular and peripheral calcified masses	Biallelic inactivating mutations in a protein that regulates cell division, motility, longevity, and inflammation

BMP, Bone morphogenetic protein; *GNAS*, guanosine diphosphate on the alpha subunit.

intracellular signal transduction pathways occurs when BMPs bind to the mutated *ACVR1* receptor. In addition, the p.Arg206His variant appears to change the signaling specificity of the *ACVR1* receptor to activin A, leading to phosphorylation of SMAD 1/5/8 rather than SMAD 2/3, triggering the formation of heterotopic endochondral bone.^{491,492} BMPs alone are able to stimulate complete endochondral osteogenesis in ectopic sites.

Patients with clinical variants of fibrodysplasia ossificans progressiva and *ACVR1* mutations other than p.Arg206His and p.Arg258Ser have been described; they have been classified as those with fibrodysplasia ossificans progressiva plus abnormalities of the brain, eye, and/or bone marrow, or those with fibrodysplasia ossificans progressiva variants—either without abnormalities of the great toes or less severe heterotopic osteogenesis; patients with mutations in codon 328 have varied in severity from classical to late-onset disease; patients with the p.Arg201Ile mutation in *ACVR1* have adult onset of extra-skeletal ossification and normal toes.^{48,493} Management of these patients is primarily symptomatic and palliative to the extent possible, although immunosuppression may diminish the intensity of the extraskelatal ossification.^{48,494}

Glucocorticoids have been used in the treatment of patients with fibrodysplasia ossificans progressiva, but efficacy is poor and side effects substantial. Inhibitors of mast cells and leukotrienes have also been used in an effort to suppress inflammation as have bisphosphonates; however, there are no conclusive clinical trials to support the use of these agents.⁴⁹⁵ Inasmuch as trauma can induce new bone formation in these patients, prevention of injury is the first principle of management. Even an intramuscular injection can provoke local painful ossification.

Avoidance of surgery is paramount as this insult can stimulate not only new bone growth locally but also at a distance. Although presently, there is no therapy for fibrodysplasia ossificans progressiva, treatments directed toward interrupting the primary pathophysiological pathway(s) of this disease, such as monoclonal antibody-mediated neutralization of the activin A receptor or interruption of the SMAD 1/5/8 signal transduction pathway (the mammalian target of rapamycin [mTOR]) may prove beneficial.⁴⁹⁵

POH (OMIM 166350) is characterized by multiple foci (trunk, extremities, or digits) of adipose tissue-associated dermal intramembranous bone formation (osteoma cutis) beginning in infancy in the absence of any local injury or inflammatory insult.^{42,48} Lesions may be asymptomatic or painful. Over time, heterotopic ossification borrows into skeletal muscle and deep connective tissue and may be incorporated into skeletal bone. POH is transmitted as an autosomal dominant trait and occurs in both boys and girls; it is caused by inactivating mutations of the *GNAS* allele usually inherited from the father.⁴⁹⁶ Paternal transmission of mutated *GNAS* is associated with significant intrauterine growth restriction and more severe clinical manifestations of POH than when the *GNAS* mutation is transmitted by the mother. Identical mutations in *GNAS* may be clinically manifested as either POH, PHP, PPHP, or primary osteoma cutis in different members of the same family (e.g., 1-bp del, 725C), all of which disorders are associated with subcutaneous (dermal) ossification. However, patients with POH do not have physical features of AHO nor are they hormone resistant. Thus diagnostic criteria for POH are: superficial osseous lesions, absence of features of AHO (PPHP), and normal responsiveness to PTH.⁴² Only

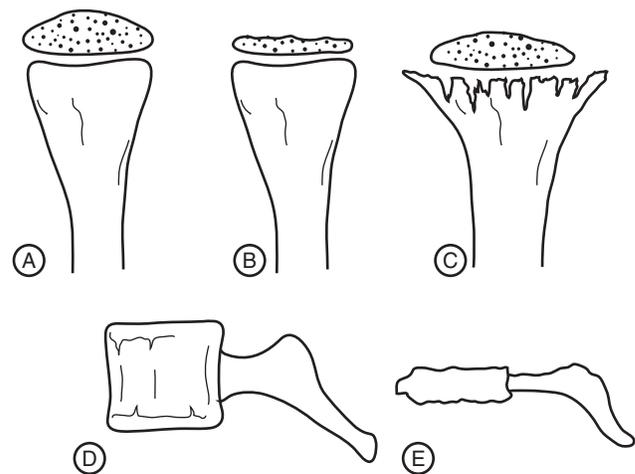
symptomatic treatment of patients with POH is currently available.

Familial tumoral calcinosis is characterized by deposition of basic calcium phosphate into periarticular spaces around the shoulder, elbow, wrist, hip, and knee joints and at times within bone itself that occurs spontaneously or after minimal but repetitive trauma to soft tissues.⁴⁹⁷ Variants of several genes (vide infra) lead to familial tumoral calcinosis with normophosphatemia or hyperphosphatemia—all with similar clinical manifestations. Familial tumoral calcinosis presents in childhood with recurrent bone pain, large, firm, nontender, subcutaneous masses; extensive periarticular masses that may compromise joint mobility; and vascular deposits of calcium phosphate. In some patients the ectopic calcifications may be confined to the eyelids; it is characterized radiographically by cortical hyperostosis, periosteal reaction, and mineral deposits around large joints, particularly hips and shoulders.⁴⁹⁸ Microscopically, there is a histiocytic response with formation of calcified bursa-like structures.⁴⁹⁹ Laboratory studies reveal either normophosphatemia or marked hyperphosphatemia and relative hypophosphaturia, the latter caused by increased renal tubular reabsorption of phosphate and inappropriately normal or elevated serum calcitriol levels, because despite hyperphosphatemia, PTH secretion is not increased and synthesis of calcitriol and intestinal calcium absorption persist. There are one normophosphatemic (OMIM 610455) and three hyperphosphatemic designated types (OMIM 211900, 617993, 617994) of familial tumoral calcinosis and also secondary forms of this disease. The disorder is caused by functional loss of FGF23 action and consequently unhindered renal tubular reabsorption of phosphate. The pathophysiology of this disorder is thus the mirror image of that associated with X-linked and autosomal dominant forms of hypophosphatemic rickets and tumor-induced osteomalacia in which there is excess FGF23 production and exaggerated activity, leading to hyperphosphaturia and consequent hypophosphatemia, rickets, and osteomalacia. Hyperphosphatemic familial tumoral calcinosis is genetically heterogeneous: type 1 (OMIM 211900) is related to inactivating variants of *GALNT3* (OMIM 601756), type 2 (OMIM 617993) to errors in *FGF23* (OMIM 605380), and type 3 (OMIM 617994) to mutations in *KL* (OMIM 604824). Most commonly present in patients with hyperphosphatemic familial tumor calcinosis are biallelic loss-of-function microdeletions, splice site, and missense or nonsense mutations in *GALNT3* (p.Arg162Stop, p.Thr272Lys, p.Cys574Gly, p.Gln592Stop).⁵⁰⁰ The product of *GALNT3* is a glycosyl transferase that initiates O-glycosylation in which N-acetylgalactosamine is the first sugar in the side chain, a step that is essential for secretion of intact, stable, and functional FGF23. Failure to O-glycosylate FGF23 at Thr178 in the Golgi apparatus permits its rapid intracellular cleavage between Arg179 and Ser180 to biologically inactive amino and carboxyl terminal fragments. Homozygous inactivating mutations in *FGF23* (p.Ser71Gly; p.Met96Thr, p.Ser129Phe) have been identified in patients with type familial tumoral calcinosis; in the absence of FGF23, renal tubular resorption of filtered phosphate is unopposed.⁵⁰¹ Serum concentrations of intact FGF23 are low or nondetectable while carboxyl terminal FGF23 levels are elevated in patients with familial tumoral calcinosis because of mutations in either *GALNT3* or *FGF23*. Therapy with an oral phosphate binder and the carbonic anhydrase inhibitor acetazolamide has resulted in hyperphosphaturia and reabsorption of ectopic calcifications without change in serum phosphate or calcium concentrations. The hyperostosis hyperphosphatemia syndrome (OMIM 610233) is a clinical variant of familial tumoral calcinosis and is also caused by mutations in *FGF23* or *GALNT3*; symptoms and signs may precede development of the more typical phenotype of familial tumoral calcinosis.⁵⁰⁰ Hyperphosphatemic familial tumoral calcinosis type 3 has been attributed to a homozygous loss-of-function mutation in *KL* (p.His193Arg)

encoding α -klotho, a cofactor necessary for the interaction of FGF23 with its receptor in the renal tubule.⁵⁰² Normophosphatemic familial tumoral calcinosis is a form of dystrophic calcification as inflammatory lesions precede ectopic calcification; it is associated with inactivating mutations (p.Arg344Stop, p.Lys1495Glu) in *SAMD9* (encoding Sterile alpha motif domain-containing protein 9, OMIM 610456), a 1589 aa protein that regulates cell division, motility, and longevity.⁵⁰³ *SAMD9* is generated in response to TNF α and IFN- γ and regulates expression of *EGR1* (OMIM 128990) encoding a transcription factor that controls expression of *TGFB1* and is involved with cell migration, proliferation, mobility, and survival.⁵⁰⁴ Scleroderma, chronic renal disease with secondary hyperparathyroidism, primary hyperparathyroidism, hypervitaminosis D, after cell lysis induced by cancer chemotherapy, subcutaneous fat necrosis, dermatomyositis, and atherosclerosis may be secondary causes of massive subcutaneous calcifications with comparable clinical findings. Treatment must be individualized and may include phosphate depletion by dietary deprivation, binding of intestinal phosphate, and induction of phosphaturia (acetazolamide), as well as surgical removal of particularly compromising masses.

OSTEOCHONDRODYSPLASIAS

The osteochondrodysplasias are pathogenetically heterogeneous malformations of cartilage and bone that are grouped according to genetic variations (e.g., *FGFR3* chondrodysplasia group; Type 2 collagen—*COL2A1*—group) or clinical characteristics, such as those involving long bone growth (epiphyseal, metaphyseal and/or diaphyseal dysplasias), long bones and vertebrae (spondyloepiphyseal and/or spondyloepimetaphyseal dysplasias), skull (cleidocranial dysplasia), hands and feet (the ectrodactylies), and other skeletal components (Fig. 20.16).⁵⁰⁵ Thus multiple genetic variations (e.g., *FGF23*, *COL1A1*) may adversely influence bone development/



Involvement	Disease Category
A+D	Normal
B+D	Epiphyseal dysplasia
C+D	Metaphyseal dysplasia
B+E	Spondyloepiphyseal dysplasia
C+E	Spondylometaphyseal dysplasia
B+C+E	Spondyloepimetaphyseal dysplasia

Fig. 20.16 Anatomic characterization of osteochondrodysplasias. (From Alanay Y, Rimoin DL. Chondrodysplasias. In: Rosen CJ (Ed.). *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* (7th ed.). Washington, DC: American Society of Bone and Mineral Metabolism; 2008:428–429.)

formation or mineralization; mutations in one gene may give rise to several different clinically defined disorders together with disorders characterized by clinical and radiographic findings that may be attributable to variations in many different genes (e.g., the acromesomelic dysplasias). Abnormal skeletal phenotypes have been classified into 42 groups based on pathogenetic variations of 436 genes and/or clinical and radiographic manifestations of the disorders.^{329,505} Groups 1 to 8 are disorders attributed to variations in a single gene (e.g., group 1 *FGFR3*; group 2 *COL2A1*; group 3 *COL11A1*; group 4 *DTDST*; group 5 *PLC*; group 6 *AGC1*; group 7 *FLNA/FLNB*; group 8 *TRPV4*). Groups 9 through 42 rely on clinical, radiographic, and genetic findings for classification; within each group variations in different genes may give rise to the same or similar osseous abnormality(ies) (e.g., group 10—multiple epiphyseal dysplasias *COMP*, *COL9A2*, *MATN3*, *COL9A1*, *SMARCAL1*; group 13—Spondyloepi-(meta)-physeal dysplasias *MATN3*, *RAB33B*, *SEDL*, *DYM*; group 17—mesomelic/rhizo-mesomelic dysplasias *SHOX*, *GPC6*, *RDR2*, *FZD2*, *WNT5A*). Group 23 encompasses variants of osteopetrosis, group 25 variants of osteogenesis imperfecta,

group 27 the lysosomal disorders, and group 30 the overgrowth syndromes. Disorders of skeletal development and function are of interest not only because of the diagnostic and therapeutic clinical challenges they present, but also because they have identified many basic physiological factors that normally regulate cartilage and bone development and function. The application of genome-wide association studies to the classification of skeletal disorders has added significant insight into the plethora of genes and intracellular signaling pathways that influence skeletal differentiation, morphology, and function.³²⁹

Table 20.13 describes some of the more commonly encountered genetic variations associated with osteochondrodystrophies. Achondroplasia (OMIM 100800), the most common of the human chondrodystrophies (1/15,000–1/40,000 live births) and its related osteochondrodystrophies (hypochondroplasia, thanatophoric dysplasia—types I and II, syndrome of severe achondroplasia-developmental delay-acanthosis nigricans [SADDAN] syndrome), are caused by gain-of-function mutations in *FGFR3* (OMIM 134934), the gene encoding fibroblast growth receptor 3. *FGFR3* is a transmembrane protein

TABLE 20.13 Genetic Variants Associated With Osteochondrodystrophies (Selected)

Gene Chromosome OMIM	Pathophysiology	Clinical Disorder (OMIM)
<i>TRIP11</i> : Thyroid hormone interactor 11 14q31-q32 604505	Coactivator of the nuclear triiodothyronine receptor; interacts with microtubules and Golgi apparatus	Achondrogenesis, type IA (200600), AR
COLLAGENOPATHIES		
<i>COL2A1</i> : Collagen, type II, alpha 1 12q13.11 120140	Subunit of collagen type II, the major collagen of cartilage comprised of three 3-alpha 1(II) chains	Achondrogenesis, type II/ Hypochondrogenesis (200610), AD Spondyloepiphyseal dysplasia congenita (183900), AD Spondyloepimetaphyseal dysplasia (184250), AD
<i>COL9A1</i> : Collagen, type IX, alpha 1 6q13 120210	Encodes a collagen that is a component of hyaline cartilage	Multiple epiphyseal dysplasia, type 6 (614135), AD Stickler syndrome type 4 (614134), AR
<i>COL10A1</i> : Collagen, type X, alpha 1 6p22.1 120110	Component of cartilage expressed at late stages of endochondral bone formation	Metaphyseal chondrodysplasia, Schmid type (156500), AD
<i>COL11A1</i> : Collagen, type XI, alpha 1 1p21.1 120280	Subunit of collagen type XI composed of two alpha 1 subunits and a modified <i>COL2A1</i> subunit; important for fibrillogenesis	Stickler syndrome type 2 (604841), AD Marshall syndrome (154780), AD
<i>FLNB</i> : Filamin B 3p14.3 603381	Cytoplasmic actin-binding protein enabling formation of and communication with the cytoskeleton; influences vertebral segmentation, endochondral ossification, joint formation	Atelosteogenesis I (108720), AD Atelosteogenesis III (108721), AD
FIBROBLAST GROWTH FACTOR RECEPTORS		
<i>FGFR1</i> : Fibroblast growth factor receptor 1 8p11.22 136350	Transmembrane tyrosine kinase receptor for FGFs	Pfeiffer syndrome (101600), AD
<i>FGFR2</i> : Fibroblast growth factor receptor 2 10q26.13 176943	Transmembrane tyrosine kinase receptor for FGFs	Apert syndrome (101200), AD Crouzon syndrome (123500), AD Jackson-Weiss syndrome (123150), AD Antley-Bixler syndrome with normal steroidogenesis (207410), AD
<i>FGFR3</i> : Fibroblast growth factor receptor 3 4p16.3 134934	Transmembrane tyrosine kinase receptor for FGFs	Achondroplasia (100800), AD Hypochondroplasia (146100), AD Thanatophoric dysplasia types I (187600) and II (187601), AD
SULFATION DISORDERS		
<i>SLC26A2</i> - Solute carrier family 26 (sulfate transporter) 5q32 606718	Also termed <i>DTDST</i> ; encodes sulfate transporter essential for normal collagen synthesis	Achondrogenesis, type IB (600972), AR Atelosteogenesis II (256050), AR Diastrophic dysplasia (222600), AR Multiple epiphyseal dysplasia, type 4 (226900), AR

TABLE 20.13 Genetic Variants Associated With Osteochondrodysostrophies (Selected)—cont'd

Gene Chromosome OMIM	Pathophysiology	Clinical Disorder (OMIM)
<i>HSPG2</i> : Heparan sulfate proteoglycan of basement membrane 1p36.12 142461	Also termed <i>perlecan</i> , a heparan sulfate proteoglycan; coreceptor for FGFR2; stabilizes basement membranes and regulates their permeability	Schwartz-Jampel type 1 myotonic chondrodysplasia (255800), AR
<i>PAPSS2</i> : 3-Prime-phosphoadenosine 5-prime-phosphosulfate synthase 2 10q23.2-23.3 603005	Enzyme that synthesizes the sulfate donor (3'-phosphoadenosine 5'-phosphosulfate) from ATP and sulfate; cofactor for adrenocortical sulfotransferase	Spondyloepimetaphyseal dysplasia (Brachyolmia type 4) (612847), AR
<i>ARSE</i> : Aryl sulfatase E Xp22.33 300180	Enzyme that removes a sulfate group from its protein	Chondrodysplasia punctata type 1 (302950), X-linked recessive
<i>PTPN11</i> : Protein tyrosine phosphatase, nonreceptor, type 11 12q24.1 176876	Encodes SHP2: a nonreceptor protein tyrosine phosphatase that acts upstream of RAS	Monoallelic loss-of-function mutations lead to metachondromatosis (156250), AD Noonan syndrome with multiple lentigenes: monoallelic gain-of-function mutations (163950), AD
OTHERS		
<i>COMP</i> : Cartilage oligomeric matrix protein 19p13.1 600310	Chondrocyte protein that binds calcium and collagen types I, II, and IX	Pseudoachondroplasia (177170), AD Multiple epiphyseal dysplasia (132400), AD
<i>TRPV4</i> : Transient receptor potential cation channel, subfamily V, member 4 12q24.11 605427	Cation channel that mediates calcium influx	Metatropic dysplasia (156530), AD Spondyloepimetaphyseal dysplasia: Maroteaux (184095), AD
<i>SHOX</i> : Short stature homeobox Xp22.33 312865	Homeobox gene transcription factor located on the pseudoautosomal region of Yp	Leri-Weill dyschondrosteosis (127300), X-linked dominant Langer dysplasia (249700), biallelic/X and Y
<i>SOX9</i> : SRY-box 9 17q24.3 608160	Transcription factor essential for chondrogenesis and testicular differentiation	Campomelic dysplasia (114290), Monoallelic because of haploinsufficiency
<i>RUNX2</i> : Runt-related transcription factor 2 6p21 600211	Osteoblast specific transcription factor	Cleidocranial dysostosis (119600), AD
<i>PTH1R</i> : PTH receptor 1 Xp22.33 168468	GPCR recognizing PTH and PTHrP with equal affinity	Inactivating mutations lead to hypocalcemia and Blomstrand osteochondrodysplasia (215045), AR Eiken syndrome (600002), AR; activating mutations result in hypercalcemia and Murk-Jansen metaphyseal chondrodysplasia (156400), AD
<i>PRKAR1A</i> : Protein kinase, cAMP-dependent regulatory, type 1, alpha 17q24.2 188830	Component of PKA response to cyclic AMP that leads to cascade of intracellular signal transduction signals in response to G α s that regulate cell division, differentiation, metabolism, apoptosis	Gain-of-function mutation leads to acrodysostosis (101800) and peripheral resistance to the biological effects of PTHrP, PTH, and TSH (de novo)
<i>POR</i> : Cytochrome P450 oxidoreductase 7q11.2 124015	Flavoprotein cofactor that donates electrons to microsomal 17 α -hydroxylase, 21-hydroxylase, and aromatase	Inactivating mutations lead to congenital adrenal hyperplasia usually with genital ambiguity in both 46XX and 46XY subjects (613571), AR; at times in association with skeletal abnormalities (Antley-Bixler syndrome: craniosynostosis, midface hypoplasia, choanal stenosis, femoral bowing, radioulnar synostosis, 201750, AR)
<i>RMRP</i> : Mitochondrial RNA-processing endoribonuclease, RNA component of 9p13.3 157660	Untranslated RNA subunit of the mitochondrial RNA-processing endoribonuclease: RNase MRP	Dependent on the severity of the biallelic loss-of-function mutation(s) the spectrum of anauxetic dysplasia (607095), cartilage hair hypoplasia (250250) emerges
<i>INPPL1</i> : Inositol polyphosphate phosphatase like-1 11q13.4 600829	Enzyme that hydrolyzes inositol-1,4,5-trisphosphate to inositol-4,5-bisphosphate: transducer of intracellular signaling	Inactivating mutations result in opsismodysplasia (258480); associated with increased skeletal synthesis of FGF23, AR

AD, Autosomal dominant; AR, autosomal recessive, ATP, adenosine triphosphate; cyclic AMP, cyclic adenosine monophosphate; GPCR, G-protein-coupled receptor; PKA, protein kinase A; PTH, parathyroid hormone; PTHrP, parathyroid hormone related protein; RNA, ribonucleic acid; TSH, thyroid stimulating hormone.

(Modified from Bonafe L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. *Am J Med Genet.* 2015;167A:2869–2892.)

with three immunoglobulin domains in the extracellular region of the receptor and two tyrosine kinase domains in its intracellular portion; the p.Gly380Arg variant within the transmembrane domain of *FGFR3* is present in 98% of achondroplastic patients. The p.Gly380Arg variant of *FGFR3* alters the conformation of the gene product enabling ligand-independent autophosphorylation of tyrosine residues 647 and 648 within its cytoplasmic domain that is then able to propagate signals through MAPK and signal transducer and activator of transcription (STAT) pathways resulting in inhibition of chondrocyte mitosis, matrix synthesis, and terminal (hypertrophic) differentiation.⁵⁰⁶ Experimentally, in mice expressing the gain-of-function mutation in *Fgfr3* associated with achondroplasia, there is enhanced bone development but decreased bone mass and increased osteoclastic activity.⁵⁰⁷ Thus *FGFR3* normally functions as a negative regulator of cartilage and bone formation. As a single gene disorder, it is possible to identify a mutation in *FGFR3* in an affected fetus by analysis of fetal cell-free DNA in maternal plasma, if the mother is unaffected. Achondroplasia is also associated with the p.Gly375Cys variant in *FGFR3*. These disorders are transmitted as autosomal dominant traits but with a high rate of spontaneous mutations (primarily of the paternal *FGFR3* allele). Clinically, achondroplasia is manifested by shortening of the proximal limbs and trunk, macrocranium, frontal bossing, and depressed nasal bridge, and complicated by increased risk for cervical cord compression and spinal stenosis.⁵⁰⁸ The birth to normal parents of a second child with achondroplasia may reflect paternal germinal mosaicism for the p/Gly380Arg mutation in *FGFR3*.⁵⁰⁹ Hypochondroplasia is related to the p.Asn540Lys (60% of patients) variant present within the proximal tyrosine kinase segment of the intracellular domain of *FGFR3*; it is a clinically less severe disorder with usually modest short limbed (rhizomelic) short stature that presents in midchildhood. Hypochondroplasia has also been associated with mutations within the distal (p.Lys650Gln) tyrosine kinase domain, as well in the immunoglobulin domains of the extracellular region (p.Ser84Leu, p.Arg200Cys) and transmembrane domain (p.Val381Glu) of *FGFR3*. Thanatophoric dysplasia type I (OMIM 187600) is related to mutations (p.Arg248Cys, p.Gly370Cys) in the extracellular ligand-binding region next to the transmembrane domain of *FGFR3* and to substitutions at the normally terminal codon 807—p.Ter807Gly/Cys/Arg—all of which lead to addition of 141 aa to the carboxyl terminus of the *FGFR3* protein. Thanatophoric dysplasia type I is manifested by severe bony malformations that include short curved femurs, malformation of the skull, and short ribs, the latter leading to respiratory insufficiency and early death; it is the consequence of the p.Arg248Cys variant in the extracellular domain of *FGFR3*. Thanatophoric dysplasia type II (OMIM 187601) is associated with a mutation (p.Lys650Glu) in the distal tyrosine kinase domain of the intracellular segment of *FGFR3* and is characterized by a cloverleaf skull and short straight femurs. The clinical phenotype of the SADDAN syndrome (OMIM 616482) is also associated with the p.Lys650Met variant of *FGFR3* whose clinical manifestations are intermediate in severity between those of thanatophoric dysplasia and achondroplasia. It is associated with significant rhizomelic short stature, acanthosis nigricans, and mild developmental challenges; acanthosis nigricans may also develop in patients with classical achondroplasia and in those with mutations in *FGFR2*.⁵¹⁰ It is noteworthy that different mutations at p.Lys650 result in three distinct phenotypes—hypochondroplasia, thanatophoric dysplasia type II, and SADDAN syndrome. *FGFR3* activating mutations have also been encountered in patients with Muenke coronal craniosynostosis (OMIM 602849; p.Pro250Arg) and Crouzon syndrome (OMIM 123500)—evidence of the genetic heterogeneity of these clinical syndromes. A loss-of-function mutation (p.Arg621His within

the distal tyrosine kinase domain) in *FGFR3* has been identified in a family whose members display the phenotype of tall stature, camptodactyly, and hearing loss.⁵¹¹ Activating mutations in *FGFR1* and *FGFR2* have been associated with chondrodysplasias complicated by premature craniosynostosis (Pfeiffer, Apert, Crouzon, Jackson-White, Antley-Bixler, and Beare-Stevenson cutis gyrata syndromes). Interestingly, mutations in *FGFR1* have also been associated with hypogonadotropic hypogonadism (OMIM 147950) and anosmia, as *FGFR1* is an essential factor for neuronal migration and angiogenesis.⁵¹² Mutations in *FGFR4* have not been associated with osteochondrodysplasias to date.

Pseudoachondroplasia (OMIM 177170) is an osteochondrodystrophy that does not become manifest until after the first year of life as the fetus and infant with this disorder appear normal.⁵¹³ Growth retardation becomes apparent in the second year of life; stature becomes progressively compromised during childhood in association with genu varum or valgus, brachydactyly, and joint laxity and severe bone and joint pain that impairs movement. Radiographs reveal inhibition of long bone growth; metaphyseal irregularities; and small, undercrossed epiphyses. Pseudoachondroplasia is the consequence of monoallelic inactivating variants in cartilage oligomeric matrix protein (*COMP*, OMIM 600310). *COMP* is a secreted glycoprotein produced by chondrocytes that has four domains—one of which is a calcium binding domain; variations in the base sequences of this domain lead to pseudoachondroplasia because of disrupted protein folding and ensuing chondrocyte malfunction while also exerting a dominant negative effect on the intact product of the normal *COMP* allele and the retention of *COMP* protein in the endoplasmic reticulum of the chondrocyte. Normally secreted *COMP* contributes to collagen strength by cross-linking several different matrix collagens (types I, II, IX, XII, XIV) and its loss impairs this property. Mutant *COMP* retained within the endoplasmic reticulum of the chondrocyte leads to intracellular inflammation and then to chondrocyte death. Experimentally in mice, the inflammatory response to retained, mutated *COMP* can be ameliorated by administration of antiinflammatory agents, such as acetylsalicylic acid with restoration of the animals' growth.⁵¹³ Whether these experimental observations can be duplicated in children with pseudoachondroplasia is as yet unknown.

In addition to the four genetic types (I–IV) of osteogenesis imperfecta attributable to variants of *COL1A1* and *COL1A2*, defects in the formation of several other types of collagen caused by mutations in *COL2A1*, *COL9A1*, *COL9A2*, *COL10A1*, *COL11A1*, and *COL11A2* result in a large number of skeletal malformations depending on the site and developmental timing of the synthetic error (see Table 20.13). Mutations in several different collagen-encoding genes (*COL2A1*, *COL9A1*, *COL9A2*, and *COL11A1*) result in variants of Stickler syndrome (OMIM 609508, 604841, 614134, 614284) characterized by abnormalities of the skeleton (epiphyseal dysplasia), face (micrognathia, cleft palate), vision (myopia, retinal detachment), and impaired hearing that may be transmitted as autosomal dominant or recessive traits. The ectrodactylies or split hand/foot malformations are characterized by absence of the second and third metacarpals/metatarsals and corresponding fingers/toes with residual first and fused fourth and fifth digits (resulting in a “claw-like” appearance of the distal limb) that may be caused by structural abnormalities (rearrangement, deletion, duplication) of chromosome 7q21.3 in which segment reside several genes that influence embryogenesis of the digits, as well as to abnormalities of other chromosomes (Xq26, 2q31, 10q24).

Atelosteogenesis type I (OMIM 108720) is a lethal skeletal disorder characterized by absent, short, or distally tapered femora and humeri, short bowed tibiae and ulnae, absent fibulae, vertebral hypoplasia, and subnormal metacarpal ossification;

this disorder has several variants; it is the result of monoallelic loss-of-function mutations in *FLNB* (OMIM 603381) encoding a cytoplasmic protein that binds actin, thereby enabling actin to form the cytoskeleton, and also facilitates intracellular communication between the cell membrane and its cytoskeleton. Abnormalities of sulfate transport, collagen matrix protein sulfation, and sulfatase activity result in several chondrodysplasias. *SLC26A2* (OMIM 606718) encodes a sulfate transporter that is mutated in patients with diastrophic dysplasia (DTD, OMIM 222600) and achondrogenesis type IB (OMIM 600972). Spondyloepimetaphyseal dysplasia/brachyolmia type 4 is caused by biallelic loss-of-function mutations (p.Thr48Arg, p.Ser438Ter) in the *PAPSS2* (OMIM 603005) encoding 3'-phosphoadenosine-5'-phosphosulfate synthase 2, an enzyme with dual activities; it catalyzes both the synthesis of adenosine 5'-phosphosulfate and its phosphorylation to 3'-phosphoadenosine 5'-phosphosulfate, the universal sulfate donor necessary for sulfation of cartilage and bone matrix proteins. Clinical manifestations of this disorder include short limbs, kyphoscoliosis, brachydactyly, and enlarged knee joints. Inactivating mutations in *ARSE* (encoding arylsulfatase E, OMIM 300180) lead to X-linked recessive brachytelephalangic chondrodysplasia punctata type 1.⁵¹⁴ This disorder is marked clinically by compromised stature attributable to rhizomelic shortening of the limbs in association with stippling of the epiphyses, craniofacial defects, atrophic and pigmented ichthyosiform skin lesions, alopecia, cataracts, and developmental delay.

Loss-of-function mutations (insertions, missense, nonsense) in *SOX9* (OMIM 608160), a transcription factor that is expressed in both developing chondrocytes where it is coexpressed with *COL2A1* and in the genital ridges during gonadal differentiation, cause both campomelic dysplasia (OMIM 114290) and male-to-female sex reversal in 75% of affected 46XY subjects. Inasmuch as *SOX9* is essential for normal expression and function of *COL2A1* during chondrogenesis, its absence leads to impaired formation of cartilage matrix and malformation of bones embryologically derived from endochondral bone. In the developing bipotential gonad of the 46XY fetus, the expression of *SOX9* is governed by *SRY* (OMIM 480000); *SOX9* expression is also essential for normal differentiation and development of Sertoli cells and other components of the testis. Clinically, campomelic dysplasia is characterized by prenatal onset of bowing of tubular bones, hypoplastic scapulae, 11 ribs, cleft palate, and micrognathia leading often to neonatal death, as well as failure of masculinization of the 46XY conceptus.

The X- and Y-linked pseudoautosomal gene *SHOX* (OMIM 312865) encodes two nuclear proteins—SHOXa with 292 aa and SHOXb with 225 aa. *SHOX* is a transcription factor expressed in late proliferative, prehypertrophic, and hypertrophic chondrocytes one of whose target genes is *NPPB* encoding natriuretic peptide precursor B (OMIM 600295) that is also expressed in late proliferative, prehypertrophic, and hypertrophic chondrocytes.⁵¹⁵ Absence of one *SHOX* functional gene is the proximate cause of the short stature of girls with Turner syndrome and some children with "idiopathic" short stature. Heterozygous microdeletions and intragenic inactivating mutations (p.Leu132Val, p.Ala170Pro, p.Arg195Stop) of *SHOX* resulting in haploinsufficiency are present in patients with Leri-Weill dyschondrosteosis (OMIM 127300) typified by mesomelic limb shortening and growth retardation, Madelung deformity of the wrist, bowing of the radius, and ulnar dislocation. The Leri-Weill phenotype has been recorded in patients with intact *SHOX* but with microdeletions of downstream segments of the X chromosome pseudoautosomal region, implying the presence of modifying genes in this region. Biallelic loss (X and Y chromosomes) of *SHOX* leads to Langer mesomelic dysplasia (OMIM 249700) typified by severe hypoplasia of the ulna and fibula and a thickened, curved radius and tibia.

Both gain- and loss-of-function variants of *PTH1R* (OMIM 168468) lead to abnormalities of bone formation and growth. Blomstrand chondrodysplasia (OMIM 215045) is an autosomal recessive disorder lethal in utero that may be identified in the fetus with short, extremely dense long bones and markedly advanced skeletal maturation, as well as somatic anomalies, such as aortic coarctation and facial anomalies. Pathologically, epiphyseal cartilage is reduced, and there are irregular columns and erratic distribution of chondrocytes within matrix. The abnormality is the result of inactivating mutations (deletions, missense mutations—p.Pro132Leu, p.Arg383Gln) of the gene encoding the PTH/PTHrP receptor. The Eiken syndrome (OMIM 600002) of epiphyseal dysplasia with markedly delayed ossification of the primary ossification centers of tubular bones, epiphyses, and pelvic symphysis is also caused by a biallelic loss-of-function mutations (p.Glu35Lys, p.Arg485Stop) in *PTH1R*.³⁸ Patients with Murk-Jansen metaphyseal chondrodysplasia (OMIM 156400) have short bowed limbs, clinodactyly, micrognathia, and deformities of the spine and pelvis, but survive to adulthood where the average adult height is 125 cm and child-bearing is possible. Characteristically, these patients have hypercalcemia, hypophosphatemia, and low or undetectable serum levels of both PTH or PTHrP, the consequences of unregulated activity of the PTH receptor caused by monoallelic activating mutations (p.His223Arg, p.Thr410Pro) of *PTH1R*. These two disorders reflect the altered functional effects of PTHrP/PTH acting through *PTH1R* in developing cartilage where these ligands normally slow chondrocyte differentiation and decrease the rate of chondrocyte apoptosis thus prolonging chondrocyte proliferation and enhancing long bone growth.

Anauxetic dysplasia (OMIM 607095) is a spondylometaphyseal dysplasia transmitted as an autosomal recessive disorder characterized by intrauterine growth retardation (birth length <40 cm), severely compromised adult height (<85 cm), hypodontia, and mild mental retardation. All bones are malformed; there are few chondrocytes in the cartilage growth plates. It is caused by loss-of-function mutations (insertions) in *RMRP* (OMIM 157660), a gene that encodes the untranslated RNA subunit of a mitochondrial RNA-processing endoribonuclease.⁵¹⁶ This enzyme is involved in: (1) the assembly of ribosomes—the structural units in which protein synthesis take place, (2) the generation of RNA primers for replication of mitochondrial DNA, and (3) the regulation of the cyclin-dependent cell cycle. The mutations that result in anauxetic dysplasia impair ribosome assembly and protein synthesis. Inactivating mutations (duplications, insertions) in *RMRP* that modestly impair both ribosome assembly and regulation of the cell cycle are present in patients with cartilage hair hypoplasia (OMIM 250250) and metaphyseal dysplasia without hypotrichosis (OMIM 250460). Thus cartilage hair hypoplasia and anauxetic dysplasia represent different manifestations and severity of inactivation of *RMRP*.

Errors in the biosynthesis of cholesterol have been associated with a number of disorders that adversely affect bone development, as well as many other systems (Table 20.14; Fig. 20.17).⁵¹⁷ Cholesterol is a constituent of the cell's plasma membrane, as well as the membranes of intracellular organelles; it covalently binds to the amino terminal and is essential for the function of Indian hedgehog, Sonic hedgehog, and Desert hedgehog, inductive factors necessary for normal development of cartilage and bone, brain, and testes, respectively. It is a precursor of steroids and bile acids. It is likely that lack of cholesterol exerts its teratological effects by impairing membrane function and/or the intracellular signaling response(s) to normal stimuli. In addition, accumulation of precursors of cholesterol may be toxic to the developing fetus. In patients with the Smith-Lemli-Opitz syndrome (OMIM 270400), inactivating mutations of the microsomal enzyme Δ^7 -dehydrocholesterol reductase (*DHCR7*; OMIM 602858) impair the final step in the synthetic pathway of cholesterol from 7-dehydrocholesterol and lead to intrauterine and postnatal growth retardation, short limbs,

TABLE 20.14 Osteochondrodysostrophies Attributable to Genetic Variants of Proteins Essential for Cholesterol Synthesis

Gene Chromosome OMIM	Pathophysiology	Clinical Disorder (OMIM)
<i>DHCR7</i> : 7-Dehydrocholesterol reductase 11q12-q13 602858	3 β -Hydroxysteroid Δ^7 reductase converts 7-dehydrocholesterol to cholesterol	Smith-Lemli-Opitz syndrome (270400), AR
<i>DHCR24</i> : 24-Dehydrocholesterol reductase 1p32.3 606418	3 β -Hydroxysteroid Δ^{24} reductase converts desmosterol to cholesterol	Desmosterolosis (602398), AR
<i>SC5DL</i> : Sterol C5-desaturase-like 11q23.3 602286	Microsomal enzyme (3 β -hydroxysteroid- Δ 5-desaturase) that converts lathosterol to 7-dehydrocholesterol, precursor of cholesterol and cholecalciferol (vitamin D)	Lathosterolosis (607330), AR
<i>EBP</i> : Emopamil-binding protein Xp11.23 300205	Dual function protein: binding protein; 3 β -Hydroxysteroid Δ^7 , Δ^8 isomerase converts cholest-8(9)-en-3 β -ol to lathosterol	Chondrodysplasia punctata type 2 (302960), X-linked dominant
<i>NSDHL</i> : NAD(P)H steroid dehydrogenase-like protein Xq28 300275	3 β -Hydroxysteroid C4 demethylase complex converts 4,4-dimethylcholest-8(9)-en-3 β -ol to cholest 8(9)-en-3 β -ol; other members of complex are <i>SC4MOL</i> (607545) and <i>HSD17B7</i> (606756)	CHILD (congenital hemidysplasia, ichthyosiform erythroderma, limbs defects) syndrome (308050), X-linked dominant
<i>LBR</i> : Lamin B receptor 1q42.12 600024	Dual function protein: promotes heterochromatin binding to inner nuclear membrane; 3 β -hydroxysteroid Δ^{14} reductase important for synthesis of cholesterol	HEM (hydrops-ectopic calcification-"moth-eaten" or Greenberg) dysplasia (215140), AR
<i>POR</i> : Cytochrome P450 oxidoreductase 1q11.2 124015	Flavoprotein electron donor to all P450 enzymes including P450c17, P450c21, P450arom	Antley-Bixler syndrome (ABS) with genital anomalies and disordered steroidogenesis, (201750), AR (ABS with exclusively skeletal anomalies [207410] because of mutation in <i>FGFR2</i> , AD)

AD, Autosomal dominant; AR, autosomal recessive.

(Modified from Forbes FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res.* 2011;52:6–34.)

syndactyly and polydactyly, characteristic facial features (blepharoptosis, anteverted nares, broad alveolar ridges, cleft palate), congenital malformations of the heart and central nervous system, microcephaly, incomplete virilization of male external genitalia, hypoplastic thumbs, developmental delay, autism, and compromised adrenocortical function, a malformation syndrome with an incidence of 1/15000 to 1/40000 births.⁵¹⁸ Serum concentrations of cholesterol are depressed whereas those of 7- and 8-dehydrocholesterol are elevated. The serum 7-dehydrocholesterol and 8-dehydrocholesterol concentrations are negatively related to the clinical severity and intellectual attainment of subjects with the Smith-Lemli-Opitz phenotype.⁵¹⁹

Desmosterolosis (OMIM 602398) is an autosomal recessive disorder with diverse clinical manifestations that include impaired growth, osteosclerosis, shortened limbs, macro- or microcephaly, cleft palate, micrognathia, thick alveolar ridges, developmental delay, and spasticity. Cranial magnetic resonance imaging reveals little white matter and a thinned to almost complete agenesis of the corpus callosum. Desmosterolosis is caused by biallelic inactivating mutations of the gene (*DHCR24*, OMIM 606418) encoding 3 β -hydroxysteroid- Δ^{24} reductase, an enzyme that converts desmosterol to cholesterol.^{517,520,521} Lathosterolosis (OMIM 607330) is characterized by microcephaly, bitemporal narrowing of the skull, cataracts, anteverted nostrils, and other physical anomalies recorded in patients with the Smith-Lemli-Opitz syndrome.⁵¹⁷ It is the result of loss-of-function mutations in the gene (*SC5DL*, OMIM 602286) encoding 3 β -hydroxysteroid- Δ 5-desaturase, an enzyme that converts lathosterol to 7-dehydrocholesterol. Chondrodysplasia punctata 2 (OMIM 302960) is an X-linked dominant disorder (Conradi-Hunermann-Happle syndrome) that is often lethal in the affected male. It is characterized clinically in the affected female by short stature with asymmetric short proximal limbs (rhizomelic dwarfism), frontal bossing, scaly and erythematous skin lesions that resemble ichthyosis in children and atrophic pigmentary lesions in adults, coarse hair with alopecia, and cataracts; reflecting

functional X-chromosomal mosaicism, in females the phenotype may vary from stillborn to mildly affected.⁵¹⁷ Radiographic examination reveals generalized osteosclerosis, irregular punctate calcification (stippling) of epiphyses of the long bones and vertebrae in children. This disorder is caused by loss-of-function mutations in emopamil-binding protein (*EBP*, OMIM 300205), the gene encoding 3 β -hydroxysteroid- Δ^8 , Δ^7 isomerase, an enzyme that converts cholesta-8(9)-en-3 β -ol to lathosterol; in the serum of subjects with chondrodysplasia punctata 2 plasma concentrations of 8-dehydrocholesterol cholest-8(9)-en-3 β -ol are elevated. This protein also binds many unrelated molecules; its genetic designation derives from its ability to bind emopamil (i.e., EBP)—a calcium ion antagonist. X-linked recessive and autosomal recessive forms of the Conradi-Hunermann-Happle syndrome have also been reported, suggesting genetic heterogeneity for this phenotype. The CHILD syndrome of congenital hemidysplasia with ichthyosiform erythroderma or nevus and limb defects is remarkable for its unilateral distribution of anomalies that are confined to one-half of the body.⁵¹⁷ It is an X-linked disorder caused by loss-of-function mutations in NADPH steroid dehydrogenase-like (*NSDHL*, OMIM 300275) encoding a sterol dehydrogenase or decarboxylase that is part of the 3 β -hydroxysteroid C-4 sterol demethylase complex that also includes the products of *SC4MOL* and *HSD17B7*. This complex converts 4,4-dimethylcholesta-8-en-3 β .ol to cholesta-8(9)-en-3 β -ol.

The often lethal Greenberg dysplasia (OMIM 215140) of hydrops-ectopic calcification-moth-eaten (HEM) skeletal dysplasia is transmitted as an autosomal recessive trait associated with short-limbed dwarfism, polydactyly, and irregularly decreased calcification of the long bones together with calcification of the larynx and trachea. It is caused by a loss-of-function mutation in the gene (*LBR*, OMIM 600024) encoding the lamin B receptor, a nuclear envelope inner membrane protein that not only binds lamin B but also has 3 β -hydroxysteroid- Δ^{14} reductase activity, an enzyme that converts 4,4-dimethylcholesta-8(9)-dien-3 β -ol

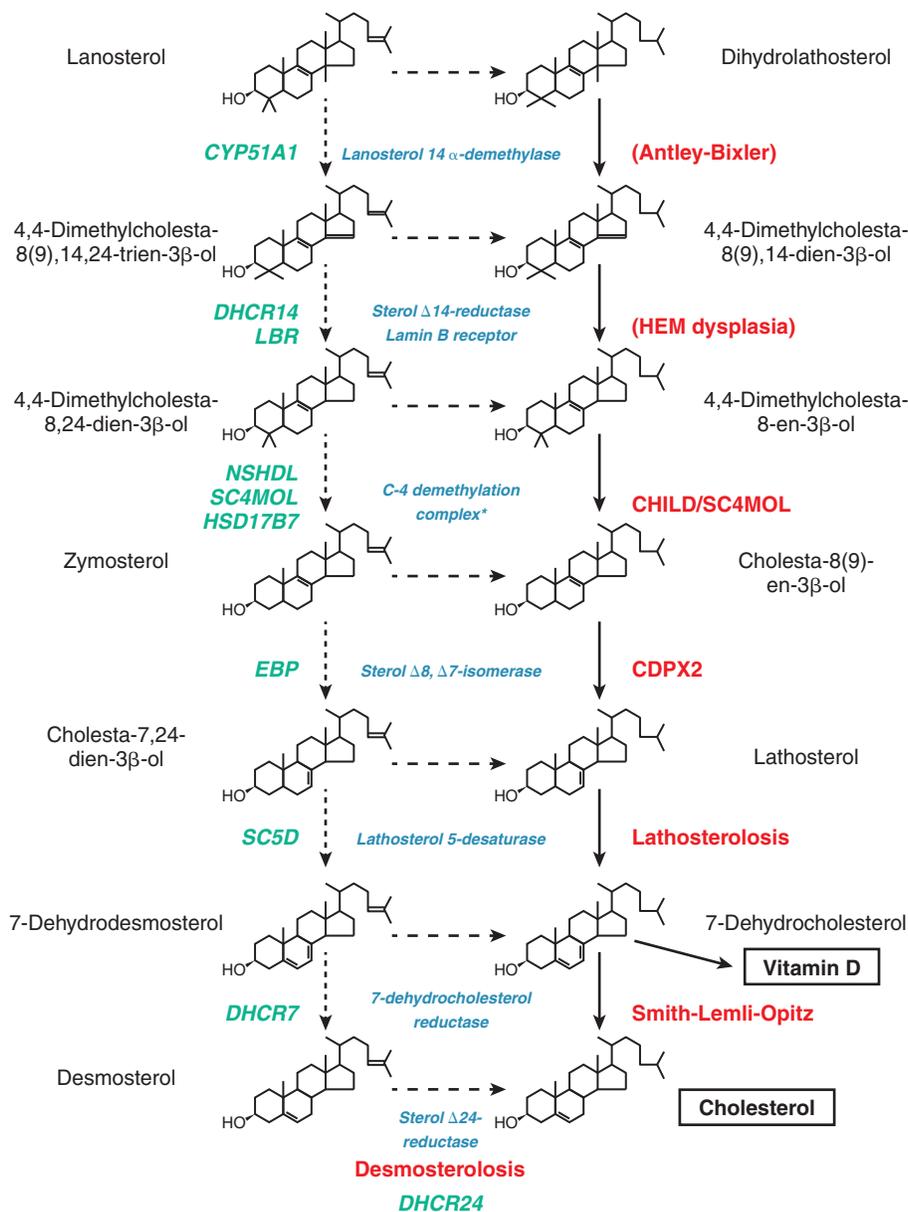


Fig. 20.17 Biosynthesis of cholesterol depicting the sites of enzyme activity lost by inactivating mutations that lead to skeletal, genital, and systemic malformations. The conversion of lanosterol to cholesterol may proceed through two pathways as depicted (see text for details). (From Forbes FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res.* 2011;52:6–34, with permission.)

to 4,4-dimethylcholesta-8-en-3 β -ol, a necessary step for normal cholesterol biosynthesis. 3 β -Hydroxysteroid- Δ ¹⁴ reductase activity is also encoded by *DHCR14*, an endoplasmic reticular enzyme that too catalyzes reduction of unsaturated C14-C15 intermediate sterols.⁵¹⁷ The HEM skeletal dysplasia is considered to be a laminopathy rather than an inborn error in cholesterol biosynthesis. The Antley-Bixler syndrome (OMIM 207410) of craniosynostosis, humeroradial synostosis, femoral bowing, midface hypoplasia, choanal stenosis/atresia, and joint contractures has been associated with heterozygous mutations in *FGFR2* (OMIM 176943). When these skeletal anomalies coexist with genital malformations and defects in steroidogenesis (OMIM 201750), biallelic loss-of-function mutations have been identified in *POR* (OMIM 124015), encoding P450 oxidoreductase, a donor of electrons for the P450 enzymes required for synthesis of adrenocortical and gonadal steroids. Thus when *POR* activity is decreased, function of the enzyme encoded by *CYP51A1* (OMIM 601637) that converts

dihydrolathosterol to 4,4-dimethylcholesta-8(9)14-dien-3 β -ol is depressed.

CONCLUDING REMARKS

Deciphering of the complex mechanisms that underlie the pathophysiology of illnesses that adversely affect the regulation of calcium, phosphate, and magnesium metabolism, chondrocyte differentiation and growth, and bone formation, mineralization, and strength has been further informed by the insights that advances in genetics, epigenetics, and proteomics have brought to these clinical problems. It may be anticipated that further clarification of the very most basic mechanisms that underlie these disorders will enable translation of these findings into the care of many of the disorders that affect our young patients.

ACKNOWLEDGMENT

In memory of Frank B. Diamond, Jr, MD.

Abbreviations			
ADHR	Autosomal dominant hypophosphatemic rickets	Mg ²⁺	Magnesium
AHO	Albright hereditary osteodystrophy	MPA	Medroxyprogesterone acetate
AMP	Adenosine monophosphate	NF-κB	Nuclear factor κB
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy	NHERF1	Sodium hydrogen exchanger regulatory factor 1
APS	Autoimmune polyendocrine syndrome	NSHPT	Neonatal severe hyperparathyroidism
ARHR	Autosomal recessive hypophosphatemic rickets	NTx	Amino terminal telopeptide of collagen type I
ASARM	Acidic serine aspartate-rich MEPE-associated motif	OIC	Osteogenesis imperfecta congenita
ATP	Adenosine triphosphate	OMIM	Online Mendelian Inheritance in Man ^a
BMC	Bone mineral content	OSTM1	Osteopetrosis-associated transmembrane protein-1
BMD	Bone mineral density	P3H1	Prolyl 3-hydroxylase 1
BMP	Bone morphogenetic protein	PDDR	Pseudovitamin D-deficiency rickets
CA	Carbonic anhydrase	PEDF	Pigment epithelium derived factor
CaSR	Calcium sensing receptor	PHEX	Phosphate-regulating endopeptidase homolog, X-linked
CBP	Collagen binding protein (HSP)	PHP	Pseudohypoparathyroidism
CKD-MBD	Chronic kidney disease-mineral and bone disorder	PICP	Carboxyl terminal propeptide of collagen type I
COL1A1	Bone collagen subunit α1(I)	POH	Progressive osseous heteroplasia
CRTAP	Cartilage-associated protein	PPHP	Pseudopseudohypoparathyroidism
DXA	Dual x-ray absorptiometry	PTH	Parathyroid hormone
DGS	DiGeorge syndrome	PTHrP	PTH-related protein
DMR	Differentially methylated region	PTHrP1	PTH/PTHrP receptor 1
FGF	Fibroblast growth factor	QCT	Quantitative computed tomography
FGFR	FGR receptor	QUS	Quantitative ultrasound
FISH	Fluorescent in situ hybridization	RANK	Receptor activator of nuclear factor κB
GDNF	Glial-derived neurotrophic factor	RANKL	RANK-ligand
GH	Growth hormone	SDS	Standard deviation score
GPCR	G-protein coupled receptor	SERM	Selective estrogen receptor modulator
GDP	Guanosine diphosphate	sFRP4	Serum frizzled related protein-4
G _{sα}	α Subunit of stimulatory G-protein (G _{sα})	SIBLING	Short integrin-binding ligand-interacting glycoprotein
GTP	Guanosine triphosphate	SOS	Speed of sound
H ⁺	Hydrogen ion	TALH	Thick ascending loop of Henle
HDR	Hypoparathyroidism, deafness, renal disease syndrome	TGFβ	Transforming growth factor β
HHC	Hereditary hypocalciuric hypercalcemia	TNF	Tumor necrosis factor
HRD	Hypoparathyroidism, retardation, dysmorphism syndrome	TNSALP	Tissue nonspecific alkaline phosphatase
HSP	Heat shock protein (CBP)	TRAP	Tartrate-resistant acid phosphatase
ICTP	Carboxyl telopeptide of collagen type I	VDDR1A	Vitamin D-dependent rickets type 1A
IFITM5	Interferon-induced transmembrane protein-5 or bone-restricted Itifm5-like protein	VDR	Vitamin D receptor
IGF	Insulin-like growth factor	VDRE	Vitamin D response element
IL	Interleukin	VEGF	Vascular endothelial growth factor
IOF	International Osteoporosis Society	VLBW	Very low birth weight
ISCD	International Society for Clinical Densitometry	WBS	Williams-Beuren syndrome
KCS	Kenny-Caffey syndrome	WINAC	WSTF including nucleotide assembly complex
LBW	Low birth weight	WHO	World Health Organization
MAPK	Mitogen-activated protein kinase	WSTF	Williams syndrome transcription factor
M-CSF	Macrophage-colony stimulating factor	XLHR	X-linked dominant hypophosphatemic rickets
MCT	Medullary carcinoma of thyroid	1,25(OH) ₂ D ₃	1,25-Dihydroxyvitamin D ₃ (calcitriol)
MEN	Multiple endocrine neoplasia	25OHD ₃	25-Hydroxyvitamin D ₃ (calcidiol)
MEPE	Matrix extracellular phosphoglycoprotein		

A, Adenine; C, cytidine; G, guanine; T, thymine.

^a<http://awww3.ncbi.nlm.nih.gov/htbin-post/Omim>.

REFERENCES

- Stokes VJ, Nielsen MF, Hannan FM, Thakker RV. Hypercalcemic disorders in children. *J Bone Mineral Res*. 2017;32:2157–2170.
- Carpenter T. Etiology of hypocalcemia in infants and children. *UpToDate*, 2018;1–17.
- Mannstadt M, Bilezikian JP, Thakker RV, et al. Hypoparathyroidism. *Nat Rev Dis Primers*. 2017;3:17055.
- Abrams SA. Neonatal hypocalcemia. *UpToDate*, 2018;1–11.
- Kovacs CS. Bone development and mineral homeostasis in the fetus and neonate: Roles of the calciotropic and phosphotropic hormones. *Physiol Rev*. 2014;94:1143–1218.
- Kurt A, Sen Y, Elkiran O, et al. Malignant infantile osteopetrosis: A rare cause of neonatal hypocalcemia. *J Pediatr Endocrinol Metab*. 2006;19:1459–1462.
- Engiz O, Kara S, Bagrut D, et al. Infantile malignant osteopetrosis: a rare cause of neonatal hypocalcemia. *J Pediatr Endocrinol Metab*. 2012;25:1205–1207.
- Whyte MP. Sclerosing bone disorders. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:412–423.
- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;457:266–281.
- Hollis BW, Wagner CL. Nutritional vitamin D status during pregnancy: reasons for concern. *CMAJ*. 2006;174:1287–1290.
- Pillar N, Pleniceanu O, Fang M, et al. A rare variant in the FHL1 gene associated with X-linked recessive hypoparathyroidism. *Hum Genet*. 2017;136:835–845.
- Shaw N. A practical approach to hypocalcaemia in children. In: Allgrove J, Shaw N, eds. *Calcium and Bone Disorders in Children and Adolescents*; 2009:73–92. Basel, Karger, *Endocr Dev*, 16.
- Baumber L, Tufarelli C, Patel S, et al. Identification of a novel mutation disrupting the DNA binding activity of GCM2 in autosomal recessive familial isolated hypoparathyroidism. *J Med Genet*. 2005;42:443–448.

14. Mirczuk SM, Bowl MR, Nesbit MA, et al. A missense *Glial Cells Missing Homolog B (GCMB)* mutations, Asn502His, causes autosomal dominant hypoparathyroidism. *J Clin Endocrinol Metab.* 2010;95:3512–3516.
15. Doyle D, Kirwin SM, Sol-Church K, Levine MA. A novel mutation in the *GCM2* gene causing severe congenital hypoparathyroidism. *J Pediatr Endocrinol Metab.* 2012;25:741–746.
16. Grigorieva IV, Mirczuk C, Gaynor KU, et al. *Gata3*-deficient mice develop parathyroid abnormalities due to dysregulation of the parathyroid-specific transcription factor *Gcm2*. *J Clin Invest.* 2010;120:2144–2155.
17. Mittleman SD, HENDY GN, Fefferman RA, et al. A hypocalcemic child with a novel activating mutation of the calcium-sensing receptor gene: Successful treatment with recombinant human parathyroid hormone. *J Clin Endocrinol Metab.* 2006;91:2474–2479.
18. Roszko KL, Ruiye DB, Mannstadt M. Autosomal dominant hypocalcemia (hypoparathyroidism) types 1 and 2. *Front Physiol.* 2016;7:458.
19. Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet.* 2003;362:1366–1373.
20. McDonald-McGinn DM, Sullivan KE. Chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/Velocardiofacial syndrome). *Medicine.* 2011;90:1–18.
21. Moon AM. Mouse models for investigating the developmental basis of human birth defects. *Pediatr Res.* 2006;59:749–755.
22. Sobin C, Kiley-Brabeck K, Dale K, et al. Olfactory disorder in children with 22q11 deletion syndrome. *Pediatrics.* 2006;118:697–703.
23. Sahoo T, Theisen A, Rosenfeld JA, et al. Copy number variants of schizophrenia susceptibility loci are associated with a spectrum of speech and developmental delays and behavior problems. *Genet Med.* 2011;13:777–784.
24. Wentzel C, Fernstrom M, Ohrner Y, et al. Clinical variability of the 22q11.2 duplication syndrome. *Europ J Med Genet.* 2008;51:501–510.
25. Carelle-Calmels N, Saugier-Verber P, Girard-Lemaire F, et al. Genetic compensation in a human genomic disorder. *N Engl J Med.* 2009;360:1211–1216.
26. Son YY, Lee B, Suh C-R, et al. A case of CHARGE syndrome featuring immunodeficiency and hypocalcemia. *J Genet Med.* 2015;12:57–60.
27. Chiu W-Y, Chen H-W, Chao H-W, et al. Identification of three novel mutations in the *GATA3* gene responsible for familial hypoparathyroidism and deafness in Chinese families. *J Clin Endocrinol Metab.* 2006;91:4587–4592.
28. Aminzadeh M, Galehdari H, Shariati G, et al. Clinical features and tubulin folding cofactor E gene analysis in Iranian patients with Sanjad-Sakati syndrome. *J Pediatr (Rio J).* 2018;S0021-7557(18):30453–30454.
29. Hershkovitz E, Parvari R, Diaz GA, Gorodischer R. Hypoparathyroidism-retardation-dysmorphism (HRD) syndrome — A review. *J Pediatr Endocrinol Metab.* 2004;17:1583–1590.
30. Parvari R, Hershkovitz E, Grossman N, et al. Mutation of *TBCE* causes hypoparathyroidism-retardation-dysmorphism and autosomal recessive Kenney-Caffey syndrome. *Nat Genet.* 2002;32:448–452.
31. Isojima T, Doi K, Mitsui J, et al. A recurrent de novo *FAM111A* mutation causes Kenny-Caffey syndrome type 2. *J Bone Miner Res.* 2014;29:992–998.
32. Chow J, Rahman J, Achermann JC, et al. Mitochondrial disease and endocrine dysfunction. *Nature Rev Endocrinol.* 2017;13:92–104.
33. Naiki M, Ochi N, Kato YS, et al. Mutations in *HADHB*, which encodes the β -subunit of mitochondrial trifunctional protein, cause infantile onset hypoparathyroidism and peripheral polyneuropathy. *Am J Med Genet.* 2014;164:1180–1187.
34. Thiele S, Mantovani G, Barlier A, et al. From pseudohypoparathyroidism to inactivating PTH/PTHrP signalling disorder (iPPSD), a novel classification proposed by the EuroPHP network. *Eur J Endocrinol.* 2016;175:P1–P17.
35. Giusti F, Cianferotti L, Masi L, Brandi ML. Blomstrand's chondrodysplasia. In: Brandi M, Brown E, eds. *Hypoparathyroidism*, Milan, Italy: Springer; 2015:389–395.
36. Thakker RV. Genetics of endocrine and metabolic disorders: Parathyroid. *Rev Endocrinol Metab Dis.* 2004;5:37–51.
37. Hoogendam J, Farih-Sips H, Wynaendts CW, et al. Novel mutations in the parathyroid hormone (PTH)/PTH-related peptide receptor type 1 causing Blomstrand osteochondrodysplasia type I and II. *J Clin Endocrinol Metab.* 2007;92:1088–1095.
38. Moirangthem A, Narayanan DL, Jacob P, et al. Report of second case and clinical and molecular characterization of Eiken syndrome. *Clin Genet.* 2018;94(5):457–460.
39. Frazier-Bowers SA, Hendricks HM, Wright JT, et al. Novel mutations in *PTH1R* associated with primary failure of eruption and osteoarthritis. *J Dent Res.* 2014;93:134–139.
40. Mantovani G. Pseudohypoparathyroidism: Diagnosis and treatment. *J Clin Endocrinol Metab.* 2011;96:3020–3030.
41. Salemi P, Skalamera Olson JM, Dickson LE, Germain-Lee EL. Ossifications in Albright hereditary osteodystrophy: Role of genotype, inheritance, sex, age, hormonal status, and BMI. *J Clin Endocrinol Metab.* 2018;103:158–168.
42. Pignola RJ, Ramaswamy G, Fong JT, et al. Progressive osseous heteroplasia: diagnosis, treatment, and prognosis. *Appl Clin Genet.* 2015;8:37–48.
43. Pinsker JE, Rogers W, McLean S, et al. Pseudohypoparathyroidism type 1a with congenital hypothyroidism. *J Pediatr Endocrinol Metab.* 2006;19:1049–1052.
44. Richard N, Abeguile G, Coudray N, et al. A new deletion ablating *NESP55* causes loss of maternal imprint of A/B *GNAS* and autosomal dominant pseudohypoparathyroidism type 1b. *J Clin Endocrinol Metab.* 2012;97:E863–E867.
45. Linglart A, Fryssira H, Hiort O, et al. *PRKAR1A* and *PDE4D* mutations cause acrodysostosis but two distinct syndromes with or without GPCR-signaling hormone resistance. *J Clin Endocrinol Metab.* 2012;97:E2328–E2338.
46. Vasava R, Tank B, Jain A. A case of acrodysostosis: a rare primary bone dysplasia. *Int J Res Med Sci.* 2018;6:2165–2168.
47. Linglart A, Menguy C, Couvineau A, et al. Recurrent *PRKAR1A* mutation in acrodysostosis with hormone resistance. *N Engl J Med.* 2011;364:2218–2219.
48. Shore EM, Kaplan FS. Inherited human diseases of heterotopic bone formation. *Nat Rev Rheumatol.* 2010;6:518–527.
49. Wan M, Li J, Herbst K, et al. *LRP6* mediates cAMP generation by G-protein coupled receptors through regulating the membrane targeting of $G\alpha_s$. *Sci Signal.* 2011;4:ra15.
50. Oskarsdottir S, Persson C, Eriksson BO, Fasth A. Presenting phenotype in 100 children with the 22q11 deletion syndrome. *Eur J Pediatr.* 2005;164:146–153.
51. Levine MA. Investigation & management of hypocalcemia. Meet the Professor. *Endocr Soc.* 2010;81–86.
52. Newfield RS. Recombinant PTH for initial management of neonatal hypocalcemia. *N Engl J Med.* 2007;356:1687–1688.
53. Kelly A, Levine MA. Hypocalcemia in the critically ill patient. *J Intens Care Med.* 2013;28:166–177.
54. Domico MB, Huynh V, Anand SK, Mink R. Severe hyperphosphatemia and hypocalcemic tetany after oral laxative administration in a 3-month-old infant. *Pediatrics.* 2006;118:1580–1583.
55. Shoback D. Hypoparathyroidism. *N Engl J Med.* 2008;359:391–403.
56. Stack Jr BC, Bimston DN, Bodenner DL, et al. Postoperative hypoparathyroidism — definitions and management. *Endocrine Pract.* 2015;21:674–685.
57. Theman TA, Collins MT, Dempster DW, et al. PTH(1-34) replacement therapy in a child with hypoparathyroidism caused by a sporadic calcium receptor mutation. *J Bone Miner Res.* 2009;24:964–973.
58. Winer KK, Sinali N, Reynolds J, et al. Long-term treatment of 12 children with chronic hypoparathyroidism: A random trial comparing synthetic human parathyroid hormone 1-34 versus calcitriol and calcium. *J Clin Endocrinol Metab.* 2010;95:2680–2688.
59. Mayer A, Ploix C, Orgiazzi J, et al. Calcium-sensing receptor antibodies are relevant markers of acquired hypoparathyroidism. *J Clin Endocrinol Metab.* 2004;89:4484–4488.
60. Carpenter TO. Disorders of mineral metabolism in childhood. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:349–353.
61. Husebye ES, Anderson MS, Kampe O. Autoimmune polyendocrine syndromes. *N Engl J Med.* 2018;378:1132–1141.

62. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab.* 2006;91:2843–2850.
63. Wolff ASB, Erichsen MM, Meager A, et al. Autoimmune polyendocrine syndrome type 1 in Norway: Phenotypic variation, autoantibodies, and novel mutations in the autoimmune regulator gene. *J Clin Endocrinol Metab.* 2007;92:595–603.
64. Orlova EM, Bukina AM, Kuznetsova ES, et al. Autoimmune polyglandular syndrome type 1 in Russian patients: Clinical variants and autoimmune regulator mutations. *Horm Res Paediatr.* 2010;73:449–457.
65. Abramson J, Giraud M, Benoist C, Mathis D. Aire's partners in the molecular control of immunological tolerance. *Cell.* 2010;140:123–135.
66. Dragin N, Bismuth J, Cizeron-Clairac G, et al. Estrogen-mediated down regulation of AIRE influences sexual dimorphism in autoimmune diseases. *J Clin Invest.* 2016;126:1525–1537.
67. Gimenez-Barcons M, Casteras A, del Pilar M, et al. Autoimmune predisposition in Down syndrome may result from a partial central tolerance failure due to insufficient intrathymic expression of AIRE and peripheral antigens. *J Immunol.* 2014;193:3872–3879.
68. Bourgault S, Baril C, Vincent A, et al. Retinal degeneration in autoimmune polyglandular syndrome type 1: a case series. *Br J Ophthalmol.* 2015;99:1536–1542.
69. Wang X, Ping F, Qi C, Xiao X. Delayed diagnosis with autoimmune polyglandular syndrome type 2 causing adrenal crisis. A case report. *Medicine.* 2016;95(e5062). 42.
70. d'Hennezel E, Dhuban KB, Torgerson T, Piccirillo C. The immunogenetics of immune dysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J Med Genet.* 2012;49:291–302.
71. Thakker RV. Hypocalcemia: pathogenesis, differential diagnosis, and management. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:213–215.
72. Downs Jr RW. Miscellaneous causes of hypocalcemia. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:227–229.
73. Di Maio S, Soliman AT, De Sanctis V, Kattamis C. Current treatment of hypoparathyroidism: theory versus reality waiting guidelines for children and adolescents. *Acta Biomed.* 2018;89:122–131.
74. Bilezikian JP, Khan A, Potts Jr JJ, et al. Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J Bone Mineral Res.* 2011;26:2317–2337.
75. Meloni A, Furcas M, Cetani F, et al. Autoantibodies against type 1 interferons as an additional diagnostic criterion for autoimmune polyendocrinopathy syndrome type I. *J Clin Endocrinol Metab.* 2008;93:4389–4397.
76. Brandi ML, Bilezikian JP, Shoback D, et al. Management of hypoparathyroidism: Summary statement and guidelines. *J Clin Endocrinol Metab.* 2016;101:2273–2283.
77. Matarazzo P, Tuli G, Fiore L, et al. Teriparatide (rhPTH) treatment in children with syndromic hypoparathyroidism. *J Pediatr Endocrinol Metab.* 2014;27:53–59.
78. Winer KK, Zhang B, Shrader JA, et al. Synthetic human parathyroid hormone 1–34 replacement therapy: A randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. *J Clin Endocrinol Metab.* 2012;97:391–399.
79. Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. *Curr Opin Pediatr.* 2010;22:508–515.
80. Hak EB, Crill CM, Bugnitz MC, et al. Increased parathyroid hormone and decreased calcitriol during neonatal extracorporeal membrane oxygenation. *Intens Care Med.* 2005;31:264–270.
81. Langman CB. Hypercalcemic syndromes in infants and children. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:209–212.
82. Garg A, Singhal R, Chaudhary SS. Neonatal hypercalcemia secondary to subcutaneous fat necrosis presenting as severe dehydration. *Indian J Pediatr Dermatol.* 2018;19:146–147.
83. Obermannova B, Banghova K, Sumnik Z, et al. Unusually severe phenotype of neonatal primary hyperparathyroidism due to a heterozygous inactivating mutation in the CASR gene. *Eur J Pediatr.* 2009;168:569–573.
84. Diaz-Thomas A, Cannon J, Iyer P, et al. A novel CASR mutation associated with neonatal severe hyperparathyroidism transmitted as an autosomal recessive disorder. *J Pediatr Endocrinol Metab.* 2014;27:851–856.
85. Lietman SA, Tenenbaum-Rakover Y, Jap TS, et al. A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab.* 2009;94:4372–4379.
86. Myashiro T, Kunii I, Manna TD, et al. Severe hypercalcemia in a 9-year-old Brazilian girl due to a novel inactivating mutation of the calcium-sensing receptor. *J Clin Endocrinol Metab.* 2004;89:5936–5941.
87. Brown EM. Editorial: Mutant extracellular calcium-sensing receptors and severity of disease. *J Clin Endocrinol Metab.* 2005;90:1246–1248.
88. Mallet E. Primary hyperparathyroidism in neonates and childhood: the French experience (1984–2004). *Horm Res.* 2008;69:180–188.
89. Levine, M.A. (2006). Spectrum of hyperparathyroidism in children. Endocrine Society 88th Annual Meeting, Boston, Meet-The-Professor, pp. 390–396.
90. Coutinho MF, Encarnacao M, Gomes R, et al. Origin and spread of a common deletion causing mucopolidosis type II: insights from patterns of haplotypic diversity. *Clin Genet.* 2011;80:273–280.
91. Csukasi F, Duran I, Barad M, et al. The PTH/PTHrP/SIK3 pathway affects skeletogenesis through altered mTOR signaling. *Sci Transl Med.* 2018;10. eaat9356.
92. Antonell A, Del Campo M, Magano LF, et al. Partial 7q11.23 deletions further implicate *GTF2I* and *GTF2IRD1* as the main genes responsible for the Williams-Beuren syndrome neurocognitive profile. *J Med Gen.* 2010;47:312–320.
93. Pober BR. Williams-Beuren syndrome. *N Engl J Med.* 2010;362:239–252.
94. Barnett C, Krebs JE. WSTF does it all: a multifunctional protein in transcription, repair, and replication. *Biochem Cell Biol.* 2011;89:12–23.
95. Fusco C, Micale L, Augello B, et al. Smaller and larger deletions of the Williams Beuren syndrome region implicate genes involved in mild facial phenotype, epilepsy and autistic traits. *Eur J Hum Genet.* 2014;22:64–70.
96. Waz WR. Williams-Beuren syndrome: renal manifestations. *UpToDate.* 2018;1–11.
97. Schlingmann KP, Kaufmann M, Weber S, et al. Mutations in *CYP24A1* and idiopathic infantile hypercalcemia. *N Engl J Med.* 2011;365:410–425.
98. Van der AN, Rooms L, Vandeweyer G, et al. Fourteen new cases contribute to the characterization of the 7q11.23 microduplication syndrome. *Eur J Med Genet.* 2009;52:94–100.
99. Schlingmann KP, Ruminska J, Kaufmann M, et al. Autosomal recessive mutations in *SLC34A1* encoding sodium-phosphate cotransporter 2A cause idiopathic infantile hypercalcemia. *J Am Soc Nephrol.* 2016;27:604–614.
100. Marks BE, Doyle DA. Idiopathic infantile hypercalcemia: case report and review of the literature. *J Pediatr Endocrinol Metab.* 2016;29:127–132.
101. Carpenter TO. *CYP24A1* loss of function: Clinical phenotype of monoallelic and biallelic mutations. *J Steroid Biochem Mol Biol.* 2017;173:337–340.
102. Tebben PJ, Milliner DS, Horst RL, et al. Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to *CYP24A1* mutations: Effects of ketoconazole therapy. *J Clin Endocrinol Metab.* 2012;97:E423–E427.
103. Tebben PJ, Singh RJ, Kumar R. Vitamin D-mediated hypercalcemia: Mechanisms, diagnosis, and treatment. *Endocr Rev.* 2016;37:521–547.
104. Silvestre C, Aragues JM, Bugalho MJ, et al. Idiopathic infantile hypercalcemia presenting in childhood but diagnosed in adulthood. *AACE Clin Case Rep.* 2018;4:e256–e262.
105. Ji H-F, Shen L. *CYP24A1* mutations in idiopathic infantile hypercalcemia. *N Engl J Med.* 2012;365:1741.

106. Demir K, Yildiz M, Bahat H, et al. Clinical heterogeneity and phenotypic expansion of NaPi-IIa-associated disease. *J Clin Endocrinol Metab.* 2017;102:4604–4614.
107. Pronicka E, Ciara E, Halat P, et al. Biallelic mutations in *CYP24A1* and *SLC34A1* as a cause of infantile idiopathic hypercalcemia (IHH) with vitamin D hypersensitivity: molecular study of 11 cases. *J Appl Genetics.* 2017;58:349–353.
108. Bhat YR, Vinayaka G, Sreelakshmi K. Antenatal Bartter syndrome: A review. *Intern J Pediatr.* 2012;2012:857136.
109. Wongsasak S, Vidmar AP, Addala A, et al. A novel *SLC12A1* gene mutation associated with hyperparathyroidism, hypercalcemia, nephrogenic diabetes insipidus, and nephrocalcinosis in four patients. *Bone.* 2017;97:121–125.
110. Distelmaier F, Herebian D, Atasever C, et al. Blue diaper syndrome and *PCSK1* mutations. *Pediatrics.* 2018;141(5). e20170548.
111. Pepin L, Colin E, Tessarech M, et al. A new case of *PCSK1* pathogenic variant with congenital proprotein convertase 1/3 deficiency and literature review. *J Clin Endocrinol Metab.* 2018;104(4):985–993.
112. Whyte MP. Hypophosphatasia: An overview for 2017. *Bone.* 2017;102:15–25.
113. Whyte MP. Hypophosphatasia. In: Glorieux FH, Jueppner H, Pettifor J, eds. *Pediatric Bone: Biology and Diseases.* 3rd ed. San Diego, CA: Academic Press; 2012:771–794.
114. Whyte MP, Greenberg CR, Salman NJ, et al. Enzyme-replacement therapy in life-threatening hypophosphatasia. *N Engl J Med.* 2012;366:904–913.
115. Baujat G, Michol C, Le Quan Sang KH, Cormier-Daire V. (2017). Perinatal and infantile hypophosphatasia: clinical features and treatment. *Arch Pediatr.* 24, 5S61–5S65.
116. Davies JH. A practical approach to the problems of hypercalcemia. In: Allgrove J, Shaw N, eds. *Calcium and Bone Disorders in Children and Adolescents;* 2009:93–114. Basel, Karger, *Endocr Dev.* 16.
117. Bilezikian JP. Primary hyperparathyroidism. *J Clin Endocrinol Metab.* 2018;103:3993–4004.
118. Markowitz ME, Underland L, Gensure R. Parathyroid disorders. *Pediatr Rev.* 2016;37:524–533.
119. Benjamin RW, Moats-Staats BM, Calikoglu A, et al. Hypercalcemia in children. *Pediatr Endocrinol Rev.* 2008;5:778–784.
120. Shane E, Irani D. Hypercalcemia: pathogenesis, clinical manifestations, differential diagnosis, and management. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:176–180.
121. Marx SJ, Lourenco DM. Familial hyperparathyroidism - Disorders of growth and secretion in hormone-secretory tissue. *Horm Metab Res.* 2017;49:805–815.
122. Magno AL, Ward BK, Ratajczak T. The calcium-sensing receptor: a molecular perspective. *Endocr Rev.* 2011;32:3–30.
123. Hannan FM, Babinsky VN, Thakker RV. Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Molec Endocrinol.* 2016;57: R127–142.
124. Marx SJ. Calcimimetic use in familial hypocalciuric hypercalcemia — A perspective in endocrinology. *J Clin Endocrinol Metab.* 2017;102:3933–3936.
125. White E, McKenna J, Cavanaugh A, Breitwieser GE. Pharmacochaperone-mediated rescue of calcium sensing receptor loss of function mutants. *Mol Endocrinol.* 2009;23:1115–1123.
126. Pidasheva S, Grant M, Canaff L, et al. Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of the *CASR* mutants retained intracellularly. *Hum Mol Genet.* 2006;15:2200–2209.
127. Brown EM. Anti-parathyroid and anti-calcium sensing receptor antibodies in autoimmune hypoparathyroidism. *Endocrinol Metab Clin NA.* 2009;38:437–446.
128. Bilezikian JP, Bandeira L, Khan A, Cusano NE. Hyperparathyroidism. *Lancet.* 2018;391:168–178.
129. Insogna KL. Primary hyperparathyroidism. *N Engl J Med.* 2018;379:1050–1059.
130. Thakker RV, Newey PJ, Walls GV, et al. Clinical Practice Guidelines for Multiple Endocrine Neoplasia type 1 (MEN1). *J Clin Endocrinol Metab.* 2012;97:2990–3011.
131. Newey PJ, Nesbit MA, Rimmer AJ, et al. Whole-exome sequencing studies of nonhereditary (sporadic) parathyroid adenomas. *J Clin Endocrinol Metab.* 2012;97:E1995–E2005.
132. Juhlin, C.C., Hoog, A. (2010). Parafibromin as a diagnostic instrument for parathyroid carcinoma — Lone ranger or part of the posse? *Intern J Endocrinol.* 2010, ID 324964.
133. Yang Y-J, Han J-W, Youn H-D, Cho E-J. The tumor suppressor, parafibromin, mediates histone H3 K9 methylation for cyclin D1 repression. *Nucl Acids Res.* 2010;38:382–390.
134. Wang Y, Ozawa A, Zaman S, et al. The tumor suppressor protein menin inhibits AKT activation by regulating its cellular localization. *Cancer Res.* 2011;71:371.
135. Francis J, Lin W, Pozenblatt-Rosen O, Meyerson M. The menin tumor suppressor protein is phosphorylated in response to DNA damage. *PLoS One.* 2011;6(1). e16119.
136. Arnold A, Marx SJ. Familial hyperparathyroidism (including MEN, FHH, and HPT-JT). In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:361–366.
137. Choi S-K, Yoon S-R, Calabrese P, Arnheim N. Positive selection for new disease mutations in the human germline: Evidence from heritable cancer syndrome multiple endocrine neoplasia type 2B. *PLoS Genet.* 2012;8(2). e1002420.
138. Araki T, Holick MF, Alfonso BD, et al. Vitamin D intoxication with severe hypercalcemia due to manufacturing and labeling errors of two dietary supplements in the United States. *J Clin Endocrinol Metab.* 2011;96:3603–3608.
139. Adams JS, Hewison M. Hypercalcemia caused by granuloma-forming disorders. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:200–202.
140. Jacobs TP, Bilezikian JP. Clinical review: rare causes of hypercalcemia. *J Clin Endocrinol Metab.* 2005;90:6316–6322.
141. Wysolmerski JJ. Miscellaneous causes of hypercalcemia. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:203–208.
142. El Saleeby CM, Grottkau BE, Friedmann AM, et al. Case 4-2011: A 4-year-old boy with back pain and hypercalcemia. *N Engl J Med.* 2011;364:552–562.
143. Hruska KA, Mathew S. Chronic kidney disease bone mineral disorder (CKD-BMD). In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:343–349.
144. Prince RL. Secondary and tertiary hyperparathyroidism. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:190–195.
145. Kollars J, Zarroug AE, van Heerden J, et al. Primary hyperparathyroidism in pediatric patients. *Pediatrics.* 2005;115:974–980.
146. Park H-J, Choi E-J, Kim J-K. A successful treatment of hypercalcemia with zoledronic acid in a 15-year-old boy with acute lymphoblastic leukemia. *Ann Pediatr Endocrinol Metab.* 2016;21: 99–104.
147. Shane E, Berenson JR. Treatment of hypercalcemia. *UpToDate.* 2019;1–19.
148. Boyce AM. Denosumab: An emerging therapy in pediatric bone disorders. *Curr Osteoporos Res.* 2017;15:283–292.
149. D'Agostino JD, Diana M, Vix M, et al. Three-dimensional virtual neck exploration before parathyroidectomy. *N Engl J Med.* 2012;367:1072–1073.
150. Fisher MM, Cabrera SM, Imel EA. Successful treatment of neonatal severe hyperparathyroidism with cinacalcet in two patients. *Endocrinol Diabetes Metab Case Rep.* 2015;150040.
151. Li H, Sun S, Chen J, et al. Genetics of magnesium disorders. *Kidney Dis.* 2017;3:85–87.
152. San-Cristobal P, Dimke H, Hoenderop JGJ, Bindels RJM. Novel molecular pathways in renal Mg^{2+} transport: a guided tour along the nephron. *Curr Opin Nephrol Hypertens.* 2010;19:456–462.
153. Viering DHM, de Baaij JHF, Walsh SB, et al. Genetic causes of hypomagnesemia, a clinical overview. *Pediatr Nephrol.* 2017;32: 1123–1135.

154. Koo WWK. Neonatal calcium, magnesium, and phosphorus disorders. In: Lifshitz F, ed. *Pediatric Endocrinology* 5th ed. New York: Informa Healthcare; 2007:497–529. Vol 2.
155. Ferre S, Hoenderop JGJ, Bindels RJM. Insight into renal Mg²⁺ transporters. *Curr Opin Nephrol Hypertens*. 2011;20:169–176.
156. Lainez S, Schlingmann KP, van der Wijst J, et al. New TRPM6 missense mutations linked to hypomagnesemia with secondary hypocalcemia. *Eur J Hum Genet*. 2014;22:497–504.
157. de Baaij JHF, Dorresteijn EM, Hannekam EAM, et al. Recurrent FXD2 p.Gly41Arg mutation in patients with isolated dominant hypomagnesemia. *Nephrol Dial Transplant*. 2015;30:952–957.
158. Adalat S, Woolf AS, Johnstone KA, et al. HNF1B mutations associate with hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2009. 20L1123–1131.
159. Ferre S, de Baaij JHF, Ferreira P, et al. Mutations in *PCBD1* cause hypomagnesemia and renal magnesium wasting. *J Am Soc Nephrol*. 2014;25:574–586.
160. Kausalya PJ, Amasheh S, Gunzel D, et al. Disease-associated mutations affect intracellular traffic and paracellular Mg²⁺ transport function of claudin-16. *J Clin Invest*. 2006;116:878–891.
161. Konrad M, Schaller A, Seelow D, et al. Mutations in the tight-junction gene claudin 19 (CLDN19) are associated with renal magnesium wasting, renal failure, and severe ocular involvement. *Am J Hum Genet*. 2006;79:949–957.
162. Groenestege WMT, Thebault S, van der Wijst J, et al. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest*. 2007;117:2260–2267.
163. Stuijver M, Lainez S, Will C, et al. CNNM2, encoding a basolateral protein required for renal Mg²⁺ handling, is mutated in dominant hypomagnesemia. *Am J Hum Genet*. 2011;88:333–343.
164. Claudemans B, van der Wijst J, Scota RH, et al. A missense mutation in the Kv1.1 voltage-gated potassium channel-encoding gene *KCNA1* is linked to human autosomal dominant hypomagnesemia. *J Clin Invest*. 2009;119:936–942.
165. van der Wijst J, Konrad M, Verkaar SAJ, et al. A de novo *KCNA1* mutation in a patient with tetany and hypomagnesemia. *Nephron*. 2018;139:359–366.
166. Kaplan W, Haymond MW, McKay S, Karaviti LP. Osteopenic effects of MgSO₄ in multiple pregnancies. *J Pediatr Endocrinol Metab*. 2006;19:1225–1230.
167. Hadj-Rabia S, Brideau G, Al-Sarraj Y, et al. Multiplex epithelium dysfunction due to CLDN10 mutation: the HELIX syndrome. *Genet Med*. 2018;20:190–201.
168. Rustico SE, Calabria AC, Garber SJ. Metabolic bone disease of prematurity. *J Clin Transl Endocrinol*. 2014;1:85–91.
169. Atabek ME, Pirgon O, Yorulmaz A, Kurtoglu S. The role of cord blood IGF-I levels in preterm osteopenia. *J Pediatr Endocrinol Metab*. 2006;19:253–257.
170. Ahmad I, Nemet D, Eliakim A, et al. Body composition and its components in preterm and term newborns: a cross-sectional, multimodal investigation. *Am J Hum Biol*. 2010;22:69–75.
171. Abrams S, the Committee on Nutrition (AAP). Calcium and vitamin D requirements of enterally fed preterm infants. *Pediatrics*. 2013;131:e1676–e1683.
172. Lam HS, So KVV, Ng PC. Osteopenia in neonates: A review. *HK J Paediatr*. 2007;12:118–124.
173. Miller ME. The bone disease of preterm birth: a biomechanical perspective. *Pediatr Res*. 2003;53:10–15.
174. Aladangady N, Coen PG, White MP, et al. Urinary excretion of calcium and phosphate in preterm infants. *Pediatr Nephrol*. 2004;19:1225–1231.
175. Funke S, Morava E, Czako M, et al. Influence of genetic polymorphisms on bone disease of preterm infants. *Pediatr Res*. 2006;60:607–612.
176. Chen W, Yang C, Chen H, Zhang B. Risk factors analysis and prevention of metabolic bone disease of prematurity. *Medicine*. 2018;97(e12861). 42.
177. Rehman MU, Narchi H. Metabolic bone disease in the preterm infant: Current state and future directions. *World J Methodol*. 2015;5:115–121.
178. Xie LF, Alos N, Cloutier A, et al. The long-term impact of very preterm birth on adult bone mineral density. *Bone Rep*. 2019;10:100189.
179. Shiff Y, Eliakim A, Shaikin-Kestenbaum R, et al. Measurement of bone turnover markers in premature infants. *J Pediatr Endocrinol Metab*. 2001;14:389–395.
180. Aly H, Moustafa MF, Amer HA, et al. Gestational age, sex, and maternal parity correlate with bone turnover in premature infants. *Pediatr Res*. 2005;57:708–711.
181. Eliakim A, Nemet D. Osteopenia of prematurity - the role of exercise in prevention and treatment. *Pediatr Endocrinol Rev*. 2005;2:675–682.
182. Rack B, Lochmuller E-M, Janni W, et al. Ultrasound for assessment of bone quality in preterm and term infants. *J Perinatol*. 2012;32:218–226.
183. Chen H-L, Lee C-L, Tseng H-I, et al. Assisted exercise improves bone strength in very low birth weight infants by bone quantitative ultrasound. *J Paediatr Child Health*. 2010;46:653–659.
184. Dutta S, Singh B, Chessell L, et al. Guidelines for feeding very low birth weight infants. *Nutrients*. 2015;7:423–442.
185. Hair AB. Approach to enteral nutrition in the premature infant. *UpToDate*. 2019:1–36.
186. Balasuriya CND, Evensen KAI, Mosti MP, et al. Peak bone mass and bone microarchitecture in adults born with low birth weights preterm or at term: A cohort study. *J Clin Endocrinol Metab*. 2017;102:2491–2500.
187. Ayadi ID, Hamida EB, Rebeh RB, et al. Perinatal lethal type II osteogenesis imperfecta: a case report. *Pan Afr Med*. 2015;21:11.
188. Sam JE, Dharmalingam M. Osteogenesis imperfecta. *Indian J Endocrinol Metab*. 2017;21:903–908.
189. Marini JC. Osteogenesis imperfecta. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:446–450.
190. Marini JC, Forlino A, Bachinger HP, et al. Osteogenesis imperfecta. *Nat Rev Dis Primers*. 2017;3. #17052.
191. Marini JC, Cabral WA, Barnes AM. Null mutations in *LEPRE1* and *CRTAP* cause severe recessive osteogenesis imperfecta. *Cell Tissue Res*. 2010;339:59–70.
192. Kang H, Aryal ACS, Marini JC. Osteogenesis imperfecta: new genes reveal novel mechanisms in bone dysplasia. *Transl Res*. 2017;181:27–48.
193. Rohrbach M, Giunta C. Recessive osteogenesis imperfecta: Clinical, radiological, and molecular findings. *Am J Med Genet Part C*. 2012;160c:175–189.
194. Bodian DL, Chan T-F, Poon A, et al. Mutation and polymorphism spectrum in osteogenesis imperfecta type II: implications for genotype-phenotype relationships. *Hum Mol Genet*. 2009;18:463–471.
195. Van Dijk FS, Huizer M, Kariminejad A, et al. Complete *COL1A1* deletions in osteogenesis imperfecta. *Genet Med*. 2010;12:736–741.
196. Rauch F, Glorieux FH. Bisphosphonate treatment in osteogenesis imperfecta: Which drug, for whom, for how long? *Ann Med*. 2005;37. 925–302.
197. Mauhin W, Habarou F, Gobin S, et al. Update on lysinuric protein intolerance, a multi-faceted disease retrospective cohort analysis from birth to adulthood. *Orphanet J Rare Dis*. 2017;12:3.
198. Font-Llitjos M, Rodriguez-Santiago B, Espino M, et al. Novel *SLC7A7* large rearrangements in lysinuric protein intolerance patients involving the same AluY repeat. *Eur J Hum Genet*. 2009;17:71–79.
199. Sheth J, Bhavsar R, Gandhi A, et al. A case of Raine syndrome presenting with facial dysmorphism and review of literature. *BMC Med Genet*. 2018;19:76.
200. Carpenter T. Overview of rickets in children. *UpToDate*. 2018;1–22.
201. Ogunkolade WB, Boucher BJ, Bustin SA, et al. Vitamin D metabolism in peripheral blood mononuclear cells is influenced by chewing “Betel nut” (*Areca catechu*) and vitamin D status. *J Clin Endocrinol Metab*. 2006;91:2612–2617.
202. Lips P, van Schoor NM, Bravenboer N. Vitamin D-related disorders. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:329–335.
203. Girgis CM, Clifton-Bligh RJ, Hamrick MW, et al. The roles of vitamin D in skeletal muscle: Form, function, and metabolism. *Endocr Rev*. 2013;34:33–83.
204. Chatterjee D, Gupta V, Sharma V, et al. A reliable and cost effective approach for radiographic monitoring in nutritional rickets. *Br J Radiol*. 2014;87. 20130648.
205. Pettifor JM. Rickets and vitamin D deficiency in children and adolescents. *Endocrinol Metab Clin NA*. 2005;34:537–553.

206. Aggarwal V, Seth A, Aneja S, et al. Role of calcium deficiency in development of nutritional rickets in Indian children: A case control study. *J Clin Endocrinol Metab.* 2012;97:3461–3466.
207. Antonucci R, Locci C, Clemente MG, et al. Vitamin D deficiency in childhood: old lessons and current challenges. *J Pediatr Endocrinol Metab.* 2018;31:247–260.
208. van Leeuwen J, Koes BW, Paulis WD, van Middelkoop M. Differences in bone mineral density between normal-weight children and children with overweight and obesity; a systematic review and meta-analysis. *Obes Rev.* 2017;18(5).
209. Munns CF, Shaw N, Kiely M, et al. Global consensus recommendations on prevention and management of nutritional rickets. *J Clin Endocrinol Metab.* 2016;101:394–415.
210. Henderson CM, Fink SL, Bassyourni H, et al. Vitamin D-binding protein deficiency and homozygous deletion of the GC gene. *N Engl J Med.* 2019;380:1150–1157.
211. Aloia JF. The 2011 report on dietary reference intake for vitamin D: Where do we go from here? *J Clin Endocrinol Metab.* 2011;96:2987–2996.
212. Carpenter T. Etiology and treatment of calciopenic rickets in children. *UpToDate.* 2018;1–19.
213. Barake M, Daher RT, Salti I, et al. 25-Hydroxyvitamin D assay variations and impact on clinical decision making. *J Clin Endocrinol Metab.* 2012;97:835–843.
214. Schnadower D, Agarwal C, Oberfield SE, et al. Hypocalcemic seizures and secondary bilateral femoral fractures in an adolescent with primary vitamin D deficiency. *Pediatrics.* 2006;118:2226–2230.
215. Ross AC, Manson JE, Abrams SA, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab.* 2011;96:53–58.
216. Rovner AJ, O'Brien KO. Hypovitaminosis D among healthy children in the United States. A review of current literature. *Arch Pediatr Adolesc Med.* 2008;162:513–519.
217. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2011;96:1911–1930.
218. Sversky AL, Kumar J, Abramowitz MK, et al. Association of glucocorticoid use and low 25-hydroxyvitamin D levels: Results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. *J Clin Endocrinol Metab.* 2011;96:3838–3845.
219. Baroncelli GI, Bertelloni S, Ceccarelli C, et al. Bone turnover in children with vitamin D deficiency rickets before and during treatment. *Acta Paediatr.* 2000;89:513–518.
220. Alemzadeh R, Kichler J, Barar G, Calhoun M. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism.* 2008;57:183–191.
221. Reinehr T, de Sousa G, Alexy U, et al. Vitamin D status and parathyroid hormone in obese children before and after weight loss. *Eur J Endocrinol.* 2007;157:225–232.
222. Censani M, Stein EM, Shane E, et al. Vitamin D deficiency is prevalent in morbidly obese adolescents prior to bariatric surgery. *ISRN Obes.* 2013. ID 284516.
223. Palermo A, Tuccinardi D, Defeudis G, et al. BMI and BMD: The potential interplay between obesity and bone fragility. *Int J Environ Res Public Health.* 2016;13:544.
224. Zakharova I, Klimov L, Kuryaninova V, et al. Vitamin D insufficiency in overweight and obese children and adolescents. *Front Endocrinol.* 2019;10:103.
225. Golden NH, Abrams SA, Committee on Nutrition. Optimizing bone health in children and adolescents. *Pediatrics.* 2014;134:e1229–e1243.
226. Yu ASL, Stubbs JR. Causes of hypophosphatemia. *UpToDate.* 2019;1–17.
227. Pettifor JM. Nutritional and drug-induced rickets and osteomalacia. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:330–338.
228. Malloy PJ, Feldman D. Genetic disorders and defects in vitamin D actions. *Endocrinol Metab Clin North Am.* 2010;39:333–346.
229. Cheng JB, Levine MA, Bell NH, et al. Genetic evidence that human CYP1B enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci.* 2004;101:7711–7715.
230. Molin A, Wiedemann A, Demers N, et al. Vitamin D-dependent rickets type 1B (25-hydroxylase deficiency): A rare condition or a misdiagnosed condition? *J Bone Miner Res.* 2017;32:1893–1899.
231. Tosson H, Rose SR. Absence of mutation in coding regions of CYP2R1 gene in apparent autosomal dominant vitamin D 25-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2012;97:E796–E801.
232. Acar S, Demir K, Shi Y. Genetic causes of rickets. *J Clin Res Pediatr Endocrinol.* 2017;9(Suppl 2):88–105.
233. Demay MB. Rickets caused by impaired vitamin D activation and hormone resistance: Pseudovitamin D deficiency rickets and hereditary vitamin D-resistant rickets. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:338–341.
234. Edouard T, Alos N, Chabot G, et al. Short and long-term outcome of patients with pseudo-vitamin D deficiency rickets treated with calcitriol. *J Clin Endocrinol Metab.* 2011;96:82–89.
235. Faiyaz-UI-Haque M, Al Dhalaan W, AlAshwal A, et al. Hereditary 1,25-dihydroxyvitamin D-resistant rickets (HVDRR): clinical heterogeneity and long-term efficacious management of eight patients from four unrelated Arab families with a loss of function VDR mutation. *J Pediatr Endocrinol Metab.* 2018;31:861–868.
236. Nicolaidou P, Tsitsika A, Papadimitriou A, et al. Hereditary vitamin D-resistant rickets in Greek children: Genotype, phenotype, and long-term response to treatment. *J Pediatr Endocrinol Metab.* 2007;20:245–430.
237. Nicolescu RC, Lombert J, Cavalier E. Vitamin D-resistant rickets and cinacalcet - one more favorable experience. *Front Pediatr.* 2018;6:376.
238. Demay MB. The hair cycle and vitamin D receptor. *Arch Biochem Biophys.* 2012;523:19–21.
239. Tiosano D, Hadad S, Chen Z, et al. Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. *J Clin Endocrinol Metab.* 2011;96:3701–3709.
240. Chen H, Hewison M, Adams JS. Functional characterization of heterogeneous nuclear ribonucleoprotein C1/C2 in vitamin D resistance: a novel response element-binding protein. *J Biol Chem.* 2006;281:39114–39120.
241. Ruppe MD, Jan de Beur SM. Disorders of phosphate homeostasis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:317–325.
242. Chesher D, Oddy M, Darbar U, et al. Outcome of adult patients with X-linked hypophosphatemia caused by PHEX gene mutations. *J Inher Metab Dis.* 2018;41:865–876.
243. Scheinman SJ, Carpenter T, Drezner MK. Hereditary hypophosphatemic rickets and tumor-induced osteomalacia. *UpToDate.* 2019;1–39.
244. Gaucher C, Walrant-Debray O, Nguyen TM, et al. PHEX analysis in 118 pedigrees reveals new genetic clues in hypophosphatemic rickets. *Hum Genet.* 2009;125:401–411.
245. Rowe PSN. Regulation of bone-renal mineral and energy metabolism: The PHEX, FGF23, DMP1, MEPE ASARM pathway. *Crit Rev Eukaryotic Gene Express.* 2012;22:61–86.
246. Staines KA, MacRae VE, Farquharson C. The importance of the SIBLING family of proteins on skeletal mineralisation and bone remodelling. *J Endocrinol.* 2012;214:241–255.
247. Martin A, David V, Laurence JS, et al. Degradation of MEPE, DMP1, and release of SIBLING ASARM-peptides (minhibins): ASARM-peptide(s) are directly responsible for defective mineralization in HYP. *Endocrinology.* 2008;149:1757–1772.
248. Addison WN, Nakano Y, Loisel T, et al. MEPE-ASARM peptides control extracellular matrix mineralization by binding to hydroxyapatite: An inhibition regulated by PHEX cleavage of ASARM. *J Bone Miner Res.* 2008;23:1638–1649.
249. Kinoshita Y, Fukumoto S. X-linked hypophosphatemia and FGF23-related hypophosphatemic diseases: Prospect for new treatment. *Endocr Rev.* 2018;39:274–291.

250. Gattineni J, Baum M. Genetic disorders of phosphate regulation. *Pediatr Nephrol*. 2012;27:1477–1487.
251. Sabbagh Y, Carpenter TO, Demay M. Hypophosphatemia leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes. *Proc Natl Acad Sci*. 2005;102:9637–9642.
252. Clinkenbeard EL, White KE. Heritable and acquired disorders of phosphate metabolism: Etiologies involving FGF23 and current therapeutics. *Bone*. 2017;102:31–39.
253. Martín A, David V, Quarles LD. Regulation and function of the FGF23/klotho endocrine pathway. *Physiol Rev*. 2012;92:131–155.
254. White KE, Larsson TE, Econs MJ. The roles of specific genes implicated as circulating factors in normal and disordered phosphate homeostasis: frizzled related protein-4, matric extracellular phosphoglycoprotein, and fibroblast growth factor 23. *Endocr Rev*. 2006;27:221–241.
255. Benet-Pages A, Lorenz-Depiereux B, Zischka H, et al. FGF23 is processed by proprotein convertases but not by PHEX. *Bone*. 2004;35:455–462.
256. Burnett SAM, Gunawardene SC, Bringhurst FR, et al. Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. *J Bone Miner Res*. 2006;21:1187–1196.
257. Hori M, Shimizu Y, Fukumoto S. Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology*. 2011;152:4–10.
258. Goji K, Ozaki K, Sadewa AH, et al. Somatic and germline mosaicism for a mutation of the PHEX gene can lead to genetic transmission of X-linked hypophosphatemic rickets that mimics an autosomal dominant trait. *J Clin Endocrinol Metab*. 2006;91:365–370.
259. Carpenter TO, Whyte MP, Imel EA, et al. Burosumab therapy in children with X-linked hypophosphatemia. *N Engl J Med*. 2018;378:1987–1998.
260. Imel EA, Glorieux FH, Whyte MP, et al. Burosumab versus conventional therapy in children with X-linked hypophosphatemia; a randomized active-controlled, open-label, phase 3 trial. *Lancet*. 2019;393.
261. Yuan B, Feng JQ, Bowman S, et al. Hexa-D-arginine treatment increases 7B2*PC2 activity in hyp-mouse osteoblasts and rescues the HYP phenotype. *J Bone Miner Res*. 2013;28:56–72.
262. Bai X, Miao D, Xiao S, et al. Cyp24 inhibition as a therapeutic target in FGF23-mediated renal phosphate wasting disorders. *J Clin Invest*. 2016;126:667–680.
263. Makitie O, Doria A, Kooh SW, et al. Early treatment improves growth and biochemical and radiographic outcome in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*. 2003;88:3591–3597.
264. Zivcinkjak M, Schnabel D, Billing H, et al. Age-related stature and linear body segments in children with X-linked hypophosphatemic rickets. *Pediatr Nephrol*. 2011;26:223–231.
265. Zivcinkjak M, Schnabel D, Staudé H, et al. Three-year growth hormone treatment in short children with X-linked hypophosphatemic rickets Effects on linear growth and body disproportion. *J Clin Endocrinol Metab*. 2011;96:E2097–E2105.
266. Carpenter TO, Imel EA, Ruppé MD, et al. Randomized trial of the anti-FGF23 antibody KRN23 in X-linked hypophosphatemia. *J Clin Invest*. 2014;124:1587–1597.
267. Brownstein CA, Adler F, Nelson-Williams C, et al. A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proc Natl Acad Sci*. 2008;105:3455–3460.
268. Watanabe R, Fujita N, Sato Y, et al. Enppi is an anti-aging factor that regulates Klotho under phosphate overload conditions. *Sci Rep*. 2017;7:7786.
269. Below JE, Earl DL, Shively KM, et al. Whole-genome analysis reveals that mutations in inositol polyphosphate phosphatase-like 1 cause opsismodysplasia. *Am J Hum Genet*. 2013;92:137–143.
270. Huber C, Fageih EA, Bartholdi D, et al. Exome sequencing identifies INPPL1 mutations as a cause of opsismodysplasia. *Am J Hum Genet*. 2013;92:144–149.
271. Tagliabracci VS, Engel JL, Wiley SL, et al. Dynamic regulation of FGF23 by Fam20C phosphorylation GalNAc-T3 glycosylation, and furin proteolysis. *PNAS*. 2014;111:5520–5525.
272. Shulman DI, Hahn G, Benator R, et al. Tumor-induced rickets: Usefulness of MR gradient echo recall imaging for tumor localization. *J Pediatr*. 2004;144:381–385.
273. Carpenter TO, Ellis BK, Insogna KL, et al. Fibroblast growth factor 7: An inhibitor of phosphate transport derived from oncogenic osteomalacia-causing tumors. *J Clin Endocrinol Metab*. 2005;90:1012–1020.
274. Ward LM, Rauch F, White KE, et al. Resolution of severe, adolescent-onset hypophosphatemic rickets following removal of an FGF-23-producing tumour of the distal ulna. *Bone*. 2004;34:905–911.
275. Segawa H, Aranami F, Kaneko I, et al. The roles of Na/Pi-II transporters in phosphate metabolism. *Bone*. 2009;45(1):S2–S7.
276. Magen D, Berger L, Coady MJ, et al. A loss-of-function mutation in NaPi-IIa and renal Fanconi's syndrome. *N Engl J Med*. 2010;362:1102–1109.
277. Courbebaisse M, Leroy C, Bakouh N, et al. A new human NHERF1 mutation decreases renal phosphate transporter NPT2a expression by a PTH-independent mechanism. *PLoS One*. 2012;7(4):e34764.
278. Levi M, Breusegem S. Renal phosphate-transporter regulatory proteins and nephrolithiasis. *N Engl J Med*. 2008;359:1171–1173.
279. Tebben PJ, Thomas LF, Kumar R. Fanconi syndrome and renal tubular acidosis. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 6th ed. Washington, DC: American Society for Bone and Mineral Research; 2006:354–358.
280. Mannstadt M, Magen D, Segawa H, et al. Fanconi-Bickel syndrome and autosomal recessive proximal tubulopathy with hypercalciuria (ARPTH) are allelic variants caused by GLUT2 mutations. *J Clin Endocrinol Metab*. 2012;97:E1978–E1986.
281. Stanescu DE, Hughes N, Kaplan B, et al. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab*. 2012;97:E2026–E2030.
282. Marable SS, Chung E, Adam M, et al. Hnf4a deletion in the mouse kidney phenocopies Fanconi renotubular syndrome. *JCI Insight*. 2018;3(14).
283. Sprecher E. Familial tumoral calcinosis: from characterization of a rare phenotype to the pathogenesis of ectopic calcification. *J Invest Dermatol*. 2010;130:652–660.
284. Covic A, Vervloet M, Massy ZA, et al. Bone and mineral disorders in chronic kidney disease: implications for cardiovascular health and ageing in the general population. *Lancet Diabetes Endocrinol*. 2018;6:315–331.
285. Ichikawa S, Imel EA, Kreiter ML, et al. A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest*. 2007;117:2684–2691.
286. Stevenson DA, Carey JC, Coburn SP. Autosomal recessive hypophosphatemia manifesting in utero with long bone deformity but showing spontaneous postnatal improvement. *J Clin Endocrinol Metab*. 2008;93:3443–3448.
287. Wenkert D, McAlister WH, Coburn SP, et al. Hypophosphatasia: nonlethal disease despite skeletal presentation in utero (17 new cases and literature review). *J Bone Miner Res*. 2011;26:2389–2398.
288. Zankl A, Mornet E, Wong S. Specific ultra-sonographic features of perinatal lethal hypophosphatasia. *Am J Med Genet A*. 2008;146A:1200–1204.
289. Whyte MP, Mumm S, Deal C. Adult hypophosphatasia treated with teriparatide. *J Clin Endocrinol Metab*. 2007;92:1203–1208.
290. Bowden SA, Foster BL. Profile of alfofosate alfa in the treatment of hypophosphatasia: design, development, and place in therapy. *Drug Design Dev Ther*. 2018;12:3147–3161.
291. Kishnani PS, Rockman-Greenberg C, Rauch F, et al. Five-year efficacy and safety of asfotase alfa therapy for adults and adolescents with hypophosphatasia. *Bone*. 2019;121:149–162.
292. Whyte MP, Fujita KP, Mosley S, et al. Validation of a novel scoring system for changes in skeletal manifestations of hypophosphatasia in newborns, infants, and children: The Radiographic Global Impression of Change Scale. *J Bone Miner Res*. 2018;33:868–874.
293. Fawaz R, Israel R. Transient hyperphosphatasemia of infancy and early childhood. *UpToDate*. 2019;1–13.
294. Schonhait BL, Rocha RA. Benign transient hyperphosphatasemia in infants, clinical series. *Rev Child Pediatr*. 2017;88:176–181.
295. Huh SY, Feldman HA, Cox JE, Gordon M. Prevalence of transient hyperphosphatasemia among healthy infants and toddlers. *Pediatrics*. 2009;124:703–709.

296. Knaus A, Pantel JT, Pendziwiat M, et al. Characterization of glycosylphosphatidylinositol biosynthesis defects by clinical features, flow cytometry, and automated image analysis. *Genome Med.* 2018;10:3.
297. Shroff R. Dysregulated mineral metabolism in children with chronic kidney disease. *Curr Opin Nephrol Hypertens.* 2011;20:233–240.
298. Chauhan V, Keleporis E, Chauhan N, Vaid M. Current concepts and management strategies in chronic kidney disease-mineral and bone disorder. *South Med J.* 2012;105:479–485.
299. Hou YC, Lu CL, Lu KC. Mineral bone disorders in chronic kidney disease. *Nephrology.* 2018;(Suppl 4):88–94.
300. Qunibi WY. Overview of chronic kidney disease - mineral and bone disorder (CKD-MBD). *UpToDate.* 2019;1–31.
301. Bueno de Oliveira R, Affonso-Moyses RM. FGF-23:state of the art. *J Bras Nefrol.* 2010;32:316–323.
302. De Rechter S, Bacchetta J, Godefroid N, et al. Evidence for bone and mineral metabolism alterations in children with autosomal dominant polycystic kidney disease. *J Clin Endocrinol Metab.* 2017;102:210–217.
303. Doyon A, Fischer D-C, Bayazit AK, et al. Markers of bone metabolism are affected by renal function and growth hormone therapy in children with chronic kidney disease. *PLoS One.* 2015;10:e0113482.
304. Shalhoub V, Shatzen EM, Ward SC, et al. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. *J Clin Invest.* 2012;122:2543–2553.
305. Reusz GS, Szabo AJ, Peter F, et al. Bone metabolism and mineral density following renal transplantation. *Arch Dis Child.* 2000;83:146–151.
306. Henriksen K, Bollerslev J, Everts V, Karsdal MA. Osteoclast activity and subtypes as a function of physiology and pathology - Implications for future treatment of osteoporosis. *Endocr Rev.* 2011;32:31–63.
307. Ralston S. Juvenile Paget's disease, familial expansile osteolysis and other genetic osteolytic disorders. *Best Pract Res Clin Rheumatol.* 2008;22:101–111.
308. Middleton-Hardie C, Zhu Q, Cundy H, et al. Deletion of aspartate 182 in OPG causes juvenile Paget's disease by impairing both protein secretion and binding to RANKL. *J Bone Miner Res.* 2006;21:438–445.
309. Polyzos SA, Anastasilakis AD, Litsas J, et al. Profound hypocalcemia following effective response to zoledronic acid treatment in a patient with juvenile Paget's disease. *J Bone Miner Metab.* 2010;28:706–712.
310. Crockett JC, Mellis DJ, Scott DJ, Helfrich MH. New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: focus on the RANK/RANKL axis. *Osteoporos Intern.* 2011;22:1–22.
311. Whyte MP, Tau C, McLister WH, et al. Juvenile Paget's disease with heterozygous duplication with *TTNFRSF11A* encoding RANK. *Bone.* 2014;68:153–161.
312. Crockett JC, Mellis DJ, Shennan KI, et al. Signal peptide mutations in RANK prevent downstream activation of NF-kappaB. *J Bone Miner Res.* 2011;26:1926–1938.
313. Guerrini MM, Sobacchi C, Cassani B, et al. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia. *Am J Hum Genet.* 2008;83:64–76.
314. Boyce AM, Gafni RI. Approach to the child with fractures. *J Clin Endocrinol Metab.* 2011;96:1943–1952.
315. Chevalley T, Bonjour JP, van Rietbergen B, et al. Fractures during childhood and adolescence in healthy boys: Relation with bone mass and strength. *J Clin Endocrinol Metab.* 2011;96:3134–3142.
316. Ferrari S, Bianchi ML, Eisman JA, et al. Osteoporosis in young adults: pathophysiology, diagnosis, and management. *Osteoporos Int.* 2012;23:2735–2748.
317. Bachrach LK, Sills IN. Clinical report - Bone densitometry in children and adolescents. *Pediatrics.* 2011;127:189–194.
318. Cohen A, Shane E. Premenopausal osteoporosis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:289–293.
319. Rauch F, Plotkin H, DiMeglio L, et al. Fracture prediction and the definition of osteoporosis in children and adolescents: The ISCD 2007 pediatric official positions. *J Clin Densit: Assess Clin Densit.* 2008;11:22–28.
320. Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: Results of the Bone Mineral Density in Childhood Study. *J Clin Endocrinol Metab.* 2011;96:3160–3169.
321. Bachrach LK, Gordon CM, AAP Section on Endocrinology. Bone densitometry in children and adolescents. *Pediatrics.* 2016;138(4). e20162398.
322. Grover M, Bachrach LK. Osteoporosis in children with chronic illness: Diagnosis, monitoring, and treatment. *Curr Osteoporos Res.* 2017;15:271–282.
323. Wasserman H, O'Donnell JM, Gordon CM. Use of dual energy X-ray absorptiometry in pediatric patients. *Bone.* 2017;104:84–90.
324. Vierucci F, Saggese G, Cimaz R. Osteoporosis in childhood. *Curr Opin Rheumatol.* 2017;29:535–546.
325. Hardy RS, Zhou H, Seibel MJ, Cooper MS. Glucocorticoids and bone: Consequences of endogenous and exogenous excess and replacement therapy. *Endocr Rev.* 2018;39:519–568.
326. Mazziotti G, Frara S, Giustina A. Pituitary diseases and bone. *Endocr Rev.* 2018;39:440–488.
327. Compston J. Glucocorticoid-induced osteoporosis: an update. *Endocrine.* 2018;61:7–16.
328. Harvey N, Dennison E, Cooper C. Epidemiology of osteoporotic fractures. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:198–203.
329. Hsu Y-H, Kiel DP. Genome-wide associations studies of skeletal phenotypes: What we have learned and where we are headed. *J Clin Endocrinol Metab.* 2012;97:E1958–E1977.
330. Wosje KS, Khoury PR, Clayton RP, et al. Adiposity and TV viewing are related to less bone accrual in young children. *J Pediatr.* 2009;154:79–84.
331. Janicka A, Wren TAL, Sanchez MM, et al. Fat mass is not beneficial to bone in adolescents and young adults. *J Clin Endocrinol Metab.* 2007;92:143–147.
332. Kindler JM, Lobene AJ, Vogel KA, et al. Adiposity, insulin resistance, and bone mass in children and adolescents. *J Clin Endocrinol Metab.* 2019;104:892–899.
333. Kessler J, Koebnick C, Smith N, Adams A. Childhood obesity is associated with increased risk of most lower extremity fractures. *Clin Orthop Relat Res.* 2012;471(4):1197–1204.
334. Fazeli PK, Klibanski A. Effects of anorexia nervosa on bone mineralization. *Endocr Rev.* 2018;39:895–910.
335. Reid IR, Baldock PA, Cornish J. Effects of leptin on the skeleton. *Endocr Rev.* 2018;39:938–959.
336. Pitukcheewanont P, Punyasavatsut N, Feuilee M. Physical activity and bone health in children and adolescents. *Pediatr Endocrinol Rev.* 2010;7:275–282.
337. Zacharin M. Current advances in bone health of disabled children. *Curr Opin Pediatr.* 2004;16:545–551.
338. Henderson JK, Kairalla JA, Barrington JW, et al. Longitudinal changes in bone density in children and adolescents with moderate to severe cerebral palsy. *J Pediatr.* 2005;146:769–775.
339. Ali O, Shim M, Fowler E, et al. Growth hormone therapy improves bone mineral density in children with cerebral palsy: A preliminary pilot study. *J Clin Endocrinol Metab.* 2007;92:932–937.
340. Paksu M, Vurucu S, Karaoglu A, et al. Osteopenia in children with cerebral palsy can be treated with oral alendronate. *Child Nerv Syst.* 2012;28:283–286.
341. Drake MT, Khosla S. Role of sex steroids in the pathogenesis of osteoporosis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:208–213.
342. Faienza MF, Ventura A, Colucci S, et al. Bone fragility in Turner syndrome: Mechanisms and prevention strategies. *Front Endocrinol.* 2006;7:34.
343. Bakalov VK, Foodim J, Bondy CA. Bone density and fractures in Turner syndrome. *Int Cong Series.* 2006;1298:160–167.
344. Nour MA, Burt LA, Perry RJ, et al. Impact of growth hormone on adult bone quality in Turner syndrome: A HR-pQCT study. *Calcif Tissue Int.* 2016;98:49–59.

345. Antoniazzi F, Monti E, Gaudino R, et al. Bone density in children treated with gonadotropin-releasing hormone analogs for central precocious puberty. *Exp Rev Endocrinol Metab.* 2010;5:285–290.
346. DiVasta AD, Gordon CM. Bone health in adolescents. *Adolesc Med.* 2006;17:639–652.
347. Buckley L, Humphrey MB. Glucocorticoid-induced osteoporosis. *N Engl J Med.* 2018;379:2547–2556.
348. Hsu E, Nanes M. Advances in treatment of glucocorticoid-induced osteoporosis. *Curr Opin Endocrinol Diabetes Obes.* 2017;24:411–417.
349. Van Staa TP. The pathogenesis, epidemiology and management of glucocorticoid-induced osteoporosis. *Calcif Tissue Int.* 2006;79:129–137.
350. Dore RK. How to prevent glucocorticoid-induced osteoporosis. *Cleveland Clin J Med.* 2010;77:529–536.
351. Allen DB. Effects of inhaled steroids on growth, bone metabolism, and adrenal function. *Adv Pediatr.* 2006;53:101–110.
352. Kelly HW, Van Natta ML, Covar RA, et al. Effect of long-term corticosteroid use on bone mineral density in children: A prospective longitudinal assessment in the Childhood Asthma Management Program (CAMP) study. *Pediatrics.* 2008;122:e53–e61.
353. Holm IA. Do short courses of oral corticosteroids and use of inhaled corticosteroids affect bone health in children? *Nat Clin Pract Endocrinol Metab.* 2009;5:132–133.
354. Wong CA, Walsh LJ, Smith CJP, et al. Inhaled corticosteroid use and bone mineral density in patients with asthma. *Lancet.* 2000;355:1399–1403.
355. Tritos NA, Klibanski A. Effects of growth hormone on bone. *Prog Mol Biol Translat Sci.* 2016;138:193–211.
356. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev.* 2008;29:535–559.
357. Tahimic CGT, Wang Y, Bikle DD. Anabolic effects of IGF-1 signaling on the skeleton. *Front Endocrinol.* 2013;4:6.
358. Bassett JHD, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. *Endocr Rev.* 2016;37:135–187.
359. Sundaraghavan V, Mazur MM, Evans B, et al. Diabetes and bone health: latest evidence and clinical implications. *Ther Adv Musculoskel Dis.* 2017;9:67–74.
360. Zhukouskaya VV, Shepelkevich AP, Chiodini I. Bone health in type 1 diabetes: Where we are now and how we should proceed. *Adv Endocrinol.* 2014. ID 982129.
361. Bechtold S, Dirlenbach I, Raile K, et al. Early manifestation of type 1 diabetes in children is a risk factor for changed bone geometry: Data using peripheral quantitative computed tomography. *Pediatrics.* 2006;118:627–634.
362. Chaiban J, Muwakkit S, Arabi A, et al. Modeling pathways for low bone mass in children with malignancies. *J Clin Densit: Assess Skeletal Health.* 2009;12:441–449.
363. George S, Weber DR, Kaplan P, et al. Short-term safety of zoledronic acid in young patients with bone disorders: An extensive institutional experience. *J Clin Endocrinol Metab.* 2015;100:463–471.
364. Ebeling P. Transplantation osteoporosis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:279–285.
365. Rovner A, Zemel BS, Leonard MB, et al. Mild to moderate cystic fibrosis is not associated with increased fracture risk in children and adolescents. *J Pediatr.* 2005;147:327–331.
366. Larussa T, Suraci E, Nazionale I, et al. Bone mineralization in celiac disease. *Gastroenterol Res Pract.* 2012;2012. ID 198025.
367. Carmo FB, Terreri MT, Succu RCM, et al. Bone mineral density and vitamin D concentration: the challenges in taking care of children and adolescents with HIV. *Braz J Infect Dis.* 2017;21:270–275.
368. Imerci A, Canbek U, Haghart S, et al. Idiopathic juvenile osteoporosis: A case report and review of the literature. *Int J Surg Case Rep.* 2015;9:127–129.
369. Rauch F, Bishop N. Juvenile osteoporosis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:264–267.
370. Hartikka H, Makiö O, Mannikko M, et al. Heterozygous mutations in the LDL receptor-related protein 5 (LRP5) gene are associated with primary osteoporosis in children. *J Bone Miner Res.* 2005;20:783–789.
371. Glass II DA, Karsenty G. Minireview: *In vivo* analysis of Wnt signaling in bone. *Endocrinology.* 2007;148:2630–2634.
372. Baron R, Rawadi G. Minireview: Targeting the Wnt/ β -catenin pathway to regulate bone formation in the adult skeleton. *Endocrinology.* 2007;148:2635–2643.
373. Ke HZ, Richards WC, Li X, Ominsky MS. Sclerostin and Dkkopf-1 as therapeutic targets in bone diseases. *Endocr Rev.* 2012;33:747–783.
374. Ai M, Heeger S, Barteks CF, et al. Clinical and molecular findings in osteoporosis-pseudoglioma syndrome. *Am J Hum Genet.* 2005;77:741–753.
375. Balesman W, Van Hul W. Minireview: The genetics of low density Lipoprotein Receptor-Related Protein 5 in bone: A story of extremes. *Endocrinology.* 2007;148:2622–2629.
376. Tuysuz B, Bursali A, Alp Z, et al. Osteoporosis-pseudoglioma syndrome: Three novel mutations in the LRP5 gene and response to bisphosphonate treatment. *Horm Res Paediatr.* 2012;77:115–120.
377. Tauer JT, Robinson M-E, Rauch F. Osteogenesis imperfecta: new perspectives from clinical and translational research. *J Bone Miner Res.* 2019;3(8). e10174.
378. Van Dijk FS, Pals G, Vam Rijn RR, et al. Classification of osteogenesis imperfecta revisited. *Eur J Med Genet.* 2010;53:1–5.
379. Lim J, Grafe I, Alexander S, Lee B. Genetic causes and mechanisms of osteogenesis imperfecta. *Bone.* 2017;102:40–49.
380. Vuorimies I. *Bisphosphonate treatment in children with osteogenesis imperfecta.* Helsinki, Finland: Benefits and concerns. Academic dissertation. Doctoral School in Health Sciences, University of Helsinki; 2017.
381. Lapunzina P, Aglan M, Temtamy S, et al. Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. *Am J Hum Genet.* 2010;87:110–114.
382. Zhou X, Zhang Z, Feng JQ, et al. Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. *Proc Natl Acad Sci.* 2010;107:12919–12924.
383. Doyard M, Bacrot S, Huber C, et al. FAM46A mutations are responsible for autosomal recessive osteogenesis imperfecta. *J Med Genet.* 2018;278–284.
384. Wang JY, Liu Y, Song LJ, et al. Novel mutations in SERPINF1 result in rare osteogenesis imperfecta type VI. *Calcif Tissue Int.* 2017;100:55–66.
385. Kannu P, Mahjoub A, Babul-Hirji R, et al. PLS3 mutations in X-linked osteoporosis: Clinical and bone characteristics of two novel mutations. *Horm Res Paediatr.* 2017;.
386. Lindahl K, Barnes AM, Fratzi-Zelman N, et al. COL1 C-propeptide cleavage site mutations cause high bone mass osteogenesis imperfecta. *Hum Mutat.* 2011;32:598–609.
387. Cabral WA, Makareeva E, Colige A, et al. Mutations near amino end of alpha-1(I) collagen cause combined osteogenesis imperfecta/Ehlers-Danlos syndrome by interfering with N-propeptide processing. *J Biol Chem.* 2005;280:19259–19269.
388. Marakeeva E, Cabral WA, Marini JC, Leikin S. Molecular mechanism of alpha-1(I)-osteogenesis imperfecta/Ehlers-Danlos syndrome: unfolding of an N-anchor domain at the N-terminal end of the type I collagen triple helix. *J Biol Chem.* 2006;281:6463–6470.
389. Cabral WA, Chang W, Barnes AM, et al. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nat Genet.* 2007;39:359–365.
390. Willaert A, Malfait F, Symoens S, et al. Recessive osteogenesis imperfecta cause by LEPRE1 mutations: clinical documentation and identification of the splice form responsible for prolyl 3-hydroxylation. *J Med Genet.* 2009;46:233–241.
391. Cabral WA, Barnes AM, Adevemo A, et al. A founder mutation in LEPRE1 carried by 1.5% of West Africans and 0.4% of African Americans causes lethal recessive osteogenesis imperfecta. *Genet Med.* 2012;14:543–551.
392. Chang W, Barnes AM, Cabral WA, et al. Prolyl 3-hydroxylase 1 and CRTAP are mutually stabilizing in the endoplasmic reticulum collagen prolyl 3-hydroxylation complex. *Hum Mol Genet.* 2010;19:223–234.
393. Van Dijk FS, Nesbitt IM, Zwikstra EH, et al. PPIB mutations cause severe osteogenesis imperfecta. *Am J Hum Genet.* 2009;85:521–527.

394. Barnes AM, Carter EM, Cabral WA, et al. Lack of cyclophilin B in osteogenesis imperfecta with normal collagen folding. *N Engl J Med*. 2010;362:521–528.
395. Pyott SM, Schwarze U, Christiansen HE, et al. Mutations in PPIB (cyclophilin B) delay type I procollagen chain association and result in perinatal lethal to moderate osteogenesis imperfecta phenotypes. *Hum Mol Genet*. 2011;20:1595–1609.
396. Morello R, Bertin TK, Chen Y, et al. CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell*. 2006;127:291–304.
397. Barnes AM, Chang W, Morello R, et al. Deficiency of cartilage-associated protein in lethal osteogenesis imperfecta. *N Engl J Med*. 2006;355:2757–2764.
398. Amor IM, Rauch F, Gruenwald K, Weis M, et al. Severe osteogenesis imperfecta caused by a small in-frame deletion in CRTAP. *Am J Med Genet Part A*. 2011;155A:2865–2870.
399. Asharani PV, Keupp K, Seemler O, et al. Attenuation of BMP1 function compromises osteogenesis leading to bone fragility in humans and zebrafish. *Am J Med Genet*. 2012;90:661–674.
400. Martinez-Glen V, Valencia M, Caparros-Martin JA, et al. Identification of a mutation causing deficient BMP1/mTLD proteolytic activity in autosomal recessive osteogenesis imperfecta. *Hum Mutat*. 2012;33:343–350.
401. Cho TJ, Lee KE, Lee SK, et al. A single recurrent mutation in the 5'-UTR of IFITM5 causes osteogenesis imperfecta type V. *Am J Hum Genet*. 2012;91:343–348.
402. Semler O, Garbes L, Keupp K, et al. A mutation in the 5'-UTR of IFITM5 creates an in-frame start codon and causes autosomal-dominant osteogenesis imperfecta type V with hyperplastic callus. *Am J Hum Genet*. 2012;91:349–357.
403. Rauch F, Moffatt P, Cheung M, et al. Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C-T mutation in all patients. *J Med Genet*. 2013;13:21–24.
404. Brizola E, Mattos EP, Ferrari J, et al. Clinical and molecular characterization of osteogenesis imperfecta type V. *Mol Syndromol*. 2015;6:164–172.
405. Farber CR, Reich A, Barnes AM, et al. A novel IFITM5 mutation in severe atypical osteogenesis imperfecta type VI impairs osteoblast production of pigment epithelium-derived factor. *J Bone Miner Res*. 2014;29:1402–1411.
406. Hanagata H, Li X, Morita H, et al. Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. *J Bone Miner Metab*. 2011;29:279–290.
407. Becker J, Semler O, Gilissen C, et al. Exome sequencing identifies truncating mutations in human *SERPINF1* in autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*. 2011;88:362–371.
408. Homan EP, Rauch F, Grafe I, et al. Mutations in *SERPINF1* cause osteogenesis imperfecta VI. *J Bone Miner Res*. 2011;26:2798–2803.
409. Venturi G, Gandini A, Monti E, et al. Lack of expression of *SERPINF1*, the gene coding for pigment epithelium-derived factor, causes progressively deforming osteogenesis imperfecta with normal type I collagen. *J Bone Miner Res*. 2012;27:723–728.
410. Rauch F, Hussein A, Roughley P, et al. Lack of circulating pigment epithelium-derived factor is a marker of osteogenesis imperfecta type VI. *J Clin Endocrinol Metab*. 2012;97:E1550–E1556.
411. Barnes AM, Cabral WA, Weis M, et al. Absence of FKBP10 in recessive type XI osteogenesis imperfecta leads to diminished collagen cross-linking and reduced collagen deposition in extracellular matrix. *Hum Mutat*. 2012;33:1589–1598.
412. Alanay Y, Avaygan H, Camacho N, et al. Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*. 2010;86:551–559.
413. Kelley BP, Malfait F, Bonafe L, et al. Mutations in FKBP10 cause recessive osteogenesis imperfecta and Bruck syndrome. *J Bone Miner Res*. 2011;26:666–672.
414. Venturi G, Monti E, Dalle Carbonare L, et al. A novel splicing mutation in FKBP10 causing osteogenesis imperfecta with a possible mineralization defect. *Bone*. 2012;50:343–349.
- 414a. Christiansen HE, Schwarze U, Pyott SM, et al. Homozygosity for a missense mutation in *SERPINH1*, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am J Hum Genet*. 2010;86:389–398.
415. Masago Y, Hosoya A, Kawasaki K, et al. The molecular chaperone Hsp47 is essential for cartilage and endochondral bone formation. *J Cell Sci*. 2012;125:1118–1128.
416. Widmer C, Gebauer JM, Brunstein E, et al. Molecular basis for the action of collagen-specific Hsp47/SERPINH1 and its structure-specific client recognition. *Proc Natl Acad Sci USA*. 2012;109:13243–13247.
417. Szymoens S, Malfait F, D'hondt S, et al. Deficiency for the ER-stress transducer OASIS causes severe recessive osteogenesis imperfecta in humans. *Orphanet J Rare Dis*. 2013;8:154.
418. Lindert U, Cabral WA, Ausavarat S, et al. *MBPTS2* mutations cause defective regulated intramembrane proteolysis in X-linked osteogenesis imperfecta. *Nat Comm*. 2016;7:11920.
419. Ichimura A, Takeshima H. *TRIC-B* mutations causing osteogenesis imperfecta. *Biol Pharm Bull*. 2016;39:1743–1747.
420. Shaheen R, Alazami AM, Alshammart MJ, et al. Study of autosomal recessive osteogenesis imperfecta in Arabia reveals a novel locus defined by *TMEM38B* mutation. *J Med Genet*. 2012;49:630–635.
421. Volodarsky M, Markus Cohen, I, et al. A deletion mutation in *TMEM38B* associated with autosomal recessive osteogenesis imperfecta. *Hum Mutat*. 2013;34(4):584–586.
422. Bianchi ML, Leonard MB, Bechtold S, et al. Bone health in children and adolescents with chronic diseases that may affect the skeleton: The 2013 ISCD pediatric official positions. *J Clin Densitomet*. 2014;17:281–294.
423. Simm PJ, Biggin A, Zacharin MR, et al. Consensus guidelines on the use of bisphosphonate therapy in children and adolescents. *J Paediatr Child Health*. 2018;54:223–233.
424. Kindler JM, Lappe JM, Gilsanz V, et al. Lumbar spine bone mineral apparent density in children: Results from the Bone Mineral Density in Childhood study. *J Clin Endocrinol Metab*. 2019;104:1283–1292.
425. Topor LS, Melvin P, Giancaterino C, Gordon CM. Factors associated with low bone density in patients referred for assessment of bone health. *Int J Pediatr Endocrinol*. 2013;2013(1):4.
426. Rauchenzauner M, Schmid A, Heinz-Erian P, et al. Sex- and age-specific reference curves for serum markers of bone turnover in healthy children 2 months to 18 years. *J Clin Endocrinol Metab*. 2007;92:443–449.
427. Nishizawa Y, Ohia H, Miora M, et al. Guidelines for the use of bone metabolic markers in the diagnosis and treatment of osteoporosis (2012 edition). *J Bone Miner Metab*. 2013;31:1–15.
428. Kleerekoper M. Overview of osteoporosis treatment. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:220–221.
429. Bachrach LK, Ward LM. Clinical Review: Bisphosphonate use in childhood osteoporosis. *J Clin Endocrinol Metab*. 2009;94:400–409.
430. Guo B, Peng S, Liang C, et al. Recent developments in bone anabolic therapy for osteoporosis. *Expert Rev Endocrinol Metab*. 2012;7:677–685.
431. Lindsay R. Estrogens and SERMS. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:234–236.
432. Zaidi M. Skeletal remodeling in health and disease. *Nat Med*. 2007;13:791–801.
433. Adami S. Calcitonin. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:250–251.
434. Haas AV, LeBoff ME. Osteoanabolic agents for osteoporosis. *J Endocr Soc*. 2018;2:922–932.
435. Bowden SA, Mahan JD. Zoledronic acid in pediatric metabolic bone disease. *Transl Pediatr*. 2017;6:256–268.
436. Rogers MJ, Crockett JC, Coxon FP, Monkkonen J. Biochemical and molecular mechanisms of action of bisphosphonates. *Bone*. 2011;49:34–41.
437. Nasomyont N, Homung LN, Gordon CM, Wasserman H. Outcomes following intravenous bisphosphonate infusion in pediatric patients: A 7-year retrospective chart review. *Bone*. 2019;121:60–67.
438. Odvina CY, Zerwekh JE, Sudhaker R, et al. Severely suppressed bone turnover: A potential complication of alendronate therapy. *J Clin Endocrinol Metab*. 2005;90:1294–1301.
439. Chan B, Zacharin M. Maternal and infant outcome after pamidronate treatment of polyostotic fibrous dysplasia and osteogenesis

- imperfecta before conception: A report of four cases. *J Clin Endocrinol Metab.* 2006;91:2107–2020.
440. Bellido T, Ali AA, Gubrij J, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: A novel mechanism for hormonal control of osteoblastogenesis. *Endocrinology.* 2005;146:4577–4583.
 441. Shapiro JR, Sponsellor PD. Osteogenesis imperfecta: questions and answers. *Curr Opin Pediatr.* 2010;21:709–716.
 442. Land C, Rauch F, Munns CF, et al. Vertebral morphometry in children and adolescents with osteogenesis imperfecta: effect of intravenous pamidronate treatment. *Bone.* 2006;39:901–906.
 443. Hoyer-Kuhn H, Franklin J, Allo G, Kron M, Netzer C, Eysel P, et al. Safety and efficacy of denosumab in children with osteogenesis imperfecta—a first prospective trial. *J Musculoskelet Neuronal Interact.* 2016;16(1):24–32.
 444. Hoyer-Kuhn H, Stark C, Franklin J, et al. Correlation of bone mineral density on quality of life in patients with osteogenesis imperfecta during treatment with denosumab. *Pediatr Endocrinol Rev.* 2017;15(Suppl 1):123–129.
 445. Rehberg M, Winzenrieth R, Hoyer-Kuhn H, et al. TBS as a tool to differentiate the impact of antiresorptives on cortical and trabecular bone in children with osteogenesis imperfecta. *J Clin Densitom.* 2019;22:229–235.
 446. Ljunggren O, Lindahl K, Rubin CJ, Kindmark A. Allele-specific gene silencing in osteogenesis imperfecta. *Endocr Dev.* 2011;21:85–90.
 447. Besio R, Forlino A. New frontiers for dominant osteogenesis imperfecta treatment: gene/cellular therapy approaches. *Adv Regener Med.* 2015;2:1.
 448. Collins MT, Riminucci M, Bianco P. Fibrous dysplasia. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:423–427.
 449. Riddle ND, Bui MM. Fibrous dysplasia. *Arch Pathol Lab Med.* 2013;137:134–138.
 450. Tafti D, Cecava ND. Fibrous dysplasia. In: *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019.*
 451. Weinstein LS. G_{α} mutations in fibrous dysplasia and McCune-Albright syndrome. *J Bone Miner Res.* 2006;21(Suppl 2):P120–P124.
 452. Boyce AM, Kelly MH, Brillante BA, et al. A randomized, double blind, placebo-controlled trial of alendronate treatment for fibrous dysplasia of bone. *J Clin Endocrinol Metab.* 2014;99:4133–4140.
 453. Majoor BC, Appelman-Dijkstra NM, Fiocco M, et al. Outcome of long-term bisphosphonate therapy in McCune-Albright syndrome and polyostotic fibrous dysplasia. *J Bone Miner Res.* 2017;32:264–276.
 454. Wang Y, Wang O, Jiang Y, et al. Efficacy and safety of bisphosphonate therapy in McCune-Albright syndrome-related polyostotic fibrous dysplasia: A single-center experience. *Endocr Pract.* 2019;24:23–30.
 455. Boyce AM, Chung WH, Yao J, et al. Denosumab treatment for fibrous dysplasia. *J Bone Miner Res.* 2012;27:1462–1470.
 456. Teti A, Econs MJ. Osteopetroses, emphasizing potential approach to treatment. *Bone.* 2017;102:50–59.
 457. Wu CC, Econs MJ, DiMeglio LA, et al. Diagnosis and management of osteopetrosis: Consensus guidelines from the Osteopetrosis Working Group. *J Clin Endocrinol Metab.* 2017;3111–3123.
 458. Van Lierop AH, Hamdy NAT, van Bezooijen RL, et al. The role of sclerostin in the pathophysiology of sclerosing bone dysplasias. *Clin Rev Bone Miner Metab.* 2012;10:108–116.
 459. Boudin E, Yorgan T, Fijalkowski I, et al. The LRP4 1170Q homozygous knock-in mouse recapitulates the bone phenotype of sclerosteosis in humans. *J Bone Miner Res.* 2017;32:1739–1749.
 460. Pangrazio A, Cassani B, Guerrini MM, et al. RANK-dependent autosomal recessive osteopetrosis: Characterization of five new cases with novel mutations. *J Bone Miner Res.* 2012;27:342–351.
 461. Waguespack SG, Hui SL, DiMeglio LA, Econs MJ. Autosomal dominant osteopetrosis: Clinical severity and natural history of 94 subjects with a chloride channel 7 gene mutation. *J Clin Endocrinol Metab.* 2007;92:771–778.
 462. Douni E, Rinotas V, Makrinou E, et al. A RANKL G278R mutation causing osteopetrosis identifies a functional amino acid essential for trimer assembly in RANKL and TNF. *Hum Mol Genet.* 2012;21:784–798.
 463. Sobacchi C, Frattini A, Guerrini NM, et al. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet.* 2007;39:960–962.
 464. Roberts CM, Angus JE, Leach IH, et al. A novel NEMO mutation causing osteopetrosis, hypohidrotic ectodermal dysplasia and immunodeficiency (OL-HED-ID). *Eur J Pediatr.* 2010;169:1403–1407.
 465. Gheorge G, Galambos C, Jain S, et al. A novel TCRG1 mutation leads to severe osteopetrosis with altered content of monocytes/macrophages in several organs. *Pediatr Develop Pathol.* 2012;15:156–159.
 466. Aker M, Rouvinski A, Hashavia S, et al. An SNX10 mutation causes malignant osteopetrosis of infancy. *J Med Genet.* 2012;49:221–226.
 467. Stattin E-L, Henning P, Klar J, et al. SNX10 gene mutation leading to osteopetrosis with dysfunctional osteoclasts. *Sci Rep.* 2017;7:3012.
 468. Weinert S, Jabs S, Supancharat C, et al. Lysosomal pathology and osteopetrosis upon loss of H+-driven lysosomal Cl- accumulation. *Science.* 2010;328:1401–1403.
 469. Sobacchi C, Villa A, Schulz A, et al. CLCN7-related osteopetrosis. In: Adam MP, Ardinger HH, Pagon RA, et al. eds. *Gene Reviews NBK1127.* Seattle: University of Washington; 2015:1–34.
 470. Kantaputra PH, Thawanaphong S, Issarangporn W, et al. Long-term survival in infantile malignant autosomal recessive osteopetrosis secondary to homozygous p.Arg526Gln mutation in CLCN7. *Am J Med Genet Part A.* 2012;158A:909–916.
 471. Lange PF, Wartosch L, Jentsch TJ, Fuhrmann JC. CIC-7 requires Ostm1 as a β -subunit to support bone resorption and lysosomal function. *Nature.* 2006;440:220–223.
 472. Maranda B, Chabot G, Decarle JC, et al. Clinical and cellular manifestations of OSTM1-related infantile osteopetrosis. *J Bone Miner Res.* 2008;23:296–300.
 473. Overholt KM, Rose MJ, Joshi S, et al. Hematopoietic cell transplantation for a child with OSTM1 osteopetrosis. *Blood Adv.* 2017;1:279–281.
 474. Shin B, Yu J, Park E-S, et al. Secretion of a truncated Osteopetrosis-associated Transmembrane Protein 1 (OSTM1) mutant inhibits osteoclastogenesis through down-regulation of the B Lymphocyte-induced Maturation Protein 1 (BLIMP1)-Nuclear Factor of Activated T Cells (NFATc1) axis. *J Biol Chem.* 2014;289:35868–35881.
 475. Tabata K, Matsunaga K, Sakane A, et al. Rubicon and PLEKHM1 negatively regulate the endocytic/autophagic pathway via a novel Rab7-binding domain. *Mol Biol Cell.* 2010;21:4162–4172.
 476. Fujiwara T, Ye S, Castro-Gomes T, et al. PLEKHH1/DEF8/RAB7 complex regulates lysosome positioning and bone homeostasis. *JCI Insight.* 2016;1(17):e86330.
 477. Demirel F, Esen I, Tunc B, Tavil B. Scarcity despite wealth: osteopetrorickets. *J Pediatr Endocrinol Metab.* 2010;23:931–934.
 478. Stewart CG. Hematopoietic stem cell transplantation in osteopetrosis. *Pediatr Clin NA.* 2010;57:171–180.
 479. Penna S, Capo V, Palagano E, et al. One disease, many genes: Implications for the treatment of osteopetroses. *Front Endocrinol.* 2019;10:85.
 480. Shroff R, Beringer O, Rao K, et al. Denosumab for post-transplant hypercalcemia in osteopetrosis. *N Engl J Med.* 2012;367:1766–1767.
 481. Whyte MP, Wenkert D, Clements KL, et al. Bisphosphonate-induced osteopetrosis. *N Engl J Med.* 2003;349:457–463.
 482. Matar HE, James LA. A challenging paediatric pathological femur fracture in pyknodysostosis (osteopetrosis acro-osteolytica): lessons learnt. *BMJ Rep.* 2014;2014. pii:bcr2014207730.
 483. Ramaiah KKK, George GB, Padiyath S, et al. Pyknodysostosis: report of a rare case with review of literature. *Imaging Sci Dent.* 2011;41:177–181.
 484. Mandal K, Ray S, Saxena D, et al. Pyknodysostosis: mutation spectrum in five unrelated Indian children. *Clin Dysmorph.* 2016;25:113–120.
 485. Williams BO. LRP5: From bedside to bench to bone. *Bone.* 2017;102:26–30.
 486. Loots GB, Kneissel M, Keller H, et al. Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. *Genome Res.* 2005;15:928–935.

487. Leupin O, Piters E, Halleux C, et al. Bone overgrowth-associated mutations in the *LRP4* gene impair sclerostin facilitator function. *J Biol Chem*. 2011;286:19489–19500.
488. Ihde LL, Forrester DM, Gottsegen CJ, et al. Sclerosing bone dysplasias: review and differentiation from other causes of osteosclerosis. *RadioGraphics*. 2011;31:1865–1882.
489. Kim Y-M, Kang E, Choi J-H, et al. Clinical characteristics and treatment outcomes in Camurati-Engelmann disease: A case series. *Medicine*. 2018;97. e0309.
490. Hammond P, Suttie M, Hennekam RC, et al. The face signature of fibrodysplasia ossificans progressiva. *Am J Med Genet Part A*. 2012;158A:1368–1380.
491. Hatsell SJ, Idone V, Wolken DM, Huang L, Kim HJ, Wang L, et al. ACVR1R206H receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. *Sci Transl Med*. 2015;7(303). 303ra137.
492. Hino K, Ikeya M, Horigome K, Matsumoto Y, Ebise H, Nishio M, et al. Neofunction of ACVR1 in fibrodysplasia ossificans progressiva. *PNAS*. 2015;112(50):15438–15443.
493. Barnett CP, Dugar M, Haan EA. Late-onset variant fibrodysplasia ossificans progressiva leading to misdiagnosis of ankylosing spondylitis. *Am J Med Genet Part A*. 2011;155A:1492–1495.
494. Kaplan FS, Glaser DL, Shore EM, et al. Hematopoietic stem-cell contribution to ectopic skeletogenesis. *J Bone Joint Surg*. 2007;89:347–357.
495. Wentworth KL, Masharani U, Hsiao EC. Therapeutic advances for blocking heterotopic ossification in fibrodysplasia progressiva. *Br J Clin Pharmacol*. 2018;85(6):1180–1187.
496. Lebrun M, Richard N, Abeguile G, et al. Progressive osseous heteroplasia: A model for the imprinting effects of GNAS inactivating mutations. *J Clin Endocrinol Metab*. 2010;95:3028–3038.
497. Fathi I, Sakr M. Review of tumoral calcinosis: A rare clinicopathological entity. *World J Clin Cases*. 2014;16:409–414.
498. Farrow EG, Imel EA, White KE. Miscellaneous non-inflammatory musculoskeletal conditions. Hyperphosphatemic familial tumoral calcinosis (FGF23, GALNT3 and Klotho). *Best Pract Res Clin Rheumatol*. 2011;25:735–747.
499. Slavin RE, Wen J, Barmada A. Tumoral calcinosis - a pathogenetic overview: a histological and ultrastructural study with report of two new cases, one in infancy. *Int J Surg Pathol*. 2012;20:462–473.
500. Ichikawa S, Baujat G, Sevahi A, et al. Clinical variability of familial tumoral calcinosis caused by novel GALNT3 mutations. *Am J Med Genet*. 2010;152A:896–903.
501. Shaw N. Tumoral calcinosis - dermatomyositis. In: Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. 7th ed. Washington DC: The American Society of Bone and Mineral Metabolism; 2008:433–435.
502. Ichikawa S, Imel EA, Kreiter ML, et al. A homozygous missense mutation in human KLOTHO causes severe tumoral calcinosis. *J Clin Invest*. 2007;117:2684–2691.
503. Chefetz I, Ben Amital D, Browning S, et al. Normophosphatemic familial tumoral calcinosis is caused by deleterious mutations in SMD9, encoding a TNF-alpha responsive protein. *J Invest Dermatol*. 2008;128:1423–1429.
504. Hershkovitz D, Gross Y, Nahum S, et al. Functional characterization of SAMD9, a protein deficient in normophosphatemic familial tumoral calcinosis. *J Invest Dermatol*. 2011;131:662–669.
505. Bonafe L, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2015 revision. *Am J Med Genet*. 2015;167A:2869–2892.
506. He L, Horton W, Hristova K. Physical basis behind achondroplasia, the most common form of human dwarfism. *J Biol Chem*. 2010;285:30103–30114.
507. Su N, Sun Q, Lu X, et al. Gain-of-function mutation in FGFR3 in mice leads to decreased bone mass by affecting both osteoblastogenesis and osteoclastogenesis. *Hum Mol Genet*. 2010;19:1199–1210.
508. Bacino CA. Skeletal dysplasias: Specific disorders. *UpToDate*. 2019;1–20.
509. Natucci F, Baffico M, Cavallari U, et al. Germline mosaicism in achondroplasia detected in sperm DNA of the father of three affected sibs. *Am J Hum Genet*. 2008;146A:784–786.
510. Hari Kumar KVS, Shaikh A, Sharma R, Prusty P. SADDAN syndrome. *J Pediatr Endocrinol Metab*. 2011;24:851–852.
511. Toydemir RM, Brassington AE, Bayrack-Toydemir P, et al. A novel mutation in *FGFR3* causes camptodactyly, tall stature, and hearing loss (CATSHL) syndrome. *Am J Hum Genet*. 2006;79:935–941.
512. Akkus G, Kotan LD, Durmaz E, et al. Hypogonadotropic hypogonadism due to novel FGFR1 mutations. *J Clin Res Pediatr Endocrinol*. 2017;9:95–100.
513. Posey KL, Hecht JT. Novel therapeutic interventions for pseudoachondroplasia. *Bone*. 2017;102:60–68.
514. Nino M, Matos-Miranda C, Maeda M, et al. Clinical and molecular analysis of arylsulfatase E in patients with brachytelephalangic chondrodysplasia punctata. *Am J Med Genet A*. 2008;146A:997–1008.
515. Marchini A, Hacker B, Marttila T, et al. BNP is a transcriptional target of the short stature homeobox gene SHOX. *Hum Mol Genet*. 2007;16:3081–3087.
516. Thiel CT, Rauch A. The molecular basis of the cartilage-hair hypoplasia-anauxetic dysplasia spectrum. *Best Pract Res Clin Endocrinol Metab*. 2011;25:131–142.
517. Forbes FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res*. 2011;52:6–34.
518. DeBarber AE, Eroglu Y, Merckens LS, et al. Smith-Lemli-Opitz syndrome. *Expert Rev Mol Med*. 2011;13:e24.
519. Thurm A, Tierney E, Farmer C, et al. Development, behavior, and biomarker characterization of Smith-Lemli-Opitz syndrome: an update. *J Neurodev Disord*. 2016;8:12.
520. Schaaf CP, Koster J, Katsonis P, et al. Desmosterolosis-phenotypic and molecular characterization of a third case and review of the literature. *Am J Med Genet A*. 2011;155A:1597–1604.
521. Zolotushko J, Flusser H, Markus B, et al. The desmosterolosis phenotype: spasticity, microcephaly and micrognathia with agenesis of the corpus callosum and loss of white matter. *Eur J Hum Genet*. 2011;19:942–946.

21

Diabetes Mellitus

Mark A. Sperling, Joseph I. Wolfsdorf, Ram K. Menon, William V. Tamborlane,
David Maahs, Tadej Battelino, Moshe Phillip

CHAPTER OUTLINE

INTRODUCTION	814
CLASSIFICATION	815
Type 1 Diabetes Mellitus	817
Type 2 Diabetes	817
TYPE 1 DIABETES MELLITUS	817
Epidemiology	817
Etiology, Pathogenesis, and Genetics	819
Prediction and Prevention	823
Insulin Biosynthesis	824
Insulin Secretion	825
Insulin Action	827
Pathophysiology	829
Clinical Manifestations of Diabetes Mellitus	830
Diagnosis	831
DIABETIC KETOACIDOSIS PATHOPHYSIOLOGY AND TREATMENT	831
Diabetic Ketoacidosis	831
Management of Diabetic Ketoacidosis	833
Where Should the Child With Diabetic Ketoacidosis be Managed?	833
Cerebral Edema	837
Treatment of Hyperosmolar Hyperglycemic Syndrome	839
Mixed Hyperosmolar Hyperglycemic Syndrome and Diabetic Ketoacidosis	840
TREATMENT OF TYPE 1 DIABETES MELLITUS	840
General Principles	840
Goals of Therapy	840
Types of Insulin	840
INSULIN REGIMENS	843
Multiple Daily Injection Regimens	843
Insulin Pump Therapy	843
CSII-Based Treatment Regimens	844
Basal Insulin Rates	845
Temporary Basal Rates and Basal Insulin Suspend	846
Bolus Insulin Dosages	846
Blood Glucose Monitoring	847
Continuous Glucose Monitoring	847
Clinical Evidence for Continuous Glucose Monitor	848
Practical Deliberations for Continuous Glucose Monitor	848
Sensor-Augmented Pump Therapy	849
Practical Deliberations for Sensor-Augmented Pump	849
AUTOMATED INSULIN DELIVERY—CLOSED-LOOP (ARTIFICIAL PANCREAS)	849
THE EMERGING DIGITAL WORLD OF DIABETES	850
The Open-Source Movement	851
Technology Challenges	851
LIVING WITH DIABETES	851
Medical Nutrition Therapy	851
Exercise	852
Residual β -Cell Function (the Honeymoon Period)	852
Hypoglycemia	852
Sick Day Management	853
Associated Autoimmune Diseases	853
Associated Psychosocial Problems	854
Outpatient Care	854
Management During Surgery	855
Nonautoimmune Type 1 Diabetes	855
COMPLICATIONS OF DIABETES	856
Chronic Metabolic Complications	856
Chronic Vascular Complications	856
Risk Factors for Complications	856
Other Risk Factors for the Development of Complications	856
Diabetic Nephropathy	856
Diabetic Retinopathy	858
Diabetic Neuropathy	859
Macrovascular Disease	860
TYPE 2 DIABETES MELLITUS	860
Typical	860
GENETIC DEFECTS OF BETA CELL FUNCTION	861
MODY Syndromes	861
Other Forms of Monogenic Diabetes	862
GENETIC DEFECTS IN INSULIN ACTION	864
Type A Insulin Resistance With Acanthosis Nigricans	864
Type B Insulin Resistance	864
ACQUIRED DEFECTS IN INSULIN ACTION	865
GENETIC SYNDROMES WITH DIABETES AND INSULIN RESISTANCE OR INSULIN DEFICIENCY	865
Gestational Diabetes	865
Neonatal Diabetes	865
Impaired Glucose Tolerance	866
Pancreas and Islet Transplantation	866
CONCLUDING REMARKS	866
References	867

INTRODUCTION

Diabetes mellitus (DM) is a syndrome of disturbed energy metabolism involving carbohydrate, protein, and fat brought about by absolute or relative deficiency of insulin secretion, in

814

the context of sensitivity or resistance to insulin actions at various tissue sites. Thus both the extent of impairment in insulin secretion and tissue sensitivity to its actions define the severity of the clinical manifestations, most commonly monitored by the extent

of inappropriate fasting and postprandial hyperglycemia. It is the degree of hyperglycemia that leads to the classical symptoms and signs of polyuria, polydipsia, and weight loss. Metabolic disturbances in protein and fat metabolism exist but, in contrast to glucose, are monitored intermittently, not routinely on a daily basis. In time, these metabolic disturbances, if uncontrolled, lead to various micro- and macrovascular complications, such as retinopathy, nephropathy, neuropathy, and large vessel obstruction of peripheral arteries and the heart, which lead to shortening of life span and lower quality of life. The severity and time of appearance of these complications are inversely related to the degree of metabolic control as judged by various parameters, chiefly the degree of hyperglycemia and glucose variability throughout the day. The closer that metabolic control approaches normal, the less likely to occur are the complications.

DM occurs when the normal constant of the product of insulin secretion times insulin sensitivity, a parabolic function termed the *disposition index* (Fig. 21.1), is inadequate to prevent hyperglycemia and its clinical consequences of polyuria, polydipsia, and weight loss. At high degrees of insulin sensitivity, small declines in the ability to secrete insulin cause only mild, clinically imperceptible defects in glucose metabolism. However, irrespective of insulin sensitivity, a minimum amount

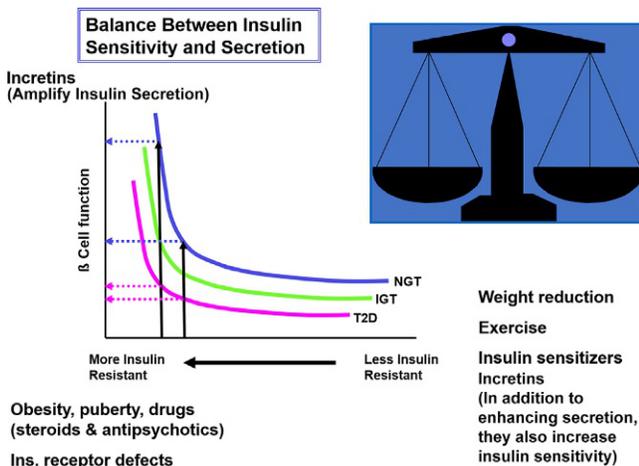


Fig. 21.1 The hyperbolic relationship of insulin resistance and beta-cell function. On the y-axis is beta-cell function as reflected in the first-phase insulin response during intravenous (IV) glucose infusion; on the x-axis is insulin sensitivity and its mirror image resistance. In a subject with normal glucose tolerance (NGT) and beta-cell reserve, an increase in insulin resistance results in increased insulin release and normal glucose tolerance. In an individual for whom the capacity to increase insulin release is compromised, increasing insulin resistance with partial or no beta-cell compensation results in progression from normal glucose tolerance, to impaired glucose tolerance (IGT), and finally to diabetes (T2D). Differences between these categories are small at high insulin sensitivity, which may be maintained by weight reduction, exercise, and certain drugs. At a critical degree of insulin resistance, because of obesity or other listed factors, only a further small increment in resistance requires a large increase in insulin output. Those that can increase insulin secretion to this extent retain normal glucose tolerance; those who cannot achieve this degree of insulin secretion (e.g., because of a mild defect in genes regulating insulin synthesis, insulin secretion, insulin action, or an ongoing immune destruction of beta cells) now unmask varying degrees of carbohydrate intolerance. The product of insulin sensitivity (the reciprocal of insulin resistance) and acute insulin response (a measurement beta-cell function) has been called the “*disposition index*.” This index remains constant in an individual with normal beta-cell compensation in response to changes in insulin resistance. Incretins are hormone such as GLP-1, which augment insulin secretion and also increase insulin sensitivity. (From Ize-Ludlow, D., & Sperling, M.A. (2005). The classification of diabetes mellitus: a conceptual framework. *Pediatr Clin North Am*, 52, 1533–1552.)

of insulin is necessary for normal metabolism. Thus near absolute deficiency of insulin must result in severe metabolic disturbance as occurs in type 1 diabetes mellitus (T1DM).¹ In contrast, with decreasing sensitivity to its action, higher amounts of insulin secretion are required for a normal disposition index. At a critical point in the disposition index curve (see Fig. 21.1), a further small decrement in insulin sensitivity requires a large increase in insulin secretion; those who can mount these higher rates of insulin secretion retain normal glucose metabolism, whereas those who cannot increase their insulin secretion because of genetic or acquired defects now manifest clinical diabetes, as typically occurs in type 2 diabetes mellitus (T2DM).²

By simultaneously considering insulin secretion and insulin action in any given individual, it becomes possible to account for the natural history of diabetes in that person (e.g., remission in a patient with T1DM or ketoacidosis in a person with T2DM). Thus DM may be the result of absolute insulin deficiency, or of absolute insulin resistance, or a combination of milder defects in both insulin secretion and insulin action.² Collectively, the syndromes of DM are the most common endocrine/metabolic disorders of childhood and adolescence. The application of molecular biological tools continues to provide remarkable insights into the etiology, pathophysiology, and genetics of the various forms of DM that result from deficient secretion of insulin or its action at the cellular level.

Morbidity and mortality stem from the metabolic derangements and from the long-term complications.³ The acute clinical manifestations are fully understood in the context of knowledge of the secretion and action of insulin.⁴ Genetic and other etiological considerations implicate autoimmune mechanisms in the evolution of the most common form of childhood diabetes, known as *type 1a diabetes*.^{5,6} Genetic defects in insulin secretion are increasingly recognized and understood as defining the causes of monogenic forms of diabetes, such as maturity-onset diabetes of youth (MODY) and neonatal DM and contributing to the spectrum of T2DM.⁷

Strong evidence that the long-term complications are related to the degree and duration of metabolic disturbances³ form the basis of standard and innovative therapeutic approaches for the spectrum of DM syndromes that include newer pharmacological formulations of insulin, delivery by traditional and more physiological means, and evolving methods to continuously monitor blood glucose to maintain it within desired limits by linking these features to algorithm-driven insulin delivery pumps for an “artificial pancreas.”

CLASSIFICATION

DM is not a single entity, but a heterogeneous group of disorders in which there are distinct genetic patterns, as well as other etiological and pathophysiological mechanisms that lead to impairment of glucose tolerance.^{2,8} Box 21.1 outlines an etiological classification of DM in children, based on the “Report of the Expert Committee on the Classification and Diagnosis of Diabetes Mellitus,” published by the American Diabetes Association (ADA) in January of 2018.⁸

Our classification is modified to reflect more accurately the major categories in childhood, including the emergence of T2DM, cystic fibrosis–related diabetes, and drug-induced diabetes—largely from the antirejection drugs cyclosporine, sirolimus, and tacrolimus (formerly FK-506). Table 21.1 presents a summary of the classification originally proposed in 1979 but incorporates the newer criteria for blood glucose values used to diagnose diabetes, impaired glucose tolerance, and gestational diabetes. Current classification of T1DM includes staging: stage 1 is the presence of two or more islet cell antibodies (ICAs), but without any discernible disturbance in glucose metabolism; stage 2 includes positive antibodies plus impaired fasting glucose or impaired glucose tolerance; stage 3 is the presence of glycemic criteria for

BOX 21.1 Etiological Classification of Diabetes Mellitus

- I. TYPE 1 DIABETES (BETA-CELL DESTRUCTION ULTIMATELY LEADING TO COMPLETE INSULIN DEFICIENCY)
 - A. Immune mediated
 - B. Idiopathic
- II. TYPE 2 DIABETES (VARIABLE COMBINATIONS OF INSULIN RESISTANCE AND INSULIN DEFICIENCY)
 - A. Typical
 - B. Atypical
- III. GENETIC DEFECTS OF BETA-CELL FUNCTION
 - A. MODY syndromes
 1. MODY 1 chromosome 20, HNF4A
 2. MODY 2 chromosome 7, glucokinase
 3. MODY 3 chromosome 12, HNF1A
 4. MODY 4 chromosome 13, IPF-1
 5. MODY 5 chromosome 17, HNF-1 β
 6. MODY 6 chromosome 2q32, neuro-D1/beta-2
 7. MODY7-14, see [Table 21.14](#)
 - B. Mitochondrial DNA mutations (includes one form of Wolfram syndrome, Pearson syndrome, Kearns-Sayre, diabetes mellitus, deafness)
 - C. Wolfram syndrome—DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, deafness): WFS1-Wolframin—chromosome 4p
 1. Wolfram locus 2—chromosome 4q22-24
 2. Wolfram mitochondrial
 - D. Thiamine responsive megaloblastic anemia and diabetes
- IV. DRUG OR CHEMICAL INDUCED
 - A. Antirejection—cyclosporine, sirolimus
 - B. Glucocorticoids (with impaired insulin secretion; e.g., cystic fibrosis)
 - C. L-Asparaginase
 - D. β -Adrenergic blockers
 - E. Vacor (rodenticide)
 - F. Phenytoin (dilantin)
 - G. Alfa-interferon
 - H. Diazoxide
 - I. Nicotinic acid
 - J. Others
- V. DISEASES OF EXOCRINE PANCREAS
 - A. Cystic fibrosis–related diabetes
 - B. Trauma—pancreatectomy
 - C. Pancreatitis—ionizing radiation
 - D. Others
- VI. INFECTIONS
 - A. Congenital rubella
 - B. Cytomegalovirus
 - C. Hemolytic-uremic syndrome
- VII. VARIANTS OF TYPE 2 DIABETES
 - A. Genetic defects of insulin action
 1. Rabson-Mendenhall syndrome
 2. Leprechaunism
 3. Lipoatrophic diabetes syndromes
 4. Type A insulin resistance—acanthosis
 - B. Acquired defects of insulin action
 1. Endocrine tumors—rare in childhood
 - C. Pheochromocytoma
 - D. Cushing
 - E. Others
 1. Antiinsulin receptor antibodies
- VIII. GENETIC SYNDROMES WITH DIABETES AND INSULIN RESISTANCE/INSULIN DEFICIENCY
 - A. Prader-Willi syndrome, chromosome 15
 - B. Down syndrome, chromosome 21
 - C. Turner syndrome
 - D. Klinefelter syndrome
 - E. Others
 1. Bardet-Biedl
 2. Alstrom
 3. Werner
- IX. GESTATIONAL DIABETES
- X. NEONATAL DIABETES
 - A. Transient—chromosome 6q24, *KCNJ11*, *ABCC8*, *INS*, *HNF1 β* , others
 - B. Permanent—agenesis of pancreas—glucokinase deficiency, homozygous, *KCNJ11*, *ABCC8*, others (see [Ch. 10](#))

ABCC8, ATP binding cassette subfamily C member 8; *DNA*, deoxyribonucleic acid; *HNF*, hepatocyte nuclear factor; *IPF*, insulin promoter factor; *KCNJ11*, potassium channel family J number 11; *MODY*, maturity-onset diabetes of the young.

TABLE 21.1 Summary of Classification of Diabetes Mellitus in Children and Adolescents

Category	Criteria
DIABETES MELLITUS	
Type 1	Typical symptoms: glucosuria, ketonuria; random plasma glucose >200 mg/dL; ≥ 2 Abs with normal glucose tolerance is now considered start of T1DM; with abnormal glucose tolerance is Stage 2; with clinical symptoms it is Stage 3
Type 2	Fasting plasma glucose >126 mg/dL with 2-hour intervening value >200 mg/dL on OGTT more than once and in the absence of precipitating factors
Other types	Type 1 or 2 criteria with genetic syndromes, including maturity-onset diabetes of the young (MODY), neonatal diabetes (NDM), drug therapy; pancreatic disease or other known causes or associations
Impaired fasting glucose	Glucose >100 mg/dL but <126 mg/dL
Impaired glucose tolerance	Fasting plasma glucose <126 mg/dL with 2-hour >140 mg/dL but <200 mg/dL on OGTT
Gestational diabetes	Two or more abnormal fasting plasma glucose levels >105 mg/dL, 1-hour >180 mg/dL, 2-hour >155 mg/dL, 3-hour > 140 mg/dL on OGTT
STATISTICAL RISK CLASSES	
Previous abnormality of glucose tolerance	Normal OGTT with previously abnormal OGTT, spontaneous hyperglycemia, or gestational diabetes
Potential abnormality of glucose tolerance	Genetic propensity (e.g., identical twin with diabetes mellitus); 2 islet cell antibodies is considered prediabetes

T1DM, Type 1 diabetes mellitus; OGTT, oral glucose tolerance tests.

diabetes together with the clinical manifestations. Among the insulin-dependent forms, severe lack of insulin secretion results most commonly from presumed autoimmune destruction of islets in genetically predisposed hosts. As noted earlier, evidence of autoimmunity may precede by months to years the clinical deterioration to abnormal glucose tolerance. This form is synonymous with **type 1a diabetes**, formerly called *juvenile-onset diabetes*.^{5,6,9,10} Severe insulin-dependent DM, clinically indistinguishable from the autoimmune form, may, however, not have any evidence of autoimmunity and can result from mitochondrial or other gene defects that interfere with **normal insulin secretion** or rarely from pancreatic agenesis.^{11–14}

The more severe forms of the MODY **syndromes**, subsequently detailed, may also require insulin.^{13,14} Clinically **similar forms of diabetes** may occur secondary to cystic fibrosis^{15,16} from toxic drugs, such as the immunosuppressive agents cyclosporine, sirolimus, and tacrolimus,^{17,18} the rodenticide Vacor,¹⁹ or streptozotocin as used for certain pancreatic islet cell tumors²⁰; with the hemolytic uremic syndrome²¹; or after pancreatectomy, such as for recurrent pancreatitis or persistent hyperinsulinemic hypoglycemia in infancy.²² Childhood insulin-dependent diabetes is generally type 1a DM.

Type 1 Diabetes Mellitus

This condition is characterized by severe insulinopenia and dependence on exogenous insulin to prevent ketosis and to preserve life. Thus it was termed *insulin-dependent DM* (IDDM). The natural history of this disease indicates that there are preketotic noninsulin-dependent phases before and after the initial diagnosis. Although the onset is predominantly in childhood, the disease may occur at any age.² Therefore such names as “juvenile diabetes,” “ketosis-prone diabetes,” and “brittle diabetes” were abandoned in favor of the term type 1 diabetes (T1D).

Type 1a diabetes is generally distinct by virtue of its association with certain histocompatibility locus antigens (HLAs) and other genetic markers the majority of which determine the response to self (or exogenous) antigens, for example, cytotoxic T-lymphocyte antigen 4 (*CTLA4*), autoimmune regulator gene (*AIRE*), variable number of tandem repeats in the insulin gene (*VNTR*), interleukin 2 receptor (*IL2R*) protein tyrosine phosphatase, and protein tyrosine phosphatase non-receptor type 22 (*PTPN22*); by the presence of circulating antibodies to cytoplasmic and cell-surface components of islet cells; of antibodies to insulin in the absence of previous exposure to exogenous injection of insulin, of antibodies to glutamic acid decarboxylase (GAD, the enzyme that converts glutamic acid to γ -aminobutyric acid found abundantly in the innervation of pancreatic islets), of antibodies to IA-2 (an islet cell-associated phosphatase) and antibodies to the zinc transporter molecule (ZnT8); by lymphocytic infiltration of islets early in the disease; and by coexistence with other autoimmune diseases.^{5,6} Occasionally, markers of autoimmunity are not found and yet there is profound insulinopenia and dependence on insulin without evidence of a mitochondrial or other genetic defect. In these cases, T1D is considered idiopathic (type 1b). With the exceptions noted, diabetes in children is usually insulin dependent and fits the type 1a category.²

Type 2 Diabetes

Persons with this subclass of diabetes (formerly known as *adult-onset diabetes*, *maturity-onset diabetes* [MOD], or *stable diabetes*)

may not be permanently insulin dependent and only occasionally develop ketosis. Some may, however, need insulin to correct symptomatic hyperglycemia—and ketosis may develop in some during severe infections or other stress. Therefore this was previously called *noninsulin-dependent DM* (NIDDM).² This form of diabetes is becoming increasingly prevalent in overweight adolescents, especially those from vulnerable groups, such as Africans, Latinos, Native Indians, and other susceptible ethnic groups.^{23,24}

T2DM is not a single entity.² T2DM may be a primary disorder, with inadequate insulin secretion caused by mutations in one of several genes encoding enzymes or transcription factors important to islet cell development and insulin secretion. Several of these defects are now part of the spectrum of the syndromes commonly associated with MODY, which has a dominant mode of inheritance.^{13,14,25} However, some patients with MODY defects may require insulin from the outset or as they grow older and become insulin resistant, exceeding their ability to compensate by increasing insulin secretion (see Fig. 21.1). A defect in the gene regulating glucose transport into the pancreatic beta cell, the GLUT2 transporter, may be responsible for another form of type 2 diabetes (T2D).⁷

Defects in glycogen synthase have also been implicated.^{26,27} A primary defect in insulin receptors—often associated with acanthosis nigricans,²⁸ postreceptor defects (including Rad [Ras associated with diabetes]),²⁹ and milder mitochondrial gene defects¹¹—also may result in T2D. Secondary causes of T2DM include excessive counterregulatory hormones, especially pharmacological doses of glucocorticoids, antibodies to the insulin receptor, and obesity with impaired insulin secretion.^{30–41}

In T2DM, the serum concentration of insulin compared with lean normal persons may be increased, normal, or moderately depressed depending on whether the defect is one of insulin action or secretion.^{28–41} The onset of T2DM commonly occurs in children generally around the time of puberty or shortly thereafter, but it is recognized that it may occur at any age and is becoming increasingly frequent in childhood and adolescence.^{23,24} In some instances, what appears to be T2DM may actually represent slowly evolving T1DM.³⁹ In T2D, there is no association with specific HLA antigens, autoimmunity, or various ICAs.⁴¹ However, several genetic abnormalities regulating insulin secretion or action are increasingly implicated in T2DM, so that the genetic architecture of T2DM is complex and remains incompletely understood.³¹

TYPE 1 DIABETES MELLITUS

Epidemiology

The prevalence of DM is highly correlated with increasing age. Available data indicate a range of one case per 1430 children at 5 years of age to one case in 360 children at 16 years.^{42–49}

Data on incidence in relation to racial or ethnic backgrounds indicate a range of more than 50 new cases annually per 100,000 population in Finland and Sardinia to about one per 100,000 in China and parts of South America^{43–46,50} (Fig. 21.2). In all examined areas, there appears to be an increasing incidence of T1DM of about 2% to 3% per year. However, the incidence of T1DM in Finland was reported to have peaked and slightly declined⁴² with similar data from Norway indicating the increase in incidence may be waning.⁴⁹

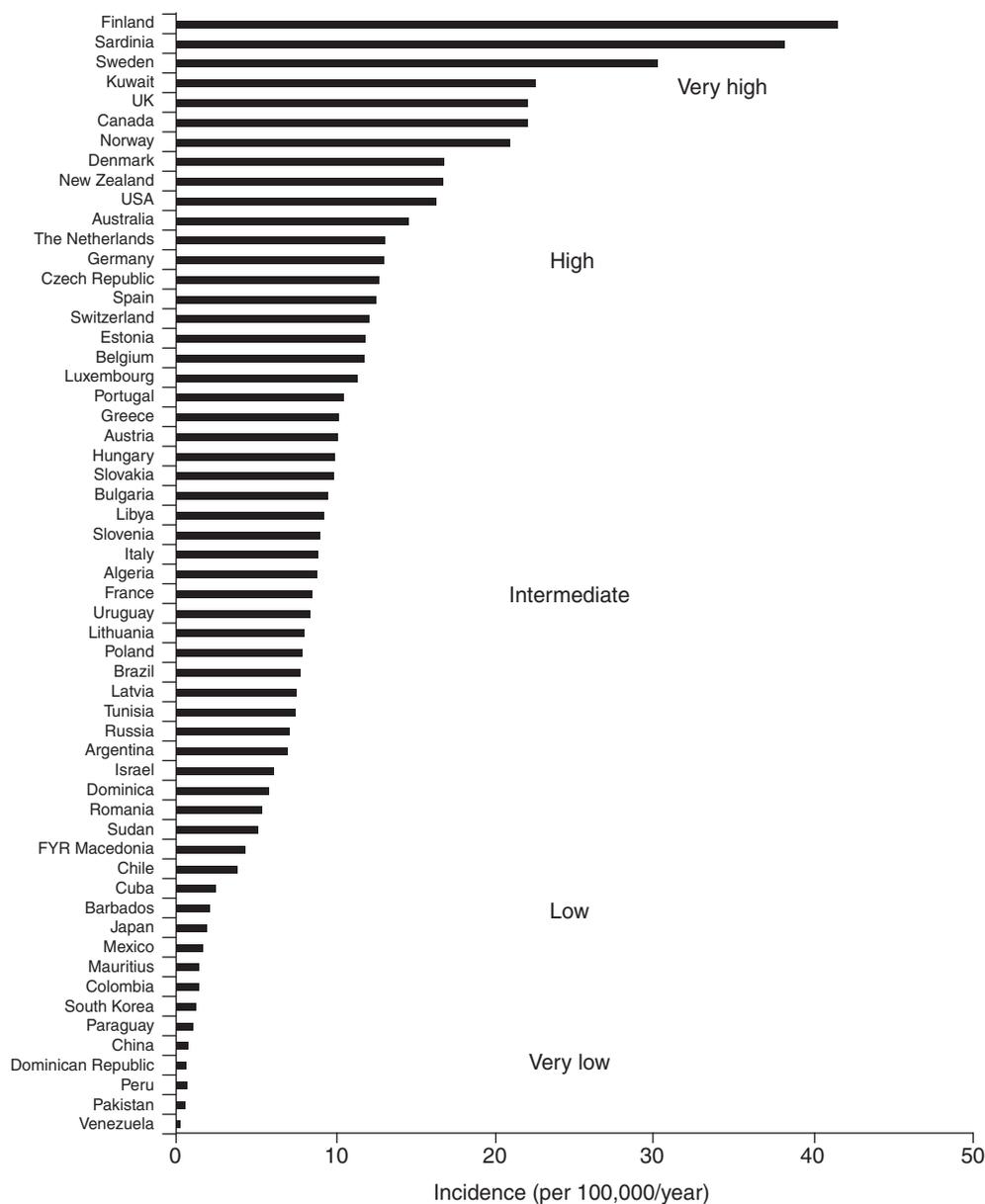


Fig. 21.2 Age-standardized incidence (per 100,000/year) of type 1 diabetes in children younger than 14 years of age in 100 populations. Data for boys and girls have been pooled. Countries are arranged in descending order according to the incidence. (Puerto Rico and the Virgin Islands are presented separately from other populations in the United States.) (From Diamond Project Group (2006). Incidence of trends of childhood type 1 diabetes worldwide 1990-1999. *Diabet Med*, 23, 857-866.)

In the United States, the occurrence of type 1 in Africans had previously been reported to be only between one-third and two-thirds of that in Caucasians.⁵⁰ More recent data suggest that the incidence of DM in African Americans is increasing.^{47,50,51} However, whether some of this reported increase in incidence among African Americans is exclusively type 1 or includes cases of type 2 presenting in ketoacidosis and thus misclassified remained unclear.⁵⁰ The most recently available information indicates an annual incidence in the United States of 20 to 25 cases per 100,000 of the childhood population⁴⁷ with a significant increase of T1DM in all groups, including African-American (see IDF Diabetes Atlas 9th Edition 2019 www.diabetesatlas.org).

Males and females appear to be almost equally affected. There is no apparent correlation with socioeconomic status. Peaks of presentation occur in two age groups: at 5 to 7 years of age and at the time of puberty. The first peak corresponds to the time of increased exposure to infectious agents coincident with the beginning of school. The latter corresponds to the pubertal growth spurt induced by increased sex steroids, as well as pubertal growth hormone secretion that antagonizes insulin action. The incidence of T1D is increasing worldwide, most prominently in certain populations (e.g., Finland) and in certain age groups (especially those aged <5 years).^{45,46} As mentioned, there appears to be a plateau in incidence reported from Finland and Norway.^{42,49} In younger patients, onset

appears to be more abrupt and the extent of immune markers is less apparent than in older children.⁵² Type 1b diabetes with abrupt onset, less evidence of autoimmunity, and indicators of viral infection (including evidence of pancreatitis) have been described in Japan.^{53,54}

Seasonal and long-term cyclical variations have been noted in the incidence of T1D. Newly recognized cases appear to occur with greater frequency in the autumn and winter in the northern and southern hemispheres.⁵⁵ Seasonal variations are most apparent in the adolescent years.⁵⁵ There is no consistent pattern linking long-term cyclicity with the incidence of viral infections; however, there was a definite increased incidence of diabetes in children with congenital rubella.^{56,57} These changing patterns in incidence and associations with viral infections suggest a potential role for viruses or other microbial agents or their products as direct or indirect triggering mechanisms for inducing T1DM in a susceptible host.⁵⁶⁻⁶¹ Detection of a low-grade enteroviral infection in the islets of Langerhans of patients with new-onset diabetes⁶² and the hyperexpression of HLA class 1 antigens on islet cells as a defining feature of T1D lend further credence to the possibility of viral infections as a potential trigger for autoimmunity leading to DM in the genetically predisposed host.⁶³

Etiology, Pathogenesis, and Genetics

The cause of the initial clinical findings in this predominant form of diabetes in childhood is the sharply diminished secretion of insulin.⁶⁴ Although basal insulin concentrations in plasma may be normal in newly diagnosed patients, insulin secretion in response to a variety of potent secretagogues is blunted and usually disappears over a period of months to years. In certain individuals considered at high risk for the development of T1D, such as the nonaffected identical twin of a diabetic, a progressive decline in insulin-secreting capacity has been noted for months to years preceding the clinical appearance of symptomatic diabetes that usually manifests when insulin-secreting reserve is 20% or less than normal for that individual (Fig. 21.3).^{5,6,64} The idealized schema of steady

decline in insulin secretion shown in Fig. 21.3 should not be construed as factual; periods of recovery and steeper declines may occur but eventually lead to significant reduction in insulin secretory capacity.

The mechanisms that lead to failure of the function of pancreatic beta cells point to autoimmune destruction of pancreatic islets in predisposed individuals. T1D has long been known to have an increased prevalence among persons with such disorders as Addison disease and Hashimoto thyroiditis, in whom autoimmune mechanisms are known to be pathogenic.⁶⁴ These conditions, as well as T1DM, are known to be associated with an increased frequency of genes involved in the regulation of immunity, including the autoimmune regulator gene *AIRE*, *PTPN22*, *CTLA4*, and the *INS* gene itself, as well as certain HLAs—in particular, DR3 and DR4.^{64,65} Located on chromosome 6, the HLA system is the major histocompatibility complex—consisting of a cluster of genes that code transplantation antigens and play a central role in immune responses.⁶⁴⁻⁷⁶

Increased susceptibility to a number of diseases has been related to one or more of the identified HLA antigens. The inheritance of HLA DR3 or DR4 confers a twofold to threefold increased risk for developing T1D. When both DR3 and DR4 are inherited, the relative risk for developing diabetes is increased 7- to 10-fold. Application of newer molecular genetic techniques has revealed further heterogeneity in the HLA D region among individuals with and without diabetes despite possessing the DR3 or DR4 markers, suggesting the participation of other susceptibility loci within these markers.⁶⁴⁻⁷⁶

Extensive genome-wide scans of markers associated with T1DM have uncovered more than 40 loci considered to confer susceptibility (Table 21.2). Some of these loci are confirmed and replicated by at least three different datasets. Others are suggestive but as yet not definitively linked. The strongest markers are those on chromosomes 6 and 11 (IDDM1 and IDDM2), respectively, linked to the HLA DQ β chain and the insulin gene itself.

In IDDM1, the homozygous absence of aspartic acid at position 57 of the HLA DQ *b* chain (non-Asp/non-Asp) confers an

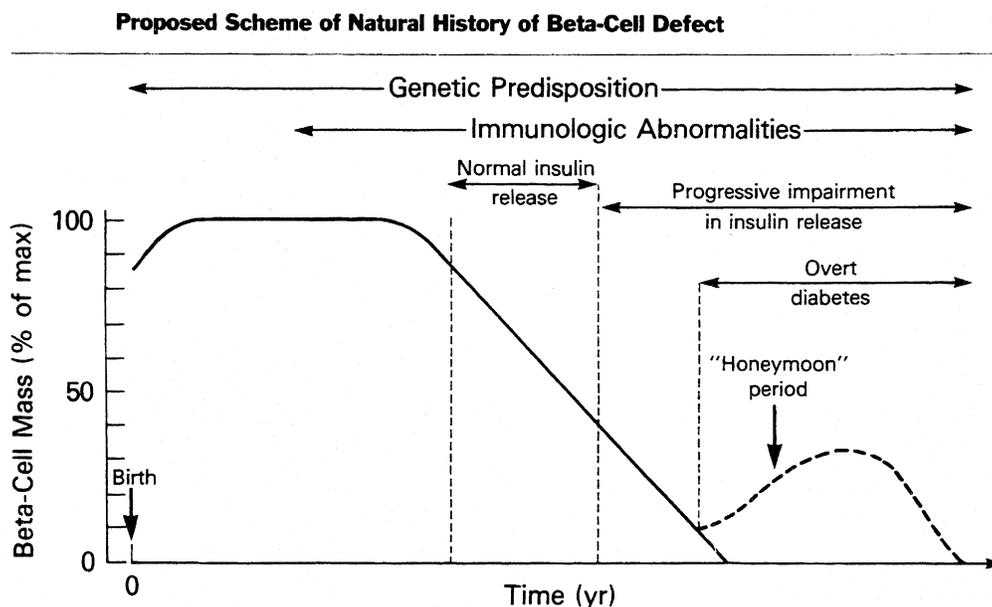


Fig. 21.3 Proposed scheme of natural history of the evolution of insulin-dependent diabetes mellitus with progressive beta-cell failure. (From Sperling, M.A. (Ed.) (1988). *Physician's guide to insulin-dependent (type 1) diabetes mellitus: diagnosis and treatment*. Alexandria, VA: American Diabetes Association.)

TABLE 21.2 Genome-Wide Association Study and Meta analysis Find More Than 40 Loci Affect Risk of Type 1 Diabetes

Loci	Approx. Relative Risk	Effect On
<i>HLA</i>	6.5	Immunity
<i>INS</i>	2.3	Insulin production, metabolism and immunity
<i>PTPN22</i>	2.0	Immunity
<i>ILR2A</i>	1.5	Immunity
<i>SH2BE</i>	1.3	Immunity
<i>ERBB3</i>	1.3	Insulin production and metabolism
<i>PTPN2</i>	1.25	Immunity
<i>CLEC16A</i>	1.20	Unknown function
<i>CTLA4</i>	1.20	Immunity
<i>IL18RAP</i>	1.20	Immunity
<i>PTPN2</i>	1.20	Immunity
<i>OCR5</i>	1.20	Immunity
<i>IFIH1</i>	1.20	Immunity
<i>CTSH</i>	1.20	Unknown
<i>CD226</i>	1.10	Immunity
<i>IL2RA</i>	1.10	Immunity
<i>PRKCCQ</i>	1.10	Immunity
<i>IL2</i>	1.10	Immunity
<i>BACH2</i>	1.10	Immunity
<i>UBASH3A</i>	1.10	Immunity
<i>RGS1</i>	1.10	Immunity
<i>IL7RA</i>	1.10	Immunity
<i>CITNF6</i>	1.10	Unknown
<i>TNFAIP3</i>	1.10	Beta-cell apoptosis protection
<i>TAGAP</i>	1.10	Immunity

(Modified from Barrett, J.C., Clayton, D.G., Concannon, P., et al. for the Type 1 Diabetes Genetics Consortium. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*, 41, 703–707.)

TABLE 21.3 Human Leukocyte Antigen DR and DQ Phenotype Frequencies in Patients With Type 1 Diabetes Mellitus and Healthy Control Subjects

Phenotype	Diabetic (%)	Nondiabetic	Odds Ratio
DR (SEROLOGY)			
DR3/DR4	33	6	8.3
DR3/DR3	7	1	9.8
DR3/DRX	7	14	0.05
DR4/DR4	26	0	—
DR4/DRX	22	16	1.5
DRX/DRX	4	63	0.02
DQ (MOLECULAR PROBES)			
Non-Asp/non-Asp	96	19	107.2
Non-Asp/Asp	4	46	0.04
Asp/Asp	0	34	0

(From Morel, P. A., Dorman, J. S., Todd, J. A., et al. (1988). Aspartic acid at position 57 of the HLA-DQ beta chain protects against type 1 diabetes: a family study. *Proc Natl Acad Sci USA*, 85, 8111.)

approximately 100-fold relative risk for developing T1D. Those who are heterozygous with a single aspartic acid at position 57 (non-Asp/Asp) are no more susceptible than individuals who contain aspartic acid on both DQ *b* chains (i.e., homozygous Asp/Asp; Table 21.3). Some studies suggest that T1DM is proportional to the gene frequency of non-Asp alleles in that population.⁷⁵ In addition, arginine at position 52 of the DQ *a* chain confers marked susceptibility to type 1.⁶⁹ Position 57 of the DQ *b* and position 52 of the DQ *a* chains are at critical locations of

the HLA molecule that permit or prevent antigen presentation to T-cell receptors and activate the autoimmune cascade (Fig. 21.4).^{66–69} The importance of these and additional amino acid substitutions in HLA DQ and DR in facilitating antigen presentation and contributing to risk for development of diabetes has been confirmed.⁷⁷

IDDM2 is a polymorphic marker near the transcription start site of the insulin gene, giving rise to VNTR at the promoter end of the insulin gene on chromosome 11. Each tandem repeat element consists of an approximately 14-bp deoxyribonucleic acid (DNA) segment with a consensus nucleotide sequence. The number of repeats ranges from about 25 to about 200, and the three classes of alleles are based on overall size. Class I insulin VNTR consists of 26 to 63 repeats and confers susceptibility, whereas class III consists of 140 to 200 or more repeats and is protective of diabetes. Together, the gene markers on chromosomes 6 and 11 (i.e., IDDM1 and IDDM2) account for 50% to 60% of the heritability of T1D. However, combinations of certain DQ alleles in association with certain DR alleles may confer susceptibility or protection to the development of T1D (see Table 21.3).

In addition, other as yet undefined genetic factors play a role because the same high-risk genotypes are about sixfold more likely to develop diabetes in an individual with a positive family history than in one without a family history without T1D (Table 21.4). Investigation of four genome-wide linkage scans in close to 1500 families with more than one affected member having T1DM identified several susceptibility loci. Of these, about 40% can still be attributed to allelic variation of HLA loci, and the influence of the VNTR in the insulin gene was confirmed.^{65,70}

In addition, the *CTLA4* gene on chromosome 2 and *PTPN22* gene on chromosome 1p13 were found to contribute significantly to predisposition to T1DM. However, the genome scan identified other potential loci conferring susceptibility on chromosomes 2q31–q33, 10p14–q11, and 16q220q24 and a locus on the long arm of chromosome 6 (6q21) distinct from the HLA region on 6p21. The precise genes in these regions that may predispose to T1DM have not been identified as yet, although some have been excluded⁶⁴ and newer candidate genes—such as *CBLB* interacting with *CTLA4*,⁷² the decay-accelerating factor gene (*daf*, a complement inhibitor),⁷³ and the interleukin 2a receptor *IL2R*—are under scrutiny.⁶⁹ Reviews of the genetics of T1DM have received considerable attention⁷⁸ and a genetic risk score predicts progression of islet cell autoimmunity and clinical diabetes in those at risk.⁷⁹ These considerations provide a rational framework for the long-recognized association of type 1 diabetes with genetic factors on the basis of the increased incidence in some families, of the concordance rates in monozygotic twins, and of ethnic and racial differences in prevalence.^{65,69,76} From multiple family pedigrees and HLA typing data, it has been estimated that if a sibling shares both HLA D haplotypes with an index case, the risk for that individual is 12% to 20%; for a sibling sharing only one haplotype, the risk for IDDM is 5% to 7%; and with no haplotypes in common, the risk is only 1% to 2%.⁷⁶ HLA typing is not recommended for routine practice, but for purposes of genetic counseling it can be safely assumed that in whites, the overall recurrence risks to siblings is approximately 6% if the proband is younger than 10 years of age and 3% if older at the time of diagnosis. The risk to offspring is 2% to 5%, with the higher risk in the offspring of a diabetic father.^{69,76}

Factors other than pure inheritance must also be involved in evoking clinical diabetes. For example, DR3 or DR4 is found in approximately 50% of the general population and non-Asp/non-Asp is found in approximately 20% of white nondiabetics in the United States. However, the risk for T1D in these subjects is only 1/10th that in an HLA-identical sibling of an index case with T1D possessing these markers.⁷⁶ Even siblings sharing

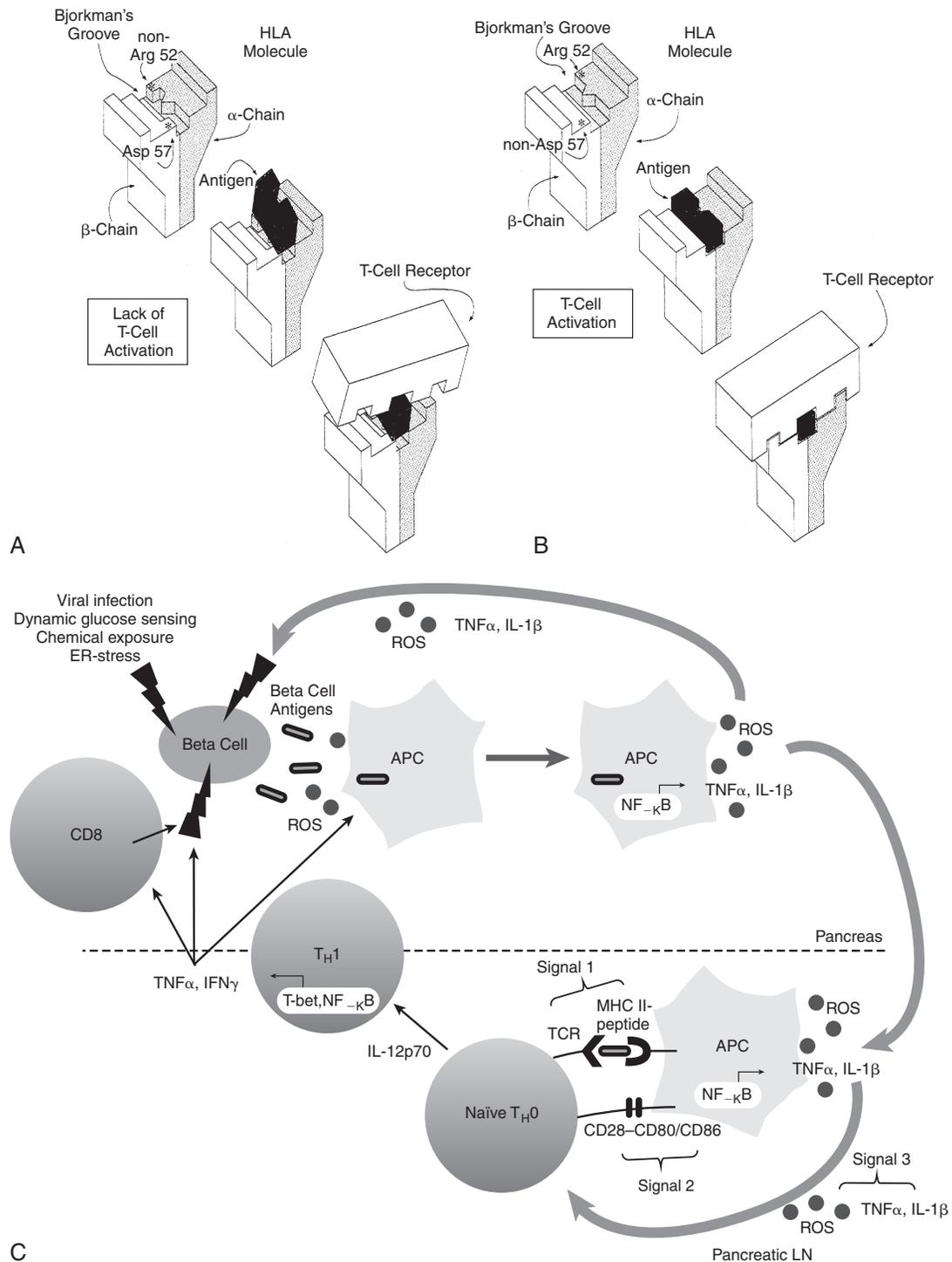


Fig. 21.4 Representation of the interaction between antigen presentation in the context of specific human leukocyte antigen (*HLA*)-DQ subtypes and the T-cell receptor. A, Presence of aspartic acid at position 57 of the DQ *b* chain and an amino acid other than arginine at position 52 of the α chain prevents antigen lodging in the Bjorkman groove. Therefore antigen presentation to the T-cell receptor is impaired—and in the absence of this “fit,” T-cell activation is prevented. B, Lack of aspartic acid at position 57 of the DQ *b* chain and arginine at position 52 of the DQ α chain permits antigen to fit and be recognized by the T-cell receptor that is now activated. C, Role of redox in the immunopathology of type 1 diabetes. An initial genetic or environmental insult to the beta cell triggers the release of beta-cell antigens, as well as the production of reactive oxygen species (ROS). Beta-cell antigens are phagocytosed, and ROS are able to stimulate redox-dependent transcription factors, such as nuclear factor (NF)- κ B, which leads to antigen presenting cell (APC) activation and cytokine secretion. ROS and proinflammatory cytokines secreted by APCs act as the third signal within the T-cell–APC immunological synapse, which occurs in the pancreatic lymph node. ROS play a critical role in the progression of naïve TH0 cells to cytokine-secreting TH1 cells. Release of interferon (IFN) γ by TH1 cells then works directly on the beta cells, as well as activating more APCs and CD8 cells, all of which can impart deleterious effects on the islets. (A and B from Trucco, M. (1995). To be or not to be Asp 57, that is the question. *Diabetes Care*, 15, 705; Faas, S., Trucco, M. (1995). The genes influencing the susceptibility to IDDM in humans. *J Endocrinol Invest*, 17, 477; C from Delmastro, M.M., & Piganelli, J.D. (2011). Oxidative stress and redox modulation potential in type 1 diabetes. *Clin Dev Immunol*, 2011.)

TABLE 21.4 Genetic Risk Estimates for Human Leukocyte Antigen Class II in Type 1 Diabetes Mellitus

High-Risk Genotypes	Risk in an Individual With This Genotype
DQB1p0302 (DQ3.2)	1 in 60
DQ3.2/DQ2 (DR3)	1 in 25
DQB1p03021 family history of IDDM	1 in 10
DQ3.2/DQ2 (DR3)1 family history of IDDM	1 in 4
Complete sharing of both HLA haplotypes	1 in 2 ^a

^aIndividual is a sibling of patient with T1DM.

(Modified from Nepom, G. T. (1995). Class II antigens and disease susceptibility. *Ann Rev Med*, 46, 17; Aly, T.A., Ide, A., Jahromi, M.M., et al. (2006). Extreme genetic risk for type 1A diabetes. *Proc Natl Acad Sci U S A*, 103, 14, 10474–10479.)

only one haplotype have a 6- to 10-fold greater risk of developing type 1 compared with the normal population (see Table 21.4).

Importantly, approximately 10% to 15% of patients with type 1 do not have HLA DR3 or DR4 (see Table 21.3).⁶⁴ Most compelling is the fact that the concordance rate among identical twins of whom one has insulin-dependent diabetes is only about 50%, suggesting the participation of environmental triggering factors or other genetic factor, such as the postnatal selection of certain autoreactive T-cell clones that bear receptors recognizing “self.” This postnatal process occurs within the thymus and implies that identical twins are not identical with respect to the T-cell receptor repertoire they possess. Identical twins discordant for diabetes have also been shown to possess differential methylation in immune effector cell types suggesting a role for epigenetics in the risk for developing diabetes.⁸⁰ Triggering factors might include viral infections.^{58–61} In animals, a number of viruses can cause a diabetic syndrome, the appearance and severity of which depend on the genetic strain and immune competence of the species of animal tested. In humans, epidemics of mumps, rubella, and coxsackie virus infections have been associated with subsequent increases in the incidence of T1D. The acute onset of DM, presumably induced by coxsackievirus B4, has been described.⁶¹ The viruses may act by directly destroying beta cells, by persisting in pancreatic beta cells as slow viral infections, or by triggering a widespread immune response to several endocrine tissues.⁶¹ Low-grade enteroviral infection in the pancreas of newly diagnosed patients with T1D and hyperexpression of HLA class 1 antigen in islet cells as a defining feature of T1DM adds to the evidence for a potential role of viral triggering of the autoimmune processes.^{62,63} A superantigen response may be involved in triggering T cells, bypassing the classic presentation by antigen-presenting cells (APCs) of the processed antigen in the context of restricted HLA molecules to T-cell receptors.⁸¹ Some viruses and certain endotoxins or exotoxins are capable of inducing a superantigen response. In addition, the virus may induce initial beta-cell damage—which results in the presentation of previously masked or altered antigenic determinants. It is also possible that the virus shares some antigenic determinants with those present on or in beta cells, including GAD, such that antibodies formed in response to the virus may interact with these shared determinants of beta cells, resulting in their destruction, an example of molecular mimicry.^{82–89}

Nitrosamines and early exposure to cow’s milk have been suggested as factors that may trigger diabetes in those genetically at risk, thus explaining the reported lower incidence of diabetes among exclusively breastfed infants. This was the basis for a primary prevention study: the trial to reduce insulin-dependent diabetes in those genetically at risk (TRIGR).^{82–89}

However, in those who were genetically at risk, the TRIGR study failed to show a significant reduction in development of diabetes via delay or exclusion of cow’s milk.⁹⁰

Antecedent stress and exposure to certain chemical toxins have also been implicated in the development of T1D. Although the rodenticide Vacor has been a cause of diabetes in individuals deliberately or inadvertently poisoned by this agent, some of these patients had ICAs, suggesting that such antibodies are secondary to islet damage or that evolving type 1 disease preceded the drug ingestion. Nitrosamines in cured meat have also been implicated in T1D, as have other environmental toxins.^{82,91}

Evidence supports an autoimmune basis for the development of T1D, but why the beta cell is the specific target remains a mystery.^{5,44} Is the pancreatic beta cell the sole target of immune destruction (homicide) or a contributor to its own demise (suicide)?^{5,44} Histological examination of pancreas from patients with type 1 who die of incidental causes has revealed lymphocytic infiltration around the islets of Langerhans. Later, the islets become progressively hyalinized and scarred, a process suggesting an ongoing inflammatory response that is possibly autoimmune.⁹² However, these changes are often patchy in distribution, so that areas that appear to contain normal beta cells are interspersed with areas of beta-cell destruction, similar to the patchy distribution of depigmentation found in vitiligo.^{64,93} Some 80% to 90% of newly diagnosed patients with T1D have ICA directed at cell-surface or cytoplasmic determinants in their islet cells. The prevalence of these antibodies decreases with the duration of established disease. In contrast, after pancreatic transplantation, ICA may reappear in patients whose sera had become negative for ICA before transplantation. Together, these findings suggest that ICA disappears as the antigens intrinsic to pancreatic islets are destroyed and reappear when fresh antigen (transplanted islets) is presented.

Studies in identical twins and in family pedigrees demonstrate that the existence of ICA may precede by months to years the appearance of symptomatic T1D.⁵ In vitro, ICA may impair insulin secretion in response to secretagogues and can be shown to be cytotoxic to islet cells—especially in the presence of complement or T cells from patients with T1D. About 80% of patients may have antibodies to GAD, and 30% to 40% of newly diagnosed patients have spontaneous antiinsulin antibodies at initial diagnosis. These antibodies may be detected months to years before clinical diabetes becomes apparent.^{1,5,66,85} A more recently described antibody, the zinc transporter, ZnT8, appears to be a major marker for progressive impairment of beta-cell function.⁵ There is also evidence of abnormal T-cell function with an alteration in the ratio of suppressor (regulatory) to killer T cells at the onset of the disease.^{5,6,10} Thus the ability of Treg to modify the activity of T effector cells in causing beta-cell destruction is an area of investigation. These findings suggest that T1D (akin to other autoimmune diseases, such as Hashimoto thyroiditis) is a disease of “autoaggression” in which autoantibodies in cooperation with complement, T cells, cytokines, FAS, and FAS ligand, and other factors, induce apoptosis or destruction of the insulin-producing islet cells.^{6–8,10}

Thus the inheritance of certain genes (such as those associated with the HLA system on chromosome 6 or other immunoregulatory or immunomodulatory genes) appears to confer a predisposition for autoimmune disease—including diabetes—when triggered by an appropriate stimulus, such as a virus.^{5,6,10} Evidence of superantigen-triggered T-cell receptor activation was discussed earlier.⁸¹

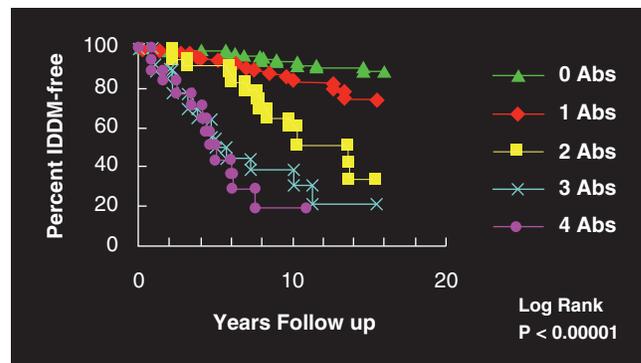
Although it is understood that some insulin-dependent diabetic patients have none of the frequently associated

HLA antigens, the evidence for an immune basis of islet cell destruction is sufficiently compelling to have fostered several studies of different immunosuppressive agents in the treatment of newly diagnosed diabetics. None of these immunosuppressive or immunomodulatory trials has had long-term positive outcome, and some agents (e.g., cyclosporine) have proven toxic to beta cells. Although newer approaches are being attempted, all must be considered as experimental and **not be** viewed as established or recommended therapy.^{85,92-100} The Diabetes Prevention Trial for T1DM [DPT1] was a multicenter randomized but nonblinded study using daily subcutaneous insulin and an annual admission for intravenous [IV] insulin infusion in first-degree relatives with proven risk factors for developing T1DM. Whereas prediction was highly accurate in identifying those most likely to develop T1DM within 5 years of entering the study, insulin injections had **no** protective effect to prevent the appearance of T1DM.⁹²⁻⁹⁹ Figs. 21.3 and 21.4 summarize current concepts of the cause of T1D as an autoimmune disease, the tendency for which is inherited and in which autoimmune destruction of beta cells is triggered by an as yet unidentified agent (possibly a virus). The slope of decline in insulin varies, and there may be periods of partial recovery such that the course of decline in insulin secretion is bumpy rather than smooth. The point at which clinical features appear corresponds to approximately 80% destruction of insulin secretory reserve. This process may take months to years in adolescent and older patients, and weeks in the very young patient in whom acute destruction by nonautoimmune mechanisms may play a significant role. Higher titers of spontaneous antiinsulin antibodies and ICA are characteristic of more active islet cell destruction, typically in the younger **patient**, and may prove useful in predicting evolving diabetes.⁹²⁻⁹⁹

Prediction and Prevention

Although no presently available single marker or test can accurately predict T1DM, evidence suggests that a combination of **immune** and genetic markers for T1D may provide predictability.^{85,92-100} Some authorities suggest that T1D is a predictable disease, but other authorities have raised objections because predictability is not as robust in their studies. Definitive preventive therapy is not available, thereby raising ethical dilemmas, and it is emphasized that the majority of new cases occur sporadically, in the absence of a positive family history in a first-degree relative.

Most predictive studies have been performed in first-degree relatives of patients with new-onset T1D.⁸⁴ Nevertheless, there is increasing evidence that the presence of high titers of islet cell, GAD, IA2, ZnT8, and insulin autoantibodies combined with a consistently diminished first-phase response of insulin to a pulse of IV glucose (corresponding to the ≤ 5 th percentile for age in insulin response) can be used to reliably predict the onset of type 1 disease.⁹²⁻¹⁰⁰



Pietropaolo M. et al. *Diabetologia* 45: 66-76, 2002
Fig. 21.5 Cumulative risk of developing clinical type 1 diabetes (T1DM) in relatives of probands with established T1DM using Ab markers alone (IAA, GAD65, IA-2, ICA). (From Pietropaolo, M. et al. (2002). Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. *Diabetologia*, 45, 66-76.)

Fig. 21.5 demonstrates that in a set of first-degree relatives, conversion to T1D was highly dependent on the number of antibodies detected in their sera. Of those with three antibodies, about half developed clinical diabetes within 5 years of follow-up. First-phase insulin response and genetic (HLA) markers may be used to augment the predictability. For example, Table 21.5 demonstrates that the relative risk of developing clinical diabetes within 4 years of detecting ICA is almost 230 among those who possess all four heterodimers in HLA DQ β that predispose to diabetes (i.e., Asp57 2/2 and Arg52 1/1).

As technological improvements continue, it is likely that population-wide screening for antibody markers (alone or combined with specific genetic markers) will be available to identify those at risk for developing T1D. Such population-wide screening would be ethically justified if prevention could be proven effective. Presently, the data are sufficiently persuasive to have fostered national trials in Europe and the United States to predict and possibly prevent the clinical onset of T1D through immune intervention strategies. The European Nicotinamide Diabetes Intervention Trial (ENDIT) was a multicenter trial that screened approximately 22,000 first-degree relatives of patients with T1D to identify 500 considered to be at high risk for developing this disease.⁹⁶⁻⁹⁹ These at-risk individuals were treated with nicotinamide or a placebo in a double-blind fashion. The results of ENDIT showed no positive protection against development of diabetes. The US DPT1 was based on promising pilot data that suggested preservation of insulin secretion and prevention of progression to DM in at-risk individuals treated with insulin.⁹⁵ Daily subcutaneous insulin, coupled with an intensive course of IV insulin every 9 months, prevented diabetes for at least 3 years in five subjects considered to be at risk because of genetic markers, islet cell and insulin

TABLE 21.5 Influence of Diabetic Heterodimers (ASP57neg Arg52pos) and Islet Cell Antibody Status on Relative Risk for Developing Diabetes After 4 Years

	ICA Negative				ICA Positive			
Diabetic heterodimers (dH)	0	1	2	4	0	1	2	4
Developed IDDM after 4 years	12	16	37	12	12	18	29	15
Relative risk	1.0	2.9	8.6	25.4	9.0	26.5	78.0	229.3

ICA, Islet cell antibody; IDDM, insulin-dependent diabetes mellitus. (Modified from Friday, R.P., Trucco, M., & Pietropaolo, M. (1999). Genetics of type 1 diabetes mellitus. *Diabetes Nutr Metab*, 12, 3.)

autoantibodies, and diminished first-phase insulin response. Among seven similar at-risk subjects who chose not to be treated, six developed insulin-dependent diabetes within 3 years. DPT1 aimed to investigate these promising pilot results in a larger carefully matched cohort and was concluded in 2001; there was no difference in the rates of developing diabetes among the placebo and insulin-treated groups. However, the ENDIT and DPT1 studies proved that large-scale multicenter studies could be successfully undertaken and that the prediction of progression to clinical diabetes was remarkably accurate. Thus in those at highest risk (such as first-degree relatives of patients with T1DM) prediction is feasible and the discovery of successful means of arresting or reversing progression to clinical diabetes is the subject of intense research.

Another study (TRIGR) involved 3000 families in whom half avoided cow's milk for the first 9 months of life to test the hypothesis that the ingestion of breast milk and avoidance of cow's milk formula (with its bovine serum albumin [BSA]) may protect participants from the appearance of diabetes.⁸⁸ As noted earlier, no benefit accrued to those who avoided cow's milk.⁹⁰ Several other studies are examining the utility of antibodies to the IL2R, CD3 antibodies, immune suppressors, such as mycophenolate mofetil, and immune modulators in preventing diabetes, including oral insulin. These studies are conducted by consortia of participating institutions (see www.trialnet.org).

In animal models, oral insulin or oral GAD had been successfully used to prevent diabetes.¹⁰¹ It was postulated that ingestion of T-lymphocyte-dependent antigens may establish immunological tolerance. Such oral strategies have been proposed and oral insulin was tested in humans but again with no definitive benefit.¹⁰¹ The subjects of primary prevention trials and secondary intervention trials to preserve residual insulin secretion at initial diagnosis are of major interest to investigators and clinicians alike. Progress is likely, but at present, all of these strategies must be viewed as experimental and not currently in the domain of daily clinical practice.^{76,100,102,103}

Of immediate relevance are the reports of a decline in the incidence of new-onset T1DM in children vaccinated against rotavirus.^{104,105} If these findings are confirmed, they would add credence to the role of viruses in initiating islet cell damage that may lead to the autoimmune cascade resulting in T1DM and the potential benefits of immunization against these, and yet to be defined other viral agents.

Insulin Biosynthesis

Insulin is synthesized on the ribosomes of pancreatic islet beta cells and released into the circulation as a molecule composed of two separate straight polypeptide chains linked by disulfide bridges between and within these chains.^{106–112} The two chains are not synthesized separately but are derived from a larger precursor, proinsulin, a single coiled chain in which the NH₂ terminus of the A chain is linked to the COOH terminus of the B chain by a connecting peptide, known as *C-peptide* (Fig. 21.6). An even larger precursor (preproinsulin, containing an additional peptide chain on the NH₂ terminus of the A chain) is first synthesized, but this additional piece (important to the initiation of synthesis) is rapidly excised. Further processing of proinsulin within the beta cell cleaves the C-peptide, consisting of 31 amino acids, from the insulin molecule at the sites indicated in Fig. 21.6.

Defects in these cleavage sites are inherited in an autosomal-dominant manner and result in insulin molecules with less-than-normal biological activity that can give rise to two types of familial hyperproinsulinemia. One defect results in B-C proinsulin, cleaved at site 1 but not at site 2 (see Fig. 21.6). This

intermediate has 50% of the biological activity of insulin, which is sufficient to prevent any abnormality in carbohydrate metabolism. The defect at site 1 yields A-C proinsulin, cleaved at site 2 but not at site 1, which has inadequate biological activity to prevent carbohydrate intolerance. A structural mutation in the proinsulin molecule, between the C-peptide and insulin, has been confirmed.^{101,106–110} In addition, a defective enzymatic conversion of a normal proinsulin molecule to insulin results in hyperproinsulinemia and mild carbohydrate intolerance.¹¹⁰

The proconvertases responsible for correct conversion of proinsulin to insulin are also involved in the processing of other hormones. Thus impaired prohormone processing may lead to severe obesity and secondary hypocortisolism owing to defective processing of proopiomelanocortin (POMC), to hypogonadotropic hypogonadism, and to diabetes insipidus.^{111–113} Native proinsulin has less than 5%, whereas C-peptide has none, of the biological activity of insulin. During synthesis, the role of C-peptide appears to be the provision of the spatial arrangement necessary in the formation of the disulfide bonds. Other defects have been described in insulin biosynthesis involving substitution of amino acids in the B chain that lead to impaired glucose tolerance in the presence of hyperinsulinemia.^{106–112,114}

The insulin gene has been cloned and localized to chromosome 11, and genetic defects in insulin synthesis may be associated with diabetes—especially the syndromes MODY 1, 3, 4, 5, and 10, the latter being insulin itself.^{7,13,14} By some estimates, MODY syndromes may constitute 2% to 5% of all lean persons developing clinical diabetes between the ages of 10 and 30 years. The MODY syndromes are briefly discussed later and in detail in Chapter 10. The association of VNTRs in the insulin gene with genetic predisposition for autoimmune T1DM was described earlier.

Under normal circumstances, only small quantities of proinsulin are released into the circulation—amounting to less than 15% of total insulin as measured by radioimmunoassay (RIA). Even smaller quantities of proinsulin intermediates are also released. However, during insulin secretion induced by all stimuli, one molecule of C-peptide is released with each molecule of insulin. Thus the plasma of normal individuals contains small amounts of proinsulin, proinsulin intermediates, and almost equimolar amounts of insulin and C-peptide. The plasma metabolic half-life of C-peptide is, however, longer than that of insulin. Therefore the molar ratio of C-peptide to insulin in peripheral plasma is always greater than 1 and the peak of C-peptide secretion or the nadir after suppression of release appears to occur later than that of insulin. Although standard RIA of insulin will also measure proinsulin, C-peptide will not be measured because it is immunologically distinct (see Fig. 21.6).

Separation of proinsulin from insulin can be achieved by chromatography to separate the larger proinsulin before assay. This is done with the use of an enzyme that degrades insulin but not proinsulin, or with the use of a C-peptide assay that will also measure proinsulin but not insulin. Because C-peptide is immunologically distinct, RIAs for this substance can be used to assess beta-cell secretory reserve even in the presence of insulin antibodies formed in response to injections of human or formerly used bovine-porcine insulin.

Endogenous insulin secretion is accompanied by C-peptide release, whereas exogenous insulin administration suppresses endogenous insulin (and therefore C-peptide) secretion in all circumstances except insulinoma. Results of standard RIA with double-antibody precipitation are high in both circumstances. These attributes are important in distinguishing abuse of individuals by injection of exogenous insulin (high insulin, low C-peptide) from insulinomas or dysregulated insulin

Human Proinsulin

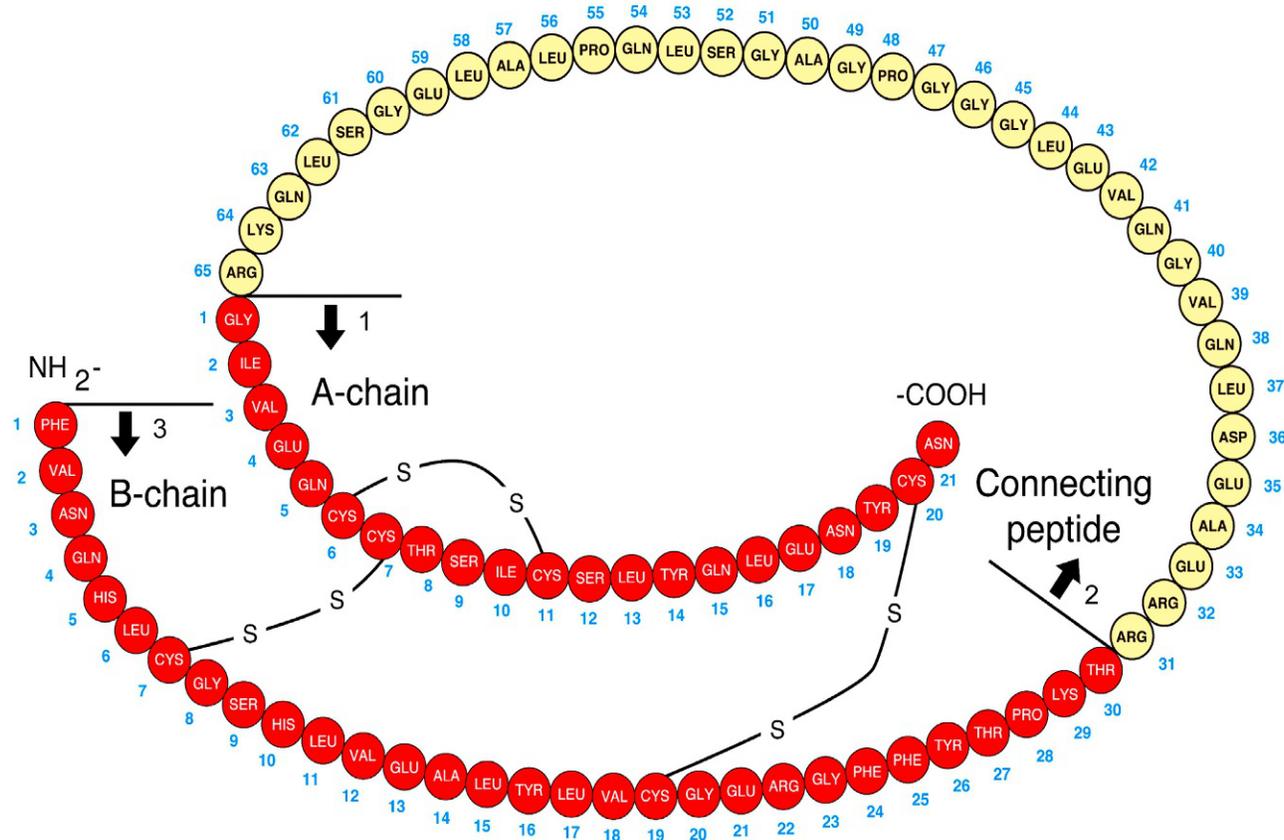


Fig. 21.6 Structure of proinsulin. Arrows 1 and 2 indicate the two sites of normal cleavage that yield insulin and C-peptide when the amino acid residues indicated in the *open circles* are removed. These cleavage points are known mutation sites, are inherited in an autosomal-dominant manner, and can yield two types of familial hyperproinsulinemia. During insulin secretion, equimolar amounts of insulin and C-peptide are released. Arrow 3 indicates the start site of the B-chain at the Amino Terminus of the pro-hormone.

secretion (high insulin, high C-peptide) in cases of hypoglycemia. Measurements of C-peptide kinetics or of urinary excretion of C-peptide can be used as an index of endogenous insulin secretion.³⁰

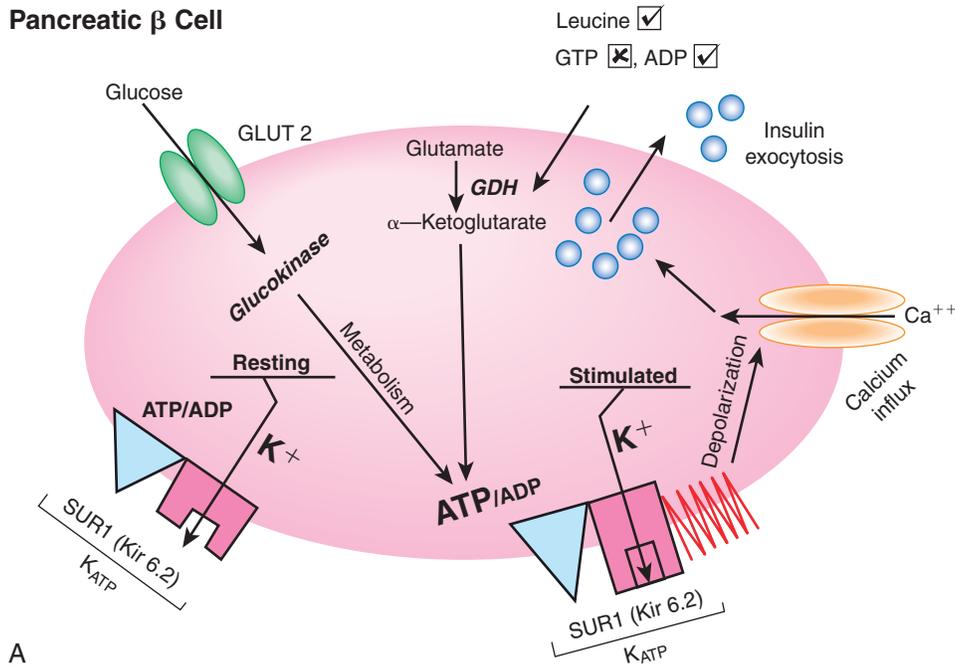
Insulin Secretion

Insulin secretion is governed by the interaction of nutrients, hormones, and the autonomic nervous system. Glucose, as well as certain other sugars metabolized by islets, stimulates insulin release. Basal and peak insulin levels are closely related to the glucose concentration, and prolonged fasting will further reduce glucose and insulin levels—which, however, remain in the measurable range at 2 to 5 mU/mL. There is evidence that a product or products of glucose metabolism may be involved in maintaining insulin secretion and that sugars *not* metabolized by islet cells do not promote insulin release.^{115–117}

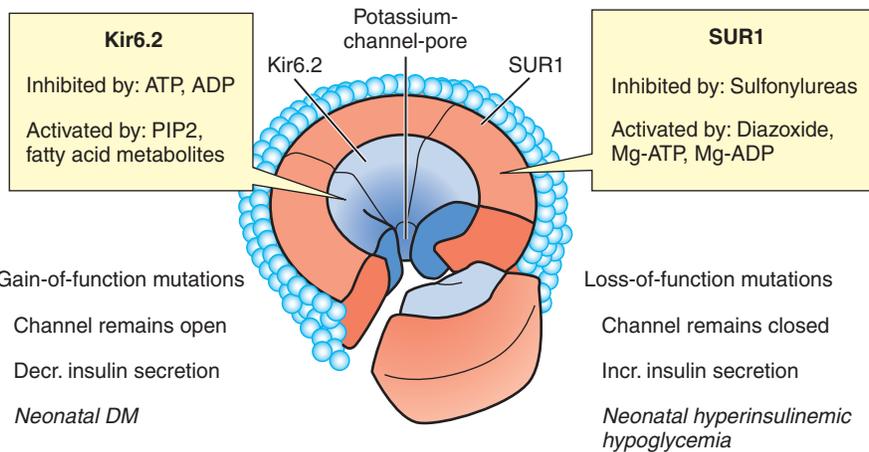
The initial steps of glucose-stimulated insulin release are depicted in Fig. 21.7 and discussed in detail in Chapters 7 and 10 in connection with mutations in the sulfonylurea receptor (SUR)-Kir6 (inward-rectifying potassium channel) complex of the adenosine triphosphate-regulated potassium channel K_{ATP}, along with the subsequent steps that may cause activation of glucose or amino acid-stimulated insulin secretion.¹¹⁸ This schema involves glucose transport into the beta cell through the GLUT2 glucose transporter and

phosphorylation of glucose by means of glucokinase. Defects in the former are associated with T2D, whereas heterozygous inactivating mutations in the latter are associated with MODY2. Homozygous mutations in glucokinase result in permanent neonatal DM (as described in detail in Chapter 10). Heterozygous inactivating mutations in glucokinase are generally associated with normal insulin release at higher glucose concentrations, and therefore with a milder type of diabetes that rarely requires treatment and is not associated with microvascular or other diabetes complications.^{13,119} After IV glucose infusion in normal persons, insulin secretion is biphasic—with an initial spike followed by a sustained plateau. It is proposed that the initial spike represents preformed insulin, whereas the sustained plateau represents newly synthesized insulin.

Cyclic adenosine monophosphate (cAMP) is involved in stimulating insulin release. Therefore agents that inhibit phosphodiesterase and reduce cAMP destruction (such as theophylline) augment insulin release. Translocation of calcium ions into the cytoplasm from the exterior, as well as from the intracellular organelles (see Fig. 21.7), plays a key role in the contractile forces that propel insulin to the cell surface.¹¹⁸ There, the membrane of the insulin vesicle fuses with the cell membrane—allowing extrusion of insulin granules into the surrounding vascular space, a process known as emiocytosis. Other ions, including potassium and magnesium, are involved in the insulin secretion.^{118,120–122} The sulfonylurea receptor is



A



B

Fig. 21.7 A, Model of insulin secretion by pancreatic beta cell. Glucose transported into the beta cell by the insulin-independent glucose transporter (*GLUT 2*) undergoes phosphorylation by glucokinase and is metabolized. This results in an increase in the adenosine triphosphate (*ATP*)/adenosine diphosphate (*ADP*) ratio with subsequent closure of the K_{ATP} channel and initiation of a cascade of events that is characterized by decreased flux of potassium across the membrane, membrane depolarization, calcium influx, and release of insulin from storage granules. Leucine stimulates insulin secretion by allosterically activating glutamate dehydrogenase (*GDH*) and by increasing the oxidation of glutamate; this increases the *ATP/ADP* ratio and closure of the K_{ATP} channel. The check mark sign (\checkmark) indicates stimulation of insulin secretion; the cross sign (\times) indicates inhibition of insulin secretion. Diazoxide inhibits insulin secretion by interacting with the sulfonylurea receptor; somatostatin and calcium channel blockers interfere with calcium signaling. B, Regulation of insulin secretion. The Kir6.2–SUR1 complex and its regulation and genetic variability. The panel shows the detailed subunit structure of the K_{ATP} channel, which is composed of four small subunits, Kir6.2, that surround a central pore and four larger regulatory subunits constituting SUR1. In the normal resting state, the potassium channel is open, modulated by the ratio of *ATP* to *ADP*. PIP2 denotes phosphatidylinositol-4,5-bisphosphate, Kir6.2 denotes the inward rectifying potassium channel 6.2, SUR1 denotes the sulfonylurea receptor 1, *ADP* is adenosine diphosphate, and *ATP* is adenosine triphosphate. As noted, in gain-of-function mutations, the channel remains open leading to decreased insulin secretion so that neonatal diabetes may result. With loss-of-function mutations, the channel remains closed, leading to persistent insulin secretion and hence is a cause of neonatal hyperinsulinemic hypoglycemia. (Modified from Sperling, M.A. (2006). *ATP-sensitive potassium channels—neonatal diabetes mellitus and beyond*. *N Engl J Med*, 355, 507–510.)

closely linked to potassium channels in the beta cell.^{118,120–122} Amino acids also stimulate insulin release, although the potency of individual amino acids varies.¹²³ A group of amino acids is more potent than any single one, and the insulin-secretory response is potentiated in the presence of glucose.¹²³ Free fatty acids and ketone bodies may also stimulate insulin release.¹²³ Insulin responses to oral glucose administration are always greater than responses to IV administration of glucose that result in the same blood glucose profile, a finding that led to the concept that gut factors (incretins) modulate and increment insulin secretion.¹²⁴ Although a variety of gut hormones participate in promoting insulin release,¹²⁴ gastrointestinal polypeptide (gip) pancreatic glucagon and the glucagon-like peptides (GLP) play a major role in stimulating insulin release.¹²⁴ These properties have found application as agents, collectively named *incretins*, in augmenting insulin secretion in persons with T2DM and in some persons with T1DM.¹²⁵

Somatotropin release-inhibiting factor (somatostatin), produced in the delta cells of islets, inhibits insulin and glucagon release and reduces splanchnic blood flow. These properties have found application to reduce insulin secretion in neonates with hyperinsulinemic hypoglycemia of infancy (see Chapter 7). Together, these factors may finely regulate nutrient intake and its disposition and form an enteroinsular axis for metabolic homeostasis.¹²⁴ In addition to these gut hormones, several other hormones modulate insulin secretion. Growth hormone is involved in insulin synthesis and storage. Persons with congenital growth hormone deficiency have subnormal basal and stimulated insulin responses, whereas in acromegaly, basal and stimulated insulin levels are increased. Human chorionic somatomammotropin (also known as *human placental lactogen*), structurally related to growth hormone, likewise affects insulin release. The stimulatory effect of each hormone on insulin secretion is antagonized by the antiinsulin effect at the peripheral level, however. Similarly, glucocorticoids and estrogens evoke greater insulin secretion while inducing peripheral insulin resistance—in part by decreasing insulin receptors on target cells.

Insulin secretion is constantly modulated by the autonomic nervous system.^{117,126} The parasympathetic arm, through the vagus, directly stimulates insulin release. Modulation of insulin secretion by the sympathetic arm depends on whether α - or β -adrenergic receptors are activated. Activation of β_2 receptors by agents, such as isoproterenol, stimulates insulin secretion by a process that involves cAMP generation. Blockade of β -adrenergic receptors by propranolol blunts basal and stimulated insulin release. Conversely, activation of α -adrenergic receptors blunts insulin secretion, and blockade of these receptors by agents, such as phentolamine, augments basal and glucose-stimulated insulin release. Epinephrine and norepinephrine stimulate predominantly α -adrenergic receptors in islets, resulting in impaired insulin secretion—as observed during stress or in patients with pheochromocytoma.¹¹⁷

In summary, in normal humans, insulin secretion is constantly modulated by the quantity, quality, and frequency of nutrient intake; by the hormonal milieu; and by autonomic impulses. The ingestion of nutrients, principally carbohydrate and protein, produces intestinal hormonal signals that prime and initiate insulin release. The entry of glucose into the beta cell, the phosphorylation of glucose, and the generation of adenosine triphosphate (ATP) by this or other nutrients result in insulin release. This sequence involves cAMP, β -adrenergic receptors, and ions—principally calcium and potassium. Glucose metabolism within the beta cell provides energy for further synthesis and release of insulin.¹¹⁸

Insulin Action

Insulin action on target cells in tissues such as liver, adipocytes, and muscle, begins by binding to specific insulin receptors located on the cell membrane. Binding to these receptors is saturable, occurs with a high energy of association (affinity), and is pH and temperature dependent.^{127–131} The insulin receptor is a heterodimeric glycoprotein consisting of two α and two β subunits linked by disulfide bonds (Fig. 21.8). The α subunit, with a molecular mass of approximately 125,000 kDa, acts as the binding site—whereas the β subunit, with a molecular mass of approximately 90,000 kDa, possesses tyrosine kinase activity for endogenous and exogenous substrates (see Figs. 21.8 and 21.9).^{132,133}

This ability to phosphorylate proteins may underlie some of the manifold actions of insulin. Among the classes of proteins phosphorylated are insulin receptor substrates 1 through 3 (considered an important insulin-signaling effector molecule) and pp 185, another substrate of the insulin receptor (see Fig. 21.9). Other insulin mediators may be involved in insulin action. This action may also be mediated in part by hydrolysis of glycan phosphoinositides in the cell plasma membrane. The insulin receptor gene has been cloned and localized to chromosome 19, whereas the structurally related insulin-like growth factor-1 (IGF-1) receptor has been localized to chromosome 15.^{127–131}

Under normal conditions, only a small proportion of the total available cell receptors need be occupied to achieve maximal biological response. Thus ordinarily there are spare receptors. Insulin receptors display two phenomena: downregulation, in which high ambient insulin concentrations reduce the number of available receptors, and negative cooperativity, in which the occupancy of a receptor reduces the affinity of adjoining receptor sites. Scatchard analysis of insulin-binding data in *in vitro* systems reveals curvilinear plots compatible with negative cooperativity or with two classes of receptors: high-affinity/low-capacity and low-affinity/high-capacity. Total receptor number and the affinities of both classes of receptor sites can be calculated with use of these Scatchard plots.

After binding to the cell surface, the receptor-insulin complex is internalized within the cell and processed by lysosomal enzymes, with release of free insulin and potential recycling of the receptor back to the cell membrane. Binding of insulin to the cell-surface receptor, perhaps with the participation of internalization that permits insulin action at the level of the nucleus, leads to the complex biochemical processes characteristic of insulin action in a given tissue. With postreceptor events assumed to be normal, however, the biological response to insulin in a tissue is a function of the number of receptor/insulin complexes formed—which in turn is directly related to the circulating insulin concentration and to the receptor concentration. Thus a reduction in receptor number could be compensated for by an increase in insulin concentration as long as the critical number of receptors necessary to produce maximal biological response remains. Conversely, reduced insulin concentration could be compensated for by an increase in receptor number, provided the minimum amount of insulin necessary to produce a maximal biological response is present.

Insulin receptors and their signaling proteins (see Figs. 21.8 and 21.9) are widely distributed in various tissues. Using targeted deletions of individual components or various combinations of components of the insulin receptor pathway has provided remarkable insight into the contribution of liver, muscle, fat, the beta cell, and brain to overall glucose homeostasis.^{127–131} Key concepts that have emerged are that the insulin receptor signal cascade on beta cells is critically important in maintaining normal insulin secretion. Thus mutations

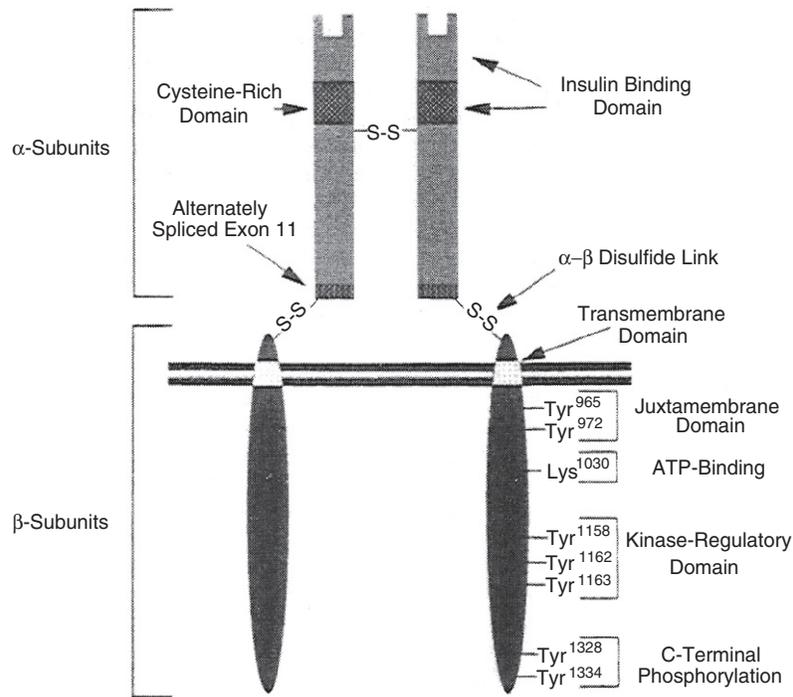


Fig. 21.8 Structure of the insulin receptor. *ATP*, adenosine triphosphate. (From Cheatham, B., Kahn, C.R (1995). Insulin action and the insulin signaling network. *Endocr Rev*, 16, 117.)

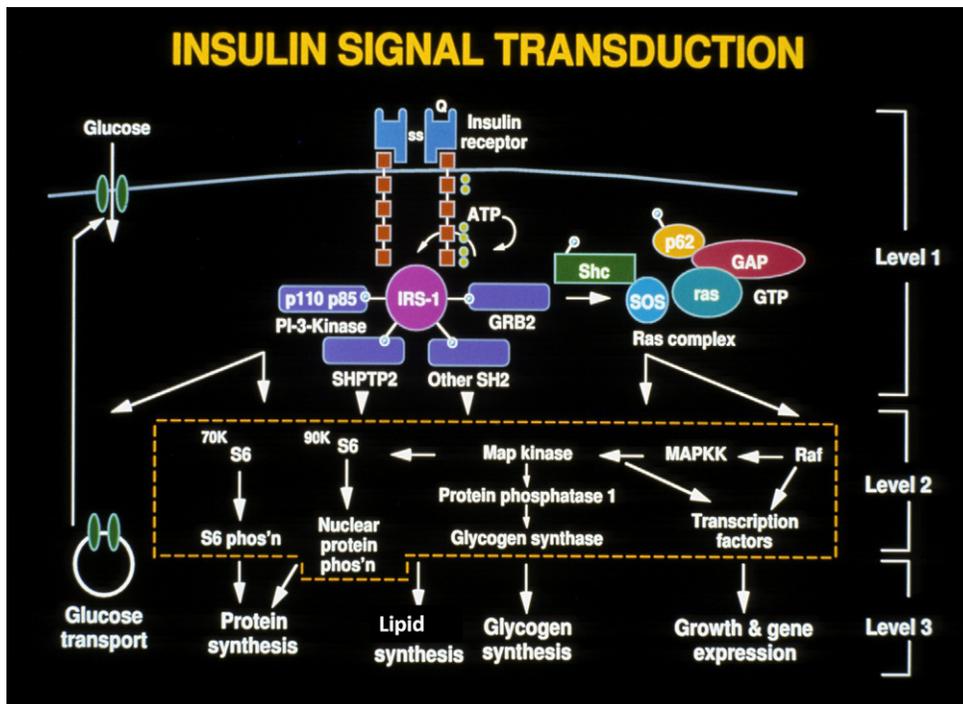


Fig. 21.9 Binding of insulin to the extracellular alpha subunit of the insulin receptor (*IR*) initiates a phosphorylation cascade first by tyrosine kinase on the intracellular beta subunit of *IR*, followed by several other phosphorylation cascades shown in level 1. These phosphorylation processes have immediate function on translocating the glucose transporter (GLUT 4) from the cytoplasm to the cell membrane thereby facilitating glucose transport across the cell membrane in cells, such as muscle, or activate other kinases, such as S6 and MAP kinases (level 2) involved in protein synthesis, lipid synthesis, and glycogen synthesis, as well as activating transcription factors involved in growth and gene expression (Level 3). *ATP*, Adenosine triphosphate; *GAP*, Ras p21 protein activator; *GRB2*, growth factor receptor bound 2; *GTP*, guanosine triphosphate; *IRS-1*, insulin receptor substrate 1; *MAP kinase*, mitogen-activated protein kinase; *MAPKK*, mitogen-activated protein kinase kinase; *p110* and *p85*, subunits of PI3 kinase (phosphoinositide 3 kinase); *p62*, nucleoporin 62; *Raf*, family of serine/threonine-specific protein kinases participating in the MAPK cascade; *S6*, ribosomal protein S6; *SH2*, Src homology region 2; *Shc*, src homology containing; *SHPTP2*, adaptor protein linking Janus kinase 2 and insulin receptor substrates; *SOS*, son of sevenless.

causing insulin resistance at the beta cell eventually lead to relative hypoinsulinemia that can interact with the insulin resistance in peripheral tissues to produce the hallmark of T2D (i.e., peripheral insulin resistance plus relative insulinopenia).

In addition, studies with targeted deletion of the insulin receptor in the brain—the so-called NIRKO mouse (neuron-specific insulin receptor knockout)—demonstrate that these animals developed obesity, increased body fat, insulin resistance with modest hyperinsulinemia, and elevated levels of triglycerides. Reproductive function in both males and females is impaired as a result of abnormal regulation of luteinizing hormone secretion, and serum leptin levels are elevated.¹³¹ Thus insulin signaling in the brain joins the emerging list of factors important in regulating energy homeostasis and reproduction.¹³¹

Primary defects in insulin receptor number or affinity may produce the same profound derangements in intermediary metabolism as deficient insulin secretion, and similar disturbances may result despite normal insulin concentration and normal receptor characteristics if postreceptor steps are defective.¹³⁴ Insulin signaling for the regulation of metabolism has been the subject of considerable research and is extensively reviewed.^{130,134} Examples of each type of defect in the individual components of this integrated system that comprises insulin biosynthesis, secretion, and action exist and can account for the metabolic abnormalities that characterize DM. An approach based on the principles of insulin biosynthesis secretion and action also permits a rational classification of DM.

Pathophysiology

Normal insulin secretion in response to feeding is exquisitely modulated by the interplay of neural, hormonal, and substrate-related mechanisms to permit controlled disposition of ingested foodstuff as energy for immediate or future use in metabolism and growth. Mobilization of energy during the fasted state depends on low plasma levels of insulin. Thus in normal metabolism, there are regular swings between the postprandial high-insulin anabolic state and the fasted low-insulin catabolic state that affect three major tissues: liver, muscle, and adipose tissue (Table 21.6).

Insulin is the key anabolic hormone that promotes the synthesis and storage of carbohydrates, lipids, and proteins while simultaneously restraining their degradation. The uptake of glucose, fatty acids, and amino acids is stimulated—as is the activity or expression of enzymes that promote glycogen, fat, and protein synthesis. Conversely, the activity or expression of enzymes that break down these metabolites is restrained.

All of these anabolic actions of insulin are reversed during the low-insulin state of starvation. T1DM, as it evolves, becomes a permanent low-insulin catabolic (starvation) state in which feeding cannot reverse but rather exaggerates these catabolic processes.

It is important to emphasize that liver is more sensitive than muscle or fat to a given concentration of insulin. That is, endogenous glucose production from the liver by means of glycogenolysis and gluconeogenesis can be restrained at insulin concentrations that do not fully augment glucose utilization by peripheral tissues. Consequently, with progressive failure of insulin secretion, the initial manifestation is postprandial hyperglycemia. Fasting hyperglycemia is a late manifestation that reflects severe insulin deficiency and indicates excessive endogenous glucose production.¹³⁵

Although insulin deficiency is the primary defect, several secondary changes that involve the stress hormones (i.e., epinephrine, cortisol, growth hormone, and glucagon) accelerate and exaggerate the rate and magnitude of metabolic decompensation.

Increased plasma concentrations of these counterregulatory hormones magnify metabolic derangements by further impairing insulin secretion (e.g., epinephrine), by antagonizing its action (e.g., epinephrine, cortisol, and growth hormone), and by promoting glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis (e.g., glucagon, epinephrine, growth hormone, and cortisol) while decreasing glucose utilization and glucose clearance (e.g., epinephrine, growth hormone, and cortisol).¹³⁶ With progressive insulin deficiency, especially with concurrently elevated stress hormones, excessive glucose production, and impairment of its utilization result in hyperglycemia with glucosuria when the renal threshold of approximately 180 mg/dL (10 mmol/L) is exceeded.

The resultant osmotic diuresis produces polyuria, urinary losses of electrolytes, dehydration, and compensatory polydipsia. These evolving manifestations, especially dehydration, represent physiological stress—resulting in hypersecretion of epinephrine, glucagon, cortisol, and growth hormone that amplifies and perpetuates metabolic derangements and accelerates metabolic decompensation. The acute stress of trauma or infection may likewise accelerate metabolic decompensation to ketoacidosis in evolving or established diabetes.¹³⁶

Hyperosmolality, commonly encountered as a result of progressive hyperglycemia, contributes to the symptomatology—especially to cerebral obtundation in diabetic ketoacidosis (DKA). Serum osmolality can be estimated with the following formula.

TABLE 21.6 Influence of Feeding (High Insulin) or Fasting (Low Insulin) on Some Metabolic Processes in Liver, Muscle, and Adipose Tissue

	High Plasma Insulin (Postprandial State)	Low Plasma Insulin (Fasted State)
Liver	Glucose uptake Glycogen synthesis Absence of gluconeogenesis Lipogenesis Absence of ketogenesis	Glucose production Glycogenolysis Gluconeogenesis Absence of lipogenesis Ketogenesis
Muscle	Glucose uptake Glucose oxidation Glycogen synthesis Protein synthesis	Absence of glucose uptake Fatty acid and ketone oxidation Glycogenolysis Proteolysis and amino acid release
Adipose tissue	Glucose uptake Lipid synthesis Triglyceride uptake	Absence of glucose uptake Lipolysis and fatty acid release Absence of triglyceride uptake

Insulin is considered the major factor governing these metabolic processes. Diabetes mellitus may be viewed as a permanent low-insulin state that if untreated results in exaggerated fasting.

$$\text{Serum osmolality (mOsm/kg)} = (\text{serum Na [mEq/L]} + \text{K [mEq/L]}) \times 2 + \text{Glucose mmol/L}$$

(Note that 1 mmol/L of glucose is equivalent to 18 mg/dL.)

Consideration of serum osmolality has important implications in the treatment of DKA. The combination of insulin deficiency and elevated plasma values of the counterregulatory hormones is also responsible for accelerated lipolysis and impaired lipid synthesis, with resulting increased plasma concentrations of total lipids, cholesterol, triglycerides, and free fatty acids. The hormonal interplay of insulin deficiency and glucagon excess shunts the free fatty acids into ketone body formation. The rate of formation of these ketone bodies, principally β -hydroxybutyrate and acetoacetate, exceeds the capacity for peripheral utilization and for their renal excretion. Accumulation of these ketoacids results in metabolic acidosis and in compensatory rapid deep breathing in an attempt to excrete excess carbon dioxide (Kussmaul respiration).

Acetone, formed by nonenzymatic conversion of acetoacetate, is responsible for the characteristic fruity odor of the breath. Ketones are excreted in the urine in association with cations and thus further increase losses of water and electrolytes (Fig. 21.10 and Table 21.7). With progressive dehydration, acidosis, hyperosmolality, and diminished cerebral oxygen use, consciousness becomes impaired—with the patient ultimately becoming comatose. Thus insulin deficiency produces a profound catabolic state—an exaggerated starvation in which all

of the initial clinical features can be explained on the basis of known alterations in intermediary metabolism mediated by insulin deficiency in combination with counterregulatory hormone excess. Because the counterregulatory hormonal changes are usually secondary, the severity and duration of the symptoms reflect the extent of primary insulinopenia. Greater details of these considerations are provided in the discussion of diabetic ketoacidosis, which follows later.^{135,136}

Clinical Manifestations of Diabetes Mellitus

The classic presentation of diabetes in children is a history of polyuria, polydipsia, polyphagia, and weight loss. Polyuria may be heralded by the recurrence of bedwetting in a previously toilet trained child and polydipsia by a child constantly requesting fluids to drink. Unexplained weight loss should raise suspicion of the existence of diabetes that should be confirmed or excluded by measurement of blood glucose concentration, first in the postprandial and later in the fasting state. The urine should also be checked for the presence of glucosuria. The duration of these symptoms varies; it often is less than 1 month, but careful history may reveal weeks of thirst, enuresis, fatigue, and weight loss. Most children who are diagnosed with T1DM have been seen by a physician within a week or so of diagnosis, but diabetes was not considered, and a glucose measurement in blood or urine was not performed.^{137,138}

An insidious onset with lethargy, weakness, and weight loss is also quite common. The loss of weight despite increased

Pathophysiology of Diabetic Ketoacidosis

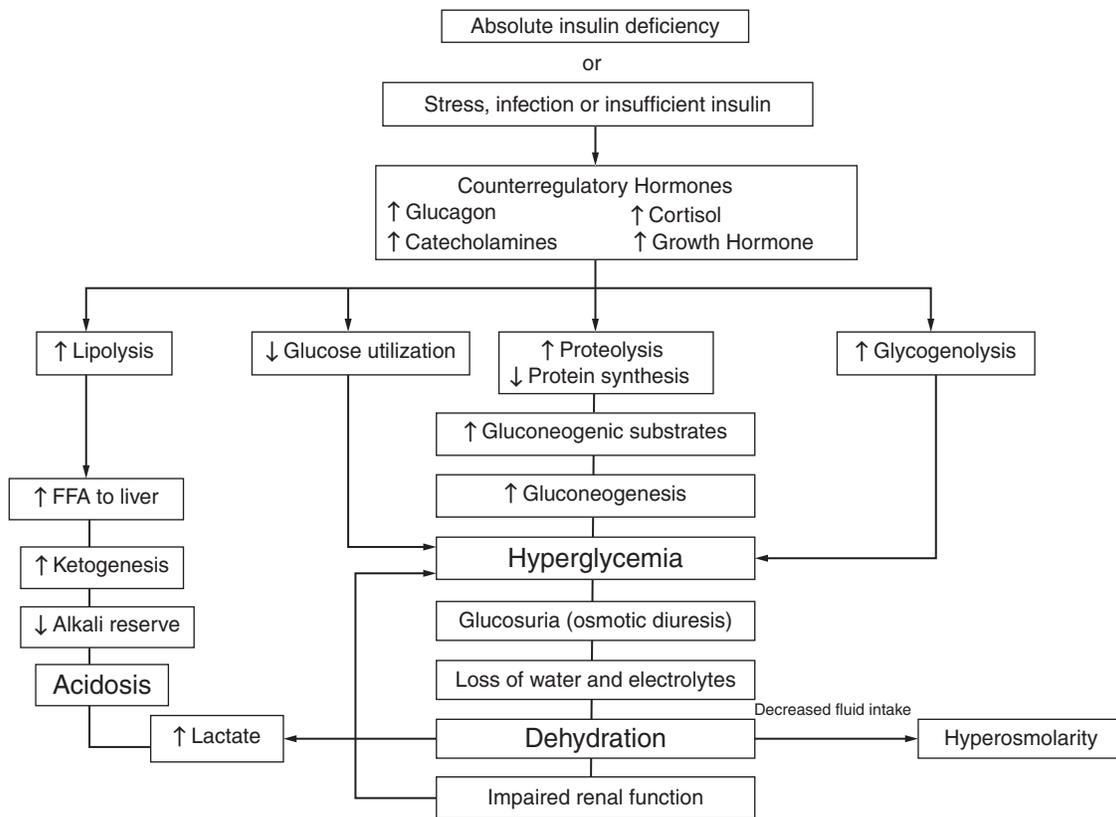


Fig. 21.10 The pathophysiology of diabetic ketoacidosis is illustrated as a function of absolute insulin deficiency or insufficient insulin in the presence of major stress, such as an infection, which leads to increases in the four major counterregulatory hormones. Together, these changes increase glucose production via glycogenolysis and gluconeogenesis, which together result in hyperglycemia, osmotic diuresis, and dehydration. Simultaneously increased lipolysis leads to ketone body production and acidosis in combination with increased lactic acid from dehydration. See text for greater detail.

TABLE 21.7 Fluid and Electrolyte Maintenance Requirements and Estimated Losses in Diabetic Ketoacidosis

Approximate Daily Maintenance Requirements		Average (range) Losses per Kg ^c
Water	Holliday-Segar method First 10 kg: 100 mL/kg/d Second 10 kg: 50 mL/kg/d Each additional 1 kg: 20 mL/kg/d alternatively Surface area method 1500 mL/m ² /d ^a	70 mL (30–100)
Sodium	2–4 mmol ^b	6 mmol (5–13)
Potassium	2–3 mmol	5 mmol (3–6)
Chloride	2–3 mmol	4 mmol (3–9)
Phosphate	1–2 mmol	0.5–2.5 mmol

Using the Holliday-Segar method, for example, for a 10-year-old child weighing 30 kg, maintenance fluid requirement is: 1000 + 500 + 200 = 1700 mL/day

^aThis method should not be used for children <10 kg.

^bMaintenance electrolyte requirements are expressed per 100 mL of maintenance fluid.

^cLosses are expressed per unit of body weight because the losses remain relatively constant in relation to total body weight.

dietary intake is readily explicable by the following example. The average healthy 10-year-old child has a daily intake of 2000 or more calories, of which approximately 50% are derived from carbohydrates. With the development of diabetes, daily losses of water and glucose may be as much as 5 L and 250 g, respectively. This represents 1000 calories lost in the urine, or 50% of average daily caloric intake. Therefore despite the child's compensatory increased intake of food and water, the calories cannot be used, excessive caloric losses continue, and increasing catabolism and weight loss ensue.

Pyogenic skin infections and candidal vaginitis in girls or candidal balanitis in uncircumcised boys are occasionally present at the time of diagnosis of diabetes. They are rarely the sole clinical manifestations of diabetes in children, and a careful history will invariably reveal the coexistence of excessive urination or recurrence of enuresis in a previously toilet-trained child, excessive thirst, and perhaps weight loss. Ketoacidosis is responsible for the initial presentation of many (about 25%–40%) diabetic children. Ketoacidosis is likely to be present more often in children younger than 5 years of age because the diagnosis may not be suspected and a history of polyuria and polydipsia may be difficult to elicit.^{137–145} The early manifestations may be relatively mild and consist of vomiting, polyuria, and dehydration.

In more prolonged and severe cases, Kussmaul respiration is present—and there is an odor of acetone on the breath. Kussmaul respiration may be confused with bronchiolitis or asthma and if erroneously treated with steroids or adrenergic agents, worsen diabetes. Abdominal pain or rigidity may be present and may mimic appendicitis or pancreatitis. Cerebral obtundation and (ultimately) coma ensue and are related to the degree of hyperosmolarity. Laboratory findings include glucosuria, ketonuria, hyperglycemia, ketonemia, and metabolic acidosis. Leukocytosis is common, and nonspecific serum amylase levels may be elevated. The serum lipase level is usually not elevated. In those with abdominal pain, it should not be assumed that these findings are evidence of a surgical emergency before a period of appropriate fluid, electrolyte, and insulin therapy to correct dehydration and acidosis. The abdominal manifestations frequently disappear after several hours of such treatment.

Diagnosis

The diagnosis of DM must be considered in children who have the following manifestations: those who have a history suggestive of diabetes, especially symptoms of polyuria with polydipsia, and failure to gain weight or a weight loss despite a healthy appetite; those who have confirmed glucosuria; and those who have clinical manifestations of metabolic acidosis with or without stupor or coma. In all instances, the diagnosis of DM is dependent on the demonstration of hyperglycemia in association with glucosuria with or without ketonuria. When classic symptoms of polyuria and polydipsia are associated with hyperglycemia and glucosuria that meet the criteria for diagnosing DM as defined by the ADA or World Health Organization (WHO), a glucose tolerance test is contraindicated.

Renal glucosuria may be an isolated congenital disorder or a manifestation of the Fanconi syndrome and other renal tubular disorders owing to severe heavy metal intoxication, ingestion of certain drugs (e.g., outdated tetracycline), or inborn errors of metabolism (e.g., cystinosis). When vomiting, diarrhea, and inadequate intake of food are complicating factors in any of these conditions, starvation ketosis may ensue and simulate DKA. The absence of hyperglycemia eliminates the possibility of diabetes. It is also important to recognize that not all urinary sugar is glucose, and infrequently galactosemia, pentosuria, and the fructosuria will require consideration as diagnostic possibilities.

The discovery of glucosuria, with or without a mild degree of hyperglycemia, during a hospital admission for trauma or infection (or even during the associated emotional upheaval) may herald the existence of diabetes. In most of these instances, the glucosuria remits during recovery.² Because this circumstance may indicate a limited capacity for insulin secretion, which is unmasked by elevated plasma concentrations of stress hormones, these patients should be rechecked at a later date for the possibility of hyperglycemia, clinical features of DM, and family history of diabetes.

A family history of DM in two preceding generations should suggest the possibility of a MODY syndrome; absence of the common antibodies to beta cells, such as IA2, GAD65, ZnT8, or ICA, strengthens the possibility of a diagnosis of MODY.^{7,13,14} Under these circumstances, a glucose tolerance test may be useful to establish a diagnosis. Glucose tolerance testing should be performed several weeks after recovery from the acute illness, with a glucose loading dose adjusted for weight. Evidence indicates that the test is most likely to be abnormal in those with HLA DR3 and DR4, in whom ICA or insulin autoantibodies are detected, or who have MODY.^{7,13,14} Transient hyperglycemia is common in patients with asthma treated with epinephrine and steroids. Further testing in such patients is not indicated. Screening procedures, such as postprandial determinations of blood glucose or oral glucose tolerance tests, have yielded low detection rates in children—even among those considered at risk, such as siblings of diabetic children. Accordingly, such screening procedures are not recommended in children.

DIABETIC KETOACIDOSIS PATHOPHYSIOLOGY AND TREATMENT

Diabetic Ketoacidosis

DKA is the entity of severe metabolic decompensation caused by the combined effects of deficiency of circulating insulin, together with increased levels of the counterregulatory hormones: catecholamines, glucagon, cortisol, and growth hormone, as well as proinflammatory cytokines^{139,146} (see Fig. 21.10). Typical

symptoms and signs are polyuria, enuresis, vomiting, dehydration, increased thirst, Kussmaul respiration, the odor of acetone on the breath, together with characteristic biochemical features, which include hyperglycemia, hyperketonemia, metabolic acidosis, and electrolyte abnormalities.^{147–149}

Severe insulin deficiency occurs as DKA evolves in previously undiagnosed T1DM, or when treated patients deliberately or inadvertently do not take insulin, especially the long-acting component of a multiple daily injection (MDI) regimen, or markedly reduce the doses of insulin, for example, during an illness, such as gastroenteritis. Patients who use an insulin pump can rapidly develop DKA when insulin delivery is interrupted for any reason¹⁵⁰ because pumps deliver only short-acting insulin and there is no long-acting component on board as there is with MDI. Relative insulin deficiency occurs when the concentrations of counterregulatory hormones increase in response to stress (e.g., sepsis, trauma, or febrile illness) and lead to metabolic decompensation despite administration of the usual dose of insulin. Although less common than in T1DM, the SEARCH for Diabetes in Youth Study in the United States reported that nearly 6% of youth with T2DM presented with DKA.¹⁵¹

The combination of absolute or relative insulin deficiency and high counterregulatory hormone concentrations causes an accelerated catabolic state with increased glucose production by the liver and kidney (via glycogenolysis and gluconeogenesis) together with impaired peripheral glucose utilization, which result in hyperglycemia and hyperosmolality (see Fig. 21.10). The combination of low insulin and high counterregulatory hormones also increases lipolysis and ketogenesis that result in hyperketonemia and metabolic acidosis from the accumulating ketoacids predominantly β -hydroxybutyrate. Hyperglycemia exceeding the usual renal threshold, approximately 10 mmol/L (180 mg/dL), causes osmotic diuresis, dehydration, and loss of electrolytes in urine, which often is aggravated by vomiting associated with severe ketosis. These changes stimulate further stress hormone production, which induces more severe insulin resistance and worsening hyperglycemia and hyperketonemia. Lactic acidosis from hypoperfusion or sepsis may contribute to the acidosis. Unless this cycle is interrupted by fluid and electrolyte therapy and administration of exogenous insulin, fatal dehydration and metabolic acidosis will ensue.

DKA is a life-threatening medical emergency that requires prompt recognition and treatment. It must be differentiated from acidosis and coma from other causes, such as hypoglycemia, uremia, gastroenteritis with metabolic acidosis, lactic acidosis, salicylate intoxication, encephalitis, and other intracranial lesions.

The biochemical criteria for the diagnosis of DKA are¹⁵²: hyperglycemia (blood glucose >11 mmol/L [200 mg/dL]), venous pH under 7.3 or serum bicarbonate less than 15 mmol/L, ketonemia or ketonuria (typically $\geq 2+$ positive, “moderate or large”), or blood beta-hydroxybutyrate (BOHB) concentration of 3 mmol/L or higher is indicative of DKA.¹⁵³ Partially treated children and children who have consumed little or no carbohydrate (anorexia or religious fasting, a low-carbohydrate high-fat diet) may have only modestly elevated blood glucose concentrations, referred to as *euglycemic ketoacidosis*.^{154,155} Recently, euglycemic ketoacidosis has been described in patients using sodium glucose transport protein 2 (SGLT2)-inhibitors (not currently approved for use in children).^{156–158} The severity of DKA is defined by the degree of acidosis: mild, venous pH 7.2 to 7.3 or serum bicarbonate 10 to 15 mmol/L; moderate, pH 7.1 to 7.2 or serum bicarbonate 5 to less than 10 mmol/L; severe, pH under 7.1 or serum bicarbonate less than 5 mmol/L.¹⁵⁹

The frequency of DKA at the time of diagnosis varies widely worldwide and inversely correlates with the regional incidence of T1DM, ranging from approximately 15% to 70% in Europe and North America. At the time of diagnosis, approximately 30% to 50% of all children have DKA even in developed countries.^{160–169} Programs that heighten awareness have decreased rates of DKA.¹⁷⁰ DKA is the leading cause of complications, death, and excessive healthcare use and costs in patients with T1DM.¹⁷¹

DKA is more common in younger children (especially <2 years of age, including infants with neonatal diabetes), often the consequence of diagnostic error and delayed treatment.^{172–175} DKA at diagnosis is also more common in ethnic minorities and in children who do not have ready access to medical care for social or economic reasons.^{164,172,176–178} In children with established T1DM the risk of DKA is 1% to 10% per patient per year.^{150,179–183} Risk is increased in children who omit insulin¹⁸⁴; children with poor metabolic control or previous episodes of DKA; intercurrent illness, especially gastroenteritis with persistent vomiting and inability to maintain hydration; children with psychiatric disorders, including those with eating disorders; children living in stressful or dysfunctional family circumstances; and peripubertal and adolescent girls. DKA is a well-recognized hazard associated with use of recreational drugs and binge alcohol consumption in adolescents and young adults.¹⁸⁵ Insulin omission or failure to follow sick day or pump failure management guidelines accounts for almost all episodes of recurrent DKA.

DKA must be distinguished from hyperglycemic hyperosmolar state (HHS), formerly referred to as *hyperosmolar nonketotic coma*, a syndrome characterized by extremely elevated serum glucose concentrations and hyperosmolality without significant ketosis.¹⁴⁵ The criteria for HHS include plasma glucose concentration over 33.3 mmol/L (600 mg/dL), venous pH greater than 7.25 (arterial pH >7.30), serum bicarbonate over 15 mmol/L, small ketonuria, absent to small ketonemia, effective serum osmolality greater than 320 mOsm/kg, obtundation, combativeness, or seizures (occur in approximately 50%).^{145,186}

Although much less common in children and adolescents than DKA, the incidence of HHS is increasing^{187–189}; a recent study found HHS at presentation in 2% of youth with T2DM (often associated with morbid obesity).¹⁹⁰ Unlike the usual symptoms of DKA (hyperventilation, vomiting and abdominal pain), which typically bring children to medical attention, the gradually increasing polyuria and polydipsia of HHS may go unrecognized, eventually resulting in profound dehydration and severe electrolyte losses at the time of presentation; fluid losses in HHS have been estimated to be twice those of DKA. Furthermore, obesity and hyperosmolality make the clinical assessment of dehydration especially challenging because hypertonicity preserves intravascular volume; thus despite severe volume depletion and electrolyte losses signs of dehydration may be less evident. Reports have described a high incidence of pre-existing neurological damage. Profound hyperglycemia may develop over a period of several days; initially, the obligatory osmotic polyuria and dehydration may be partially compensated for by increased fluid intake. However, with progression, thirst becomes impaired, possibly the result of altered function of the hypothalamic thirst center caused by hyperosmolality. In some instances, this is the consequence of a preexisting defect in the hypothalamic osmoregulatory mechanism.

The characteristic features of HHS and DKA may overlap. Some patients with HHS, especially those with severe dehydration and prerenal azotemia, have mild or moderate acidosis because of hypoperfusion and lactic acidosis. Conversely, children with T1DM may have severe hyperglycemia, especially if

BOX 21.2 Aims of Therapy in Diabetic Ketoacidosis

1. Expand intravascular volume
2. Correct derangements in fluid, electrolyte, and acid-base status
3. Correct intermediary metabolism with insulin
4. Identify and treat any precipitating event, such as infection

high-carbohydrate-containing beverages have been consumed to quench thirst.¹⁹¹

The low production of ketones is mainly attributed to hyperosmolarity, which in vitro blunts the lipolytic effect of epinephrine, and to the concomitant antilipolytic effect of residual insulin. Blunting of lipolysis by the therapeutic use of β -adrenergic blockers may also contribute to the syndrome. A key difference between DKA and HHS is the degree of insulinopenia, which is nearly absolute in DKA, whereas in HHS there is sufficient residual insulin activity to limit lipolysis in adipose tissue but inadequate insulin to suppress gluconeogenesis and permit normal peripheral glucose utilization at a time of increased glucose production induced by the increased concentrations of stress or counterregulatory hormones. Depression of consciousness correlates with the degree of hyperosmolarity in both HHS and DKA, and hemoconcentration predisposes to cerebral arterial and venous thromboses^{145,188} (Box 21.2).

Management of Diabetic Ketoacidosis

Treatment of DKA consists of three basic elements: meticulous monitoring of vital signs and biochemical responses; replacement of fluids and electrolytes, with the volume and content based on an estimate of the degree of dehydration and daily maintenance requirements; and provision of IV insulin (see Table 21.7). Treatment should be instituted as soon as the clinical diagnosis is confirmed. Acute management should follow the general guidelines for Pediatric Advanced Life Support (PALS),^{192,193} with particular attention to the following: immediately measure blood glucose and blood BOHB concentrations with bedside meters¹⁵³ or measure urine ketones with test strips (that measure only acetoacetic acid) if bedside blood ketone measurements are not available. Perform a clinical evaluation to identify a possible infection. Weigh the patient and, if body surface area is used for fluid therapy calculations, measure height or length to determine surface area. The current weight should be used for fluid calculations.

Clinical estimation of the degree of dehydration is imprecise^{194–196}; useful signs include prolonged capillary refill time (normal capillary refill is ≤ 1.5 –2 seconds), abnormal skin turgor (“tenting” or inelastic skin), dry mucus membranes, sunken eyes, absent tears, weak pulses, and cool extremities. More signs of dehydration tend to be associated with more severe dehydration¹⁹⁷; weak or impalpable peripheral pulses, hypotension, and oliguria suggest 10% or greater dehydration.

Assess level of consciousness using Glasgow Coma Scale (GCS)—Table 21.8.¹⁹⁸ In the unconscious or severely obtunded patient without normal airway protective reflexes, secure the airway and empty the stomach by continuous nasogastric suction to prevent pulmonary aspiration. Intubation should be avoided if possible; an increase of pCO₂ during or following intubation above the level that the patient had been maintaining may cause cerebrospinal fluid (CSF) pH to decrease and contribute to worsening of cerebral edema.¹⁹⁹

Give oxygen to patients with circulatory impairment or shock. A cardiac monitor should be used for continuous electrocardiographic monitoring to assess T waves for evidence of hyper- or hypokalemia.^{200,201}

A second peripheral IV catheter should be placed for repetitive blood sampling. Unless absolutely necessary, avoid inserting a central venous catheter because of the high risk of thrombosis, especially in the very young child.

Give antibiotics to febrile patients after obtaining appropriate cultures of body fluids.

Bladder catheterization usually is not necessary, but if the child is unconscious or unable to void on demand (e.g., infants and very ill young children) the bladder should be catheterized.

Obtain a blood sample for laboratory measurement of:

- Serum or plasma glucose
- Serum BOHB
- Electrolytes (including serum bicarbonate)
- Blood urea nitrogen, creatinine
- Serum osmolality
- Venous pH, pCO₂
- Hemoglobin, hematocrit. An increased white blood cell count in response to stress is characteristic of DKA and is not indicative of infection²⁰²
- Albumin, calcium, phosphate, magnesium concentrations (if possible)
- Urine ketones (if serum BOHB has not been measured)
- Obtain appropriate specimens for culture (blood, urine, throat) if there is evidence of infection (e.g., fever)
- If laboratory measurement of serum potassium is delayed, perform an electrocardiogram (ECG) for baseline evaluation of potassium status^{200,201}
- Although not essential for management of DKA per se, hemoglobin (Hb)A1c may be useful in specific patients as it provides information about the duration of hyperglycemia

Where Should the Child With Diabetic Ketoacidosis be Managed?

Successful management of DKA and HHS requires meticulous monitoring and recording of the patient’s clinical and biochemical response to treatment so that timely adjustments in treatment can be made when indicated by the patient’s clinical or laboratory data. After initial resuscitation, the child should receive care in a unit that has written guidelines and experienced nursing staff trained in management of DKA in children and adolescents. Children with severe DKA and those at increased risk for cerebral injury (e.g., <5 years of age, severe acidosis, low pCO₂, high blood urea nitrogen) should be treated in an intensive care unit (ICU).^{152,203}

Clinical and Biochemical Monitoring

- Hourly (more frequently as indicated) vital signs including heart rate, respiratory rate, blood pressure
- Hourly (more frequently as indicated) neurological observations (GCS; see Table 21.8) for warning signs and symptoms of cerebral injury (see later in section on cerebral edema)
- Amount of administered insulin
- Hourly (or more frequently as indicated) fluid input and output
- Laboratory tests: serum glucose, electrolytes, blood urea nitrogen, calcium, magnesium, phosphate, hematocrit, and blood gases should be repeated 2 to 4 hourly, or more frequently, as clinically indicated, in more severe cases
- Blood BOHB concentrations, if available, every 2 to 4 hours^{204–208}
- Point-of-care BOHB measurements correlate well with a reference method up to 3 mmol/L, but are not accurate above 5 mmol/L^{206,209}

TABLE 21.8 Glasgow Coma Scale

Best Eye Response	Best Verbal Response	Best Verbal Response (Nonverbal Children)	Best Motor Response
1. No eye opening	1. No verbal response	1. No response	1. No motor response
2. Eyes open to pain	2. No words, only incomprehensible sounds; moaning	2. Inconsolable, irritable, restless, cries	2. Extension to pain (decerebrate posture)
3. Eyes open to verbal command	3. Words, but incoherent ^a	3. Inconsistently consolable and moans; makes vocal sounds	3. Flexion to pain (decorticate posture)
4. Eyes open spontaneously	4. Confused, disoriented conversation ^b	4. Consolable when crying and interacts inappropriately	4. Withdrawal from pain
	5. Oriented, normal conversation	5. Smiles, oriented to sound, follows objects and interacts	5. Localizes pain
			6. Obeys commands

The Glasgow Coma Scale (GCS) consists of three parameters and is scored between 3 and 15; 3 being the worst and 15 the best.⁵⁵ One of the components of the GCS is the best verbal response, which cannot be assessed in nonverbal young children. A modification of the GCS was created for children too young to talk.

^aInappropriate words, random, or exclamatory articulated speech, but no sustained conversational exchange.

^bAttention can be held; patient responds to questions coherently, but there is some disorientation and confusion.

- Useful calculations:
 - Anion gap = $\text{Na} - (\text{Cl} + \text{HCO}_3)$; normal is 12 ± 2 mmol/L. In DKA the anion gap is typically 20 to 30 mmol/L; an anion gap greater than 35 mmol/L suggests concomitant lactic acidosis
 - “Corrected sodium” = measured Na + $2([\text{plasma glucose} - 5.6]/5.6)$ mmol/L or measured Na + $2([\text{plasma glucose} - 100]/100)$ mg/dL
 - Effective osmolality (mOsm/kg) = $2 \times (\text{plasma Na}) + \text{plasma glucose mmol/L}$ ²¹⁰

Fluids and Electrolyte Therapy

Fluid and electrolyte replacement therapy is a cornerstone of DKA management. The aims are to restore circulating volume, replace sodium and the extracellular and intracellular water deficits, improve glomerular filtration, and enhance clearance of glucose and ketones from the blood. Patients with DKA have a deficit in extracellular fluid (ECF) volume usually in the range of 5% to 10% of body weight.^{211,212} Shock with hemodynamic compromise is rare in pediatric DKA. In moderate DKA assume 5% to 7% and in severe DKA 7% to 10% dehydration. It is useful to calculate the corrected sodium concentration (earlier formula) to help assess the magnitude of the deficit of sodium and water.¹⁴⁸ The corrected sodium represents the expected serum sodium concentration in the absence of hyperglycemia. As the plasma glucose concentration decreases (in response to fluid and insulin), the measured serum sodium concentration should increase and the corrected sodium concentration should slowly decrease or remain in the normal range. A rapid and ongoing rise in serum sodium concentration may indicate loss of free water in the urine from diabetes insipidus, an agonal complication of cerebral edema.

There has been considerable controversy concerning the rate of fluid or sodium administration and a possible role of rapid administration of hypotonic fluid in the development of cerebral edema.^{213–215} A recent randomized clinical trial compared acute and long-term neurological outcomes in 1389 episodes in 1255 children with DKA treated with slower versus more rapid fluid administration using either 0.45% saline or 0.9% saline.²¹⁶ Neither the rate nor the sodium chloride content of IV fluids significantly influenced neurologic outcomes, suggesting that a range of fluid infusion rates and fluid sodium content may be safely used to treat DKA in children. Therefore an assumed fluid deficit between 5% and 10% of body weight should be replaced over 24 to 48 hours, together with maintenance fluids, using fluids with a sodium content between 0.45% and 0.9% saline. Most importantly, clinicians should not restrict fluid administration if clinical signs suggest the need for circulatory volume expansion.

Resuscitation Fluids. Immediately begin volume expansion with 0.9% saline 10 mL/kg infused over 30 to 60 minutes to restore the peripheral circulation. If tissue perfusion is poor, the initial fluid bolus is given more rapidly (e.g., over 15–30 minutes) and a second fluid bolus may be needed to ensure adequate tissue perfusion.

Deficit Replacement Fluids. Subsequent fluid management can be with 0.45% to 0.9% saline or a balanced salt solution (Ringer’s lactate, Hartmann’s solution or Plasmalyte).^{217–222} In addition to providing the usual daily maintenance fluid requirement, replace the estimated fluid deficit at an even rate over 24 to 48 hours^{152,219,223} (Table 21.9 provides an illustrative example). When plasma glucose concentrations fall during treatment, vascular volume will decrease; therefore it is essential to provide sufficient fluid and salt to maintain adequate tissue perfusion. Except for severely ill individuals, oral intake typically begins within 24 hours.²²³ Although rehydration is generally planned to occur over longer periods, in a study of 635 episodes of DKA the mean time to correction of DKA and complete restoration of the circulation was 11.6 ± 6.2 hours. Any remaining deficits are replenished by oral intake once DKA has resolved and patients are transitioned to subcutaneous insulin.²²³ Satisfactory outcomes have also been reported using an alternative simplified method: after initial fluid administration of 20 mL/kg of normal saline, 0.675% saline (3/4 normal saline, 115.5 mmol sodium) is infused at 2 to 2.5 times the usual maintenance rate of fluid administration regardless of the degree of dehydration, and decreased to 1 to 1.5 times the maintenance rate after 24 hours, or earlier if acidosis resolved.²²¹

Clinical assessment of hydration status and calculated effective osmolality are useful guides to fluid and electrolyte therapy. The aim is gradually to reduce serum effective osmolality to normal.^{223–225} There should be a concomitant increase in serum sodium concentration by 0.5 mmol/L for each 1 mmol/L decrease in glucose concentration. The sodium content of the fluid should be increased if measured serum sodium concentration is low and does not rise appropriately as the plasma glucose concentration falls.^{218,220,225,226}

The use of large amounts of chloride-rich fluids (combined with preferential renal excretion of ketones over chloride) may be associated with the rapid development of hyperchloremia^{227–229} (defined as a ratio of chloride:sodium $[\text{Cl}^-:\text{Na}^+] > 0.79$)²³⁰ and hyperchloremic metabolic acidosis.^{228,231–233} Hyperchloremic acidosis is more common in patients who receive large amounts of 0.9% sodium chloride than in those who receive fluid containing 0.45% sodium chloride.^{216,234} The acidifying effect of chloride can mask recognition of resolution of ketoacidosis when total base deficit is used to monitor biochemical improvement.²²⁹ When hyperchloremia develops,

TABLE 21.9 Fluid and Electrolyte Therapy for Diabetic Ketoacidosis: Recommendations for Replacement of Fluid and Electrolytes

Replacement Fluids	Approximate Accumulated Losses With 10% Dehydration	Approximate Requirements for Maintenance (36 Hours)	Approximate Totals for Replacement and Maintenance (36 Hours)		
Water (mL)	3000	2250	5250		
Sodium (mmol)	300	70	370		
Potassium (mmol)	180	65	245		
Chloride (mmol)	180	65	245		
Phosphate (mmol)	75	35	110		
REPLACEMENT SCHEDULE (CONTINUOUS INTRAVENOUS INFUSION)					
Approximate Duration	Fluid (Composition)	Sodium mmol	Potassium mmol	Chloride mmol	Phosphate mmol
Hour 1	600 mL 0.9% NaCl (isotonic saline)	92	—	92	—
Hours 2–6 (200 mL/h for 5 hours)	1000 mL 0.9% NaCl plus 40 mmol KCl/L	154	40	194	—
Hour 7–12 (200 mL/h for 6 hours)	1200 mL 0.45% NaCl with 20 mmol/L potassium chloride and 20 mmol/L potassium phosphate	92	48	101	24
Subtotal initial 12 hours	2800 mL	338	88	387	24
Next 24 hours 100 mL/h	2400 mL 5% glucose in 0.45% NaCl with 20 mmol/L potassium chloride and 20 mmol/L potassium phosphate	185	96	233	48
Total over 36 hours	5200 mL	523	184	620	72

a persisting base deficit or low bicarbonate concentration can be erroneously interpreted as being caused by ongoing ketosis.²³⁵ Measurement of bedside BOHB levels will prevent any confusion and demonstrate that ketoacidosis has resolved. Hyperchloremic acidosis resolves spontaneously. Although the anion gap is useful to track resolution of ketosis, it has two limitations in this setting: it is unable to differentiate a mixed metabolic acidosis (hyperchloremic and ketotic), and the degree of hyperchloremic acidosis is not quantifiable. Normally, the difference between the serum sodium and chloride concentrations is 30 to 35 mmol/L. To partition the chloride component of the base deficit, the following formula has been proposed to enable clinicians to track resolution of ketoacidosis at the bedside: chloride-induced base deficit = (plasma sodium – plasma chloride – 32).²²⁹ The chloride load can be reduced by not giving potassium as potassium chloride (use potassium acetate instead) and by using fluids, such as Ringer's lactate or Plasmalyte, in which a portion of the chloride is replaced, respectively, by lactate or acetate.²³⁶ Table 21.9 illustrates fluid and electrolyte therapy for a 30-kg child with severe DKA estimated to be 10% dehydrated.

Insulin Therapy

Rehydration alone causes a marked decrease in blood glucose concentration^{237,238}; however, insulin therapy is essential to suppress glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis; promote normal cellular glucose utilization; and to normalize blood glucose concentrations.²³⁹ Start continuous insulin infusion, 0.05 to 0.1 U/kg/h^{240,241} administered by IV infusion controlled by an infusion pump, at least 1 hour after starting fluid replacement therapy; that is, after the patient has received initial volume expansion.²¹⁵ An IV bolus should not be used at the start of therapy; it is unnecessary,^{242,243} may increase the risk of cerebral edema,^{215,225,244} can precipitate shock by rapidly decreasing osmotic pressure, and can exacerbate hypokalemia. The dose of insulin should usually remain at 0.05 to 0.1 U/kg/h at least until resolution of DKA (pH >7.30, serum bicarbonate >15 mmol/L, BOHB <1 mmol/L, or closure of the anion gap), which invariably takes longer than normalization of blood glucose concentrations.²³⁵ If the insulin effect is adequate serum BOHB should decrease by

approximately 0.5 mmol/L per hour.²⁰⁸ If the patient shows marked sensitivity to insulin (e.g., some young children with DKA, patients with HHS, and some older children with established diabetes), the insulin dose may be decreased provided that metabolic acidosis continues to resolve. For example, if a young child is receiving 0.05 U/kg/h, it may be necessary to reduce the insulin dose to 0.03 U/kg/h to prevent hypoglycemia despite the addition of IV glucose. For mild and moderate DKA (pH >7.1–7.2), 0.05 U/kg/h (0.03 U/kg/h for age <5 years with mild DKA) is usually sufficient to resolve the acidosis.

To prevent hypoglycemia, 5% glucose should be added to the IV fluid when the plasma glucose falls to approximately 14 to 17 mmol/L (250–300 mg/dL) or sooner if the rate of fall is precipitous. It may be necessary to use 10% or even 12.5% dextrose to prevent hypoglycemia while continuing to infuse insulin to correct the metabolic acidosis.

In circumstances where continuous IV administration is not possible and in patients with uncomplicated DKA, hourly or 2-hourly subcutaneous (SC) rapid-acting insulin analog is safe and as effective as IV regular insulin infusion. Initial dose SC: 0.3 U/kg, followed 1 hour later by SC insulin lispro or aspart at 0.1 U/kg every hour, or 0.15 to 0.20 U/kg every 2 to 3 hours.^{245–247} If blood glucose falls to under 14 mmol/L (250 mg/dL) before DKA has resolved, reduce SC insulin lispro or aspart to 0.05 U/kg/h to keep blood glucose around 11 mmol/L (200 mg/dL) until resolution of DKA. SC administration of short-acting insulin (regular) every 4 hours is also a safe and effective alternative to IV insulin infusion in children with pH 7.0 or over. A suggested starting dose is 0.8 to 1 U/kg/24 h; the calculated 24-hour dose is divided by 6 to provide an insulin dose injected every 4 hours. Doses are increased or decreased by 10% to 20% based on the blood glucose level before the next insulin injection (e.g., if a child weighs 45 kg: $45 \times 0.8 = 36$ units; the starting dose is 6 units).²⁴⁸

Potassium Replacement

Children with DKA have total body potassium deficits on the order of 3 to 6 mmol/kg.^{211,212,249,250} Intracellular potassium is depleted from transcellular shifts caused by hypertonicity (increased plasma osmolality causes solvent drag in which water and potassium are drawn out of cells), acidosis, and

glycogenolysis and proteolysis secondary to insulin deficiency also cause potassium efflux from cells.¹⁴⁸ Vomiting and osmotic diuresis are major causes of potassium loss from the body, and secondary hyperaldosteronism in response to volume depletion promotes urinary potassium excretion. Despite total body potassium depletion, serum potassium levels may be normal, increased, or decreased at presentation.²⁵¹ Renal dysfunction reduces potassium excretion and contributes to hyperkalemia²⁵¹; whereas, insulin administration and correction of acidosis drives potassium back into the cells and rapidly decreases serum potassium levels.²⁵² An abrupt decrease in serum potassium concentration can predispose to a cardiac arrhythmia. Therefore potassium replacement is required regardless of the serum potassium concentration, except when there is renal failure.²⁵³ If the patient has hypokalemia, start potassium replacement (20 mmol/L should be used) at the time of initial volume expansion and before starting insulin therapy. Otherwise, start replacing potassium after initial volume expansion and concurrent with starting insulin therapy. If the patient has hyperkalemia, defer potassium replacement therapy until urine output has been documented. If immediate serum potassium measurements are unavailable, an ECG may help to determine whether the child has hyper- or hypokalemia.^{200,201} Prolongation of the PR interval, T wave flattening and inversion, ST depression, prominent U waves, and apparent long QT interval (because of fusion of the T and U waves) indicate hypokalemia. Tall, peaked, symmetrical, T waves and shortening of the QT interval are signs of hyperkalemia. The initial potassium concentration in the infusion should be 40 mmol/L, and subsequent potassium replacement therapy should be based on serum potassium measurements. Potassium phosphate may be used together with potassium chloride or acetate; for example, 20 mmol/L potassium chloride and 20 mmol/L potassium phosphate or 20 mmol/L potassium phosphate and 20 mmol/L potassium acetate. Administration of potassium exclusively as potassium chloride contributes to the risk of hyperchloremic metabolic acidosis, whereas administration entirely as potassium phosphate can result in hypocalcemia. The maximum recommended rate of IV potassium administration is usually 0.5 mmol/kg/h. If hypokalemia persists despite a maximum rate of potassium replacement, then the rate of insulin infusion can be reduced. Profound hypokalemia (<2.5 mmol/L) in untreated DKA is rare and necessitates vigorous potassium replacement, while delaying the start of insulin therapy until serum potassium levels are above 2.5 mmol/L to reduce the risk of cardiopulmonary and neuromuscular compromise.²⁵⁴

Phosphate

Osmotic diuresis causes phosphaturia and depletion of intracellular phosphate.¹⁴⁸ Plasma phosphate levels fall after starting treatment, exacerbated by insulin, which promotes entry of phosphate into cells.²⁵⁵ Phosphate depletion has been associated with a variety of metabolic disturbances.²⁵⁶ Phosphate is essential for the formation of 2,3-diphosphoglycerate (2,3-DPG), which governs the oxygen dissociation curve.²⁵⁷ Deficiency of 2,3-DPG shifts the oxygen dissociation curve to the left (i.e., more oxygen is retained by hemoglobin and less is available to tissues, a situation that predisposes to lactic acidosis). Acidosis per se shifts the hemoglobin-oxygen dissociation curve toward the right (Bohr effect) and thus partially "compensates" for 2,3-DPG deficiency. Because acidosis is corrected by the provision of insulin, the effects of 2,3-DPG deficiency may no longer be compensated for and release of oxygen to tissues may again be impaired. Exogenous phosphate contributes to the formation of new 2,3-DPG, permitting the oxygen dissociation curve to shift to the right, which facilitates release of

oxygen to tissues. Despite the earlier pathophysiological considerations, prospective studies have not shown clinical benefit from routine phosphate replacement.²⁵⁸

Clinically significant hypophosphatemia may occur if IV therapy without food consumption is prolonged beyond 24 hours. Severe hypophosphatemia with cellular phosphate depletion is uncommon but can have severe consequences depending on the severity and chronicity of the phosphate depletion. Symptoms usually do not occur until plasma phosphate is less than 1 mg/dL (0.32 mmol/L). Although severe hypophosphatemia can occur during treatment of DKA, symptoms are uncommon because it usually is acute, typically without antecedent chronic phosphate deficiency. Many organ systems can be affected.²⁵⁶ Manifestations include: metabolic encephalopathy, impaired myocardial contractility, respiratory failure because of weakness of the diaphragm, muscle dysfunction with proximal myopathy, dysphagia and ileus, and rare hematological effects include hemolysis, decreased phagocytosis and granulocyte chemotaxis, defective clot retraction, and thrombocytopenia. Acute hypophosphatemia in a patient with preexisting severe phosphate depletion can lead to rhabdomyolysis.^{256,259} Severe hypophosphatemia associated with any of the earlier symptoms should be treated. Potassium phosphate salts may be safely used as an alternative to or combined with potassium chloride or acetate, provided that careful monitoring of serum calcium is performed to avoid hypocalcemia.^{260,261}

Acidosis

Even severe acidosis is reversible by fluid and electrolyte therapy and insulin replacement; insulin arrests ketoacid production and allows ketoacids to be metabolized, which generates bicarbonate. Treatment of hypovolemia improves tissue perfusion and renal function, thereby increasing the excretion of organic acids. Controlled trials have not shown a clinical benefit from bicarbonate administration.²⁶² Concern regarding the therapeutic administration of bicarbonate centers on four considerations: shifting the hemoglobin-oxygen dissociation curve to the left may diminish the release of oxygen to tissues and predispose to lactic acidosis; bicarbonate administration accelerates the entry of potassium into cells and may cause hypokalemia²⁶³; provision of bicarbonate according to the calculated base deficit overcorrects and may result in alkalosis; and bicarbonate may lead to paradoxical worsening of cerebral acidosis (and cause cerebral depression) during the time when the plasma pH is being restored to normal because HCO_3^- combines with H^+ ; H_2CO_3 then dissociates to CO_2 and H_2O . Whereas HCO_3^- slowly crosses the blood-brain barrier, CO_2 diffuses freely, thereby potentially exacerbating cerebral acidosis.²⁶⁴

Severe acidosis decreases respiratory minute volume, may cause hypotension by peripheral vasodilation, impairs myocardial function, and may cause insulin resistance. For these reasons, bicarbonate administration may be beneficial in the rare patient with life-threatening hyperkalemia or unusually severe acidosis (vpH <6.9) and compromised cardiac contractility.²⁶⁵ If bicarbonate is considered necessary, cautiously give 1 to 2 mmol/kg over 60 minutes. Bicarbonate must not be given by bolus infusion because it may precipitate cardiac arrhythmias.

Transition to Subcutaneous Insulin Injections

The transition to SC insulin and introduction of oral fluids should occur when substantial clinical improvement has occurred and DKA has resolved. Absence of ketonuria should not be used as an endpoint for determining resolution of DKA and clinicians must appreciate that persistent ketonuria

for 1 or more days may not reliably reflect the clinical improvement and should not be interpreted as evidence of a poor therapeutic response. Measurement of urine ketones with test strips (based on chemical reaction with nitroprusside), which measure acetoacetate and acetone (produced by nonenzymatic decarboxylation of acetoacetate) but not BOHB. The usual ratio of BOHB to acetoacetate is approximately 3:1 or 4:1 during fasting, but is commonly greater than or equal to 8:1 in DKA. With correction of acidosis, BOHB is oxidized to acetoacetate and persistent ketonuria typically occurs for several hours after serum BOHB levels have returned to normal.²⁰⁸

When ketoacidosis has resolved, oral intake is tolerated, and the change to SC insulin is planned, a dose of basal (long- or intermediate-acting) insulin should be administered in addition to rapid- or short-acting insulin. There may be benefits to earlier administration of a dose of long-acting insulin while the patient is still receiving IV insulin infusion. For example, in one study, 0.3 U/kg of SC insulin glargine was given in the first 6 hours of management and led to faster resolution of DKA.²⁶⁶ Another retrospective uncontrolled study showed that coadministration of insulin glargine (approximately 0.4 U/kg) early in the course of DKA treatment was well tolerated, did not increase the risk of hypoglycemia, but was associated with more frequent hypokalemia.²⁶⁷ To prevent rebound hyperglycemia, the first SC injection should be given 15 to 30 minutes (with rapid-acting insulin) or 1 to 2 hours (with regular insulin) before stopping the insulin infusion to allow sufficient time for the insulin to be absorbed. The blood glucose level should be monitored before and 2 hours after each meal, and the insulin dose adjusted to maintain blood glucose concentration in the range of 80 to 180 mg/dL. The specific insulin regimen, dose, and type of SC insulin should be according to local preferences and circumstances (see section Insulin Therapy).

The essential steps in managing DKA are summarized in Boxes 21.3 and 21.4.

Cerebral Edema

Although most patients appear to recover uneventfully from an episode of DKA, clinically overt cerebral edema manifesting as an abrupt decline in neurological status occurs in 0.5% to 1% of all episodes of DKA and is the major cause of morbidity and mortality, accounting for 60% to 90% of all DKA deaths.^{268,269}

In a retrospective analysis involving almost 7000 episodes of DKA, the following risk factors were identified: low partial pressure of arterial carbon dioxide ($p\text{CO}_2$) and high serum urea nitrogen concentrations at presentation, and treatment with bicarbonate.²⁶⁸ In 5% of children, radiographically apparent cerebral edema was apparent before initiation of therapy,²⁶⁸ and in another study one in five episodes of cerebral edema was evident at initial presentation before any fluid or insulin therapy could have contributed to water accumulation in the brain.²⁷⁰ These biochemical risk factors may simply reflect the initial severity of biochemical derangements. Thus avoidance of DKA (especially in young children) by early recognition of the signs and symptoms of DM is currently the only way to prevent cerebral edema, and patients with the clinical and biochemical features described earlier require even more careful monitoring.

Despite treatment with hyperosmolar agents (see later), approximately 20% to 25% of patients die and 15% to 35% of survivors have permanent neurological disabilities.^{268–270}

BOX 21.4 Complications of Therapy

- Cerebral edema
- Hypokalemia
- Hyperchloremic acidosis
- Hypoglycemia
- Inadequate rehydration

BOX 21.3 A Synopsis of the Principles of Management of Diabetic Ketoacidosis

1. Confirm diagnosis

Obtain:

- Blood glucose
- Blood beta-hydroxybutyrate (BOHB)
- Serum electrolytes
- Blood gas analysis: venous pH, $p\text{CO}_2$, HCO_3^-

Consider:

- Urine microscopy/culture
- Chest radiograph
- Blood culture
- Throat culture
- Electrocardiogram

Indications for admission to an intensive care unit:

- $\text{pH} \leq 7.00$
- Age ≤ 2 years
- Unconscious
- Blood glucose ≥ 1000 mg/dL

2. Begin intravenous fluids.

- 10–20 mL/kg of 0.9% (normal) saline (NaCl) over 30–60 minutes

3. Reassess the patient: what precipitated this episode?

- Delayed diagnosis
- Infection
- Noncompliance
- Trauma

4. Follow protocol guidelines in Table 21.9. Begin insulin 0.05–0.1

U/kg/h with hour 2 of IV therapy as outlined in Table 21.9

5. Measure glucose every 2 hours; electrolytes and acid-base status every 2 to 4 hours for the first 24 hours

6. Continue treatment with IV insulin as long as acidosis persists.

When plasma glucose approaches 300 mg/dL (17 mM) add 5% glucose to IV fluid and consider reducing insulin to 0.05 U/kg/h

7. If acidosis is not improving despite fluids and insulin infusion of 0.1 U/kg/h, consider:

- Severe sepsis causing lactic acidosis and/or insulin degradation
- Error in preparing intravenous insulin or insulin dose

8. In children age < 10 years (and especially age < 5 years), anticipate possible clinical cerebral edema after 4 to 6 hours of treatment (see Table 21.10 for symptoms and signs)

9. If cerebral edema is clinically apparent:

- Reduce rate of IV fluid administration; adjust rate to ensure maintenance of normal blood pressure
- Give mannitol 0.5 to 1 g/kg IV over 10 to 15 minutes (3% hypertonic saline 2.5–5 mL/kg is an acceptable alternative)
- Repeat in 30 minutes if inadequate response
- Elevate the head of the bed to 30 degrees and keep head in midline position

Although most patients do not exhibit any obvious neurological decline during treatment of DKA, subtle alterations of neurological function are often detectable after recovery, including deficits in memory, attention, and intelligence quotient.^{271–274} Changes in cerebral morphology have also been demonstrated by magnetic resonance imaging (MRI).^{272,275,276}

The cause of cerebral edema is controversial. An earlier theory posited that rapid fluid administration, which abruptly reduces serum osmolality, results in osmotic brain swelling (cytotoxic edema), and many treatment protocols advocate slow rehydration with isotonic fluids.^{152,226,277} It has been observed, however, that the degree of cerebral edema that develops during DKA correlates with the degree of dehydration and hyperventilation at presentation, but not with initial osmolality or osmotic changes during treatment.²⁷⁸ More recent investigations have not shown associations between the rate of fluid administration and brain injury^{268,270} and a large multicenter prospective randomized controlled trial showed that neither the salt content of the hydrating fluid (0.45% vs. 0.9% saline) nor its rate of administration influenced the development of cerebral edema or affected neurocognitive outcomes.²¹⁶ Studies of children and rodent models have found similarities between DKA-related brain injury and ischemia-reperfusion injury: low cerebral blood flow and brain cell swelling, low levels of high-energy phosphates in the brain and elevated lactate levels during untreated episodes of DKA, and cerebral hyperemia and vasogenic edema during treatment of DKA.^{278–280} MRI studies have shown a decrease in the apparent diffusion coefficient after recovery, indicating more pericellular and not intracellular water during treatment.²⁸¹ In addition, symptomatic and even fatal brain injury has been reported to occur before initiation of treatment for DKA.²⁸² The severity of cerebral hypoperfusion per se is unlikely to be sufficient to cause brain injury; therefore other factors probably contribute. DKA is also known to be associated with marked increases in systemic inflammatory cytokines and chemokines that may contribute to brain injury by activating cerebrovascular endothelia and increase leukocyte adhesion^{283–287}; and increased levels of metalloproteinase may promote blood-brain barrier dysfunction.²⁸⁷ A current alternative hypothesis posits that cerebral hypoperfusion (from dehydration and hyperventilation) and the effects of reperfusion together with neuroinflammation and cerebrovascular endothelial dysfunction are primarily involved in causing DKA-related brain injury.^{268,281,283,288,289} A prolonged duration and more severe biochemical derangements before therapy may predispose to cerebral ischemia. Younger children would be more prone because of the higher metabolic rate and oxygen requirement of a child's brain relative to an adult's brain. In addition, there may be a longer time for idiogenic osmoles to accumulate in the brain. An unexplained conundrum is that many children have evidence of raised intracranial pressure on imaging studies (e.g., computed tomography or MRI) consistent with subclinical cerebral edema frequently associated with mild mental status abnormalities, but only a minority develop clinically overt cerebral edema.^{290,291}

Clinically significant cerebral edema usually becomes apparent within the first 12 hours after therapy has started and when there has been improvement in blood glucose level, acid-base status, and state of hydration, but can be present at initial presentation or, rarely, 24 to 48 hours after the start of treatment.^{268,292,293} A previously alert patient may become drowsy, complain of severe headache, develop abnormal neurological findings, progress to coma, and have respiratory arrest with herniation of the brainstem. A constellation of bedside

TABLE 21.10 A Method of Clinical Diagnosis of Cerebral Edema Based on Bedside Evaluation of Neurologic State²⁹⁴

Diagnostic Criteria	Major Criteria	Minor Criteria
<ul style="list-style-type: none"> Abnormal motor or verbal response to pain Decorticate or decerebrate posture Cranial nerve palsy (especially III, IV, VI) Abnormal neurogenic respiratory pattern (grunting, tachypnea, Cheyne-Stokes respiration, apnea) 	<ul style="list-style-type: none"> Altered mentation/fluctuating level of consciousness Sustained heart rate deceleration (more than 20 bpm) not attributable to improved intravascular volume or sleep) Age-inappropriate incontinence^a 	<ul style="list-style-type: none"> Vomiting Headache Lethargy or being not easily aroused from sleep Diastolic blood pressure >90 mmHg Age <5 years

Signs that occur before treatment should not be considered in the diagnosis of cerebral edema. One diagnostic criterion, two major criteria, one major and two minor criteria have a 92% sensitivity and specificity of 96% (false positive rate 4%).

^aRapidly increasing serum sodium concentration suggests loss of free water in the urine from diabetes insipidus (consider that it may have been caused by interruption of blood flow to the pituitary gland because of cerebral herniation).

clinical observations may facilitate the recognition of developing cerebral edema (Table 21.10).²⁹⁴ Although useful, it should be noted that the criteria proposed by Muir and colleagues have not been independently corroborated.

Survival and neurological outcome are markedly improved with prompt recognition and intervention with mannitol or hypertonic saline, respiratory support by means of endotracheal intubation, and hyperventilation. Greater awareness, early recognition, and prompt treatment account for the reported decline in mortality²⁹⁵ (see Table 21.10 and Box 21.5).

BOX 21.5 Other Uncommon or Rare Causes of Morbidity and Mortality Associated With Diabetic Ketoacidosis

- Hypocalcemia, hypomagnesemia
- Severe hypophosphatemia
- Hypochloremic alkalosis
- Central nervous system complications: dural sinus thrombosis, basilar artery thrombosis, intracranial hemorrhage, cerebral infarction
- Venous thrombosis
- Pulmonary embolism
- Sepsis
- Rhinocerebral or pulmonary mucormycosis
- Aspiration pneumonia
- Pulmonary edema
- Adult respiratory distress syndrome (ARDS)
- Pneumothorax, pneumomediastinum, and subcutaneous emphysema
- Rhabdomyolysis
- Ischemic bowel necrosis
- Acute kidney injury including renal failure
- Acute pancreatitis

Treatment of Hyperosmolar Hyperglycemic Syndrome

The goal of initial fluid therapy is to expand the intra- and extravascular volume and restore normal renal perfusion. During therapy decreasing serum osmolality (from increased glucosuria and insulin-mediated glucose uptake) causes movement of water out of the intravascular space resulting in decreased intravascular volume; pronounced osmotic diuresis may continue for many hours in patients with extremely increased plasma glucose concentrations. Early in the course of treatment of HHS, urinary fluid losses may be considerable and, because intravascular volume may decrease rapidly, the rate of fluid replacement should be more rapid than is recommended for DKA to prevent vascular collapse. The initial bolus should be 20 mL/kg or more of isotonic saline (0.9% NaCl) and a fluid deficit of approximately 12% to 15% of body weight should be assumed. Additional fluid boluses should be given rapidly, if necessary, to restore adequate tissue perfusion. Thereafter, 0.45% to 0.75% NaCl should be administered to replace the deficit over 24 to 48 hours aiming for a gradual decline in corrected serum sodium concentration and serum osmolality. Because isotonic fluids are more effective in maintaining circulatory volume, isotonic saline should be restarted if perfusion and hemodynamic status appear inadequate as serum osmolality declines. Serum sodium concentrations should be measured frequently and the sodium concentration in IV fluids adjusted to promote a gradual decline in corrected serum sodium concentration. Mortality has been associated with failure of the corrected serum sodium concentration to decline with treatment, which may be an indication for hemodialysis.¹⁸⁷

Although there are no data to indicate an optimal rate of decline in serum sodium concentration, 0.5 mmol/L per hour has been recommended for hypernatremic dehydration.²⁹⁶ With adequate rehydration alone (i.e., before commencing insulin therapy), serum glucose concentrations should decrease by 4.1 to 5.5 mmol/L (75–100 mg/dL) per hour.^{297,298} A more rapid rate of decline in serum glucose concentration is typical during the first several hours of treatment when an expanded vascular volume leads to improved renal perfusion. If there is a continued rapid fall in serum glucose (>5 mmol/L, 90 mg/dL per hour) after the first few hours, consider adding 2.5% or 5% glucose to the rehydration fluid. Failure of the expected decrease of plasma glucose concentration should prompt reassessment and evaluation of renal function. Unlike treatment of DKA, replacement of urinary losses is recommended.²⁴⁰ The typical urine sodium concentration during an osmotic diuresis approximates 0.45% saline; however, when there is concern about the adequacy of circulatory volume, urinary losses may be replaced with a fluid containing a higher sodium concentration.

Insulin Therapy

Whereas tissue hypoperfusion in HHS commonly causes lactic acidosis, ketosis usually is minimal and early insulin administration is unnecessary. Fluid administration alone causes a marked decline in serum glucose concentration as a result of dilution, improved renal perfusion leading to increased glucosuria, and increased tissue glucose uptake with improved circulation. The osmotic effect of glucose within the vascular space contributes to the maintenance of blood volume; therefore a rapid fall in serum glucose concentration and osmolality after insulin administration may lead to circulatory compromise and venous thrombosis unless fluid replacement is adequate. Patients with HHS also have extreme potassium deficits; a rapid insulin-induced shift of potassium to the intracellular space can

trigger an arrhythmia. Insulin administration should be initiated when or if serum glucose concentration is no longer declining at a rate of at least 3 mmol/L (~50 mg/dL) per hour with fluid administration alone. In patients with more severe ketosis and acidosis, however, insulin administration should be initiated earlier. Continuous administration of insulin at a rate of 0.025 to 0.05 U/kg/h can be used initially, with the dosage titrated to achieve a decrease in serum glucose concentration of 3 to 4 mmol/L (~50–75 mg/dL) per hour. Insulin boluses are not recommended.

Electrolytes. Deficits of potassium, phosphate, and magnesium are also greater in HHS than in DKA. Potassium replacement (40 mmol/L) should begin as soon as serum potassium concentration is within the normal range and adequate renal function has been confirmed. Higher rates of potassium administration may be necessary after starting an insulin infusion. Serum potassium concentrations should be monitored every 2 to 3 hours along with electrocardiographic monitoring. Hourly potassium measurements may be necessary if the patient has hypokalemia. Bicarbonate therapy is contraindicated; it increases the risk of hypokalemia and may adversely affect tissue oxygen delivery. Severe hypophosphatemia may lead to rhabdomyolysis, hemolytic uremia, muscle weakness, and paralysis. Although administration of phosphate is associated with a risk of hypocalcemia, an IV solution that contains a 50:50 mixture of potassium phosphate and another potassium salt (potassium chloride or potassium acetate) generally permits adequate phosphate replacement while avoiding clinically significant hypocalcemia. Serum phosphate concentrations should be measured every 3 to 4 hours. Patients with HHS frequently have large magnesium deficits, but there are no data to determine whether replacement of magnesium is beneficial. Replacement of magnesium should be considered in the occasional patient who experiences severe hypomagnesemia and hypocalcemia during therapy. The recommended dose is 25 to 50 mg/kg per dose for 3 to 4 doses given every 4 to 6 hours with a maximum infusion rate of 150 mg/min and 2 g/h.

Complications. Venous thrombosis associated with use of central venous catheters is a common complication in HHS.²⁹⁹ Prophylactic use of low-dose heparin has been suggested in adults, but there are no data to indicate benefit from this practice. Heparin treatment should be reserved for children who require central venous catheters for physiological monitoring or venous access and are immobile for more than 24 to 48 hours.¹⁴⁵ Rhabdomyolysis (myalgia, weakness, and dark urine) may occur in children with HHS resulting in acute kidney failure, severe hyperkalemia, hypocalcemia, and muscle swelling causing compartment syndrome.³⁰⁰ Monitoring creatine kinase concentrations every 2 to 3 hours is recommended for early detection. For unknown reasons, several children with HHS have had clinical manifestations consistent with malignant hyperthermia, a condition associated with a high mortality rate.^{301–303} Patients with a fever associated and a rise in creatine kinase concentrations may be treated with dantrolene, which reduces release of calcium from the sarcoplasmic reticulum and stabilizes calcium metabolism within muscle cells. Nonetheless, of the three reported patients with HHS treated with dantrolene only one survived.^{301,303} Altered mental status is common when serum osmolality exceeds 330 mOsm/kg; however, cerebral edema is rare. Among 96 cases of HHS reported in the literature in 2010, including 32 deaths, there was only one instance of cerebral edema.¹⁸⁷ A decline in mental status after hyperosmolality has improved with treatment is unusual and should be promptly investigated.

BOX 21.6 Principles of Management of Hyperosmolar Hyperglycemic State

- Rapidly infuse isotonic saline (0.9% NaCl) ≥ 20 mL/kg
- Assume a fluid deficit of 12% to 15% of body weight
- If necessary, additional fluid boluses should be given rapidly, to restore peripheral perfusion
- Thereafter, use 0.45% to 0.75% NaCl and aim to replace fluid deficit over 24 to 48 hours
- Start insulin infusion when plasma glucose is no longer declining ≥ 50 mg/dL per hour with fluid alone
- In patients with mixed HHS and DKA, initiate insulin earlier
- IV insulin at 0.025 to 0.05 U/kg/h; titrate dosage to achieve a decrease in plasma glucose of around 50–75 mg/dL/h
- Begin potassium replacement (40 mmol/L) as soon as serum potassium concentration is within normal range and adequate renal function has been established

DKA, Diabetic ketoacidosis; *HHS*, hyperglycemic hyperosmolar state.

Mixed Hyperosmolar Hyperglycemic Syndrome and Diabetic Ketoacidosis

Treatment must take into consideration potential complications of both DKA and HHS. Mental status must be closely monitored and frequent reassessment of circulatory status and fluid balance is absolutely necessary to guide therapy. To maintain an adequate circulatory volume, the rate of fluid and electrolyte administration usually exceeds that required for the typical case of DKA. Insulin is essential to resolve ketosis and arrest hepatic gluconeogenesis; however, insulin infusion should be deferred until after the patient has received an initial fluid bolus and the circulation has been stabilized. Serum potassium and phosphate concentrations should be carefully monitored as described earlier for HHS (Box 21.6).

TREATMENT OF TYPE 1 DIABETES MELLITUS

General Principles

Optimal management of the child with T1DM (and T2DM) requires an integrated approach, taking into account the overall level of functioning of the child and family, the nutritional and lifestyle patterns specific to that child, and attention to the overall developmental stages of childhood and adolescence. There is no one appropriate insulin regimen or meal plan. The overriding principle should be that the diabetes care plan should fit wherever possible into the surrounding home and school environments, and that the primary childhood tasks of education, socialization, growth, and maturity continue unhindered by the extra responsibilities that diabetes care entails.

This daunting task of assisting families in managing diabetes is best accomplished by a multidisciplinary team, consisting of physicians, nurse educators and practitioners, dietitians, and mental health professionals, all trained and experienced in the nuances of diabetes care in children. Children with diabetes should be seen by the team at frequent intervals (usually every 3 months in established patients) for assessment of glycemic control, growth, and development; evaluation for related disorders and complications; education; troubleshooting; problem-solving; and screening for adjustment problems that may affect diabetes or the overall health of the child.

Goals of Therapy

The Diabetes Control and Complications Trial (DCCT) established that intensive glycemic management leading to near-normal glucose and A1c levels significantly reduced the risk of developing long-term complications and the benefits of this reduced risk outweighed the threefold increase in the risk of severe hypoglycemia.³⁰⁴ Slowing the development of early retinopathy was observed in the DCCT subgroup of intensively treated adolescents, as well as in adults in the study.³⁰⁵ In the Epidemiology of Diabetes Interventions and Complications (EDIC) Study, the observational follow-up to the DCCT, a continued increase in risk of complications was seen in the former conventional treatment group years after the end of the randomized controlled trial despite improved metabolic control in the former conventional treatment group, indicating that near normal glucose and A1c levels should be achieved and maintained in patients with T1DM as early in the course of the disease as possible.³⁰⁶ As a result of this evidence, current recommendations mandate that youth with T1DM should aim to achieve plasma glucose and HbA1c levels as close to normal as possible, as early in the course of the disease as possible, and with as few severe hypoglycemic events as possible.

With respect to specific targets, the ADA treatment guidelines indicate that HbA1c targets should be less than 7.5%¹ and the International Society of Pediatric and Adolescent Diabetes (ISPAD) lowered this to under 7%,³⁰⁷ although both sets of guidelines emphasize individualizing targets for patients and families to also minimize hypoglycemia and enhance quality of life. Previously, higher HbA1c levels were suggested for very young children because of the potential risk of recurrent hypoglycemia on the developing brain. There are many problems with this approach, including increasing evidence that chronic hyperglycemia is also detrimental to the developing brain³⁰⁸ and that lower HbA1c can be achieved without increased risk of severe hypoglycemia in children younger than 6 years of age.³⁰⁹

Types of Insulin

Currently available insulins are classified based on their duration of action as rapid, short, intermediate, and long-acting, and each is available in a concentration of 100 U/mL (U-100). A higher concentration (U-500) of human regular insulin is available for the patient who has severe insulin resistance. Appropriate dilutions can be prepared for younger patients requiring low doses. The development of recombinant DNA technology to synthesize human insulin and human insulin analogs has changed the face of insulin treatment.

The aim of insulin replacement therapy is to simulate the normal pattern of insulin secretion as closely as possible (Fig. 21.11). This aim can best be achieved through the use of basal-bolus therapy using MDI or continuous subcutaneous insulin infusion (CSII) pump therapy. Until recently, options for insulin formulations were limited. Today, there are more than 10 varieties of biosynthetic human and analog insulins, including human regular insulin, human neutral protamine Hagedorn (NPH) insulin, long-acting insulin analogs, rapid-acting insulin analogs, and several kinds of premixed insulins (see Table 21.11).

Human Regular Insulin

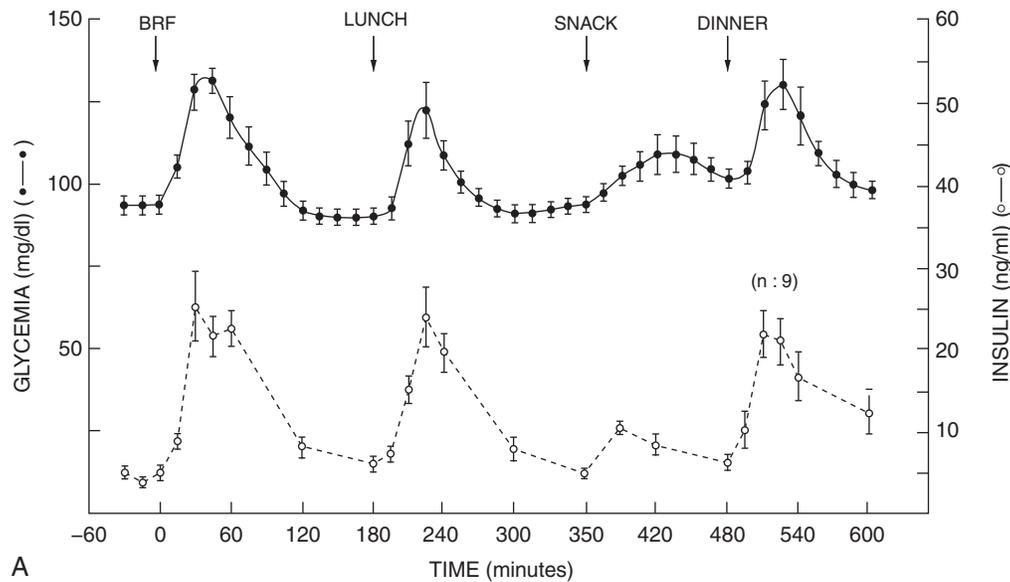
Human regular insulin was a mainstay of insulin management of youth with T1D until the early 2000s when the advent of rapid-acting insulin analogs virtually eliminated its use in children and adolescents, except for IV administration. The delayed absorption and prolonged duration of action of the large

premeal bolus doses of regular insulin that are required by adolescents with T1D to overcome the insulin resistance of puberty contributed to problems with hyper- and hypoglycemia in this age group.³⁰⁵ Regular insulin remains the insulin of choice for IV infusion in the treatment of DKA. A special U-500 (500 units/mL) formulation of regular insulin is available for use in patients with severe insulin resistance who require very large daily doses of insulin.

Rapid-Acting Analogs

Lispro (Eli Lilly), aspart (Novo Nordisk), and glulisine (Sanofi) insulins are produced by amino acid substitutions in the C-terminal region of the B chain that reduce the affinity of insulin molecules to self-aggregate into hexamers. These modifications allow more rapid absorption of the analog into the bloodstream after subcutaneous injection. A new fast-acting

insulin, Fiasp (Fast insulin aspart, Novo Nordisk) has recently been approved for use in children. Compared with regular insulin, the faster absorption of rapid-acting analogs results in higher and sharper peaks and shorter duration of action, pharmacokinetic and pharmacodynamic effects that reduce the risk of late postprandial hypoglycemia and temper early postmeal hyperglycemia.^{310,311} Puberty does not appear to alter the pharmacokinetics of premeal boluses of rapid analogs, but the insulin resistance of puberty reduces the ability of these insulins to stimulate glucose metabolism.³¹² Rapid-acting insulin may be safely mixed with intermediate-acting insulins, but mixing with long-acting insulin is not recommended, as such mixing markedly attenuates the peak action of the rapid-acting analog.^{313,314} All three rapid-acting analogs may be used in an insulin pump, and studies have shown them to be safe and effective.^{315,316} Lispro and aspart insulin may be diluted to concentrations less than U-100, such as U-50 or U-25, using diluents obtained from



A Pharmacokinetic profiles of human insulin and insulin analogs

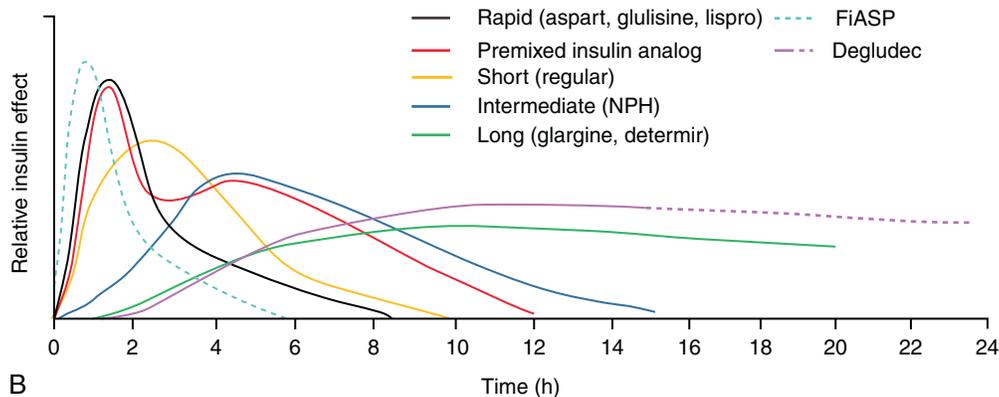


Fig. 21.11 A, Representation of the normal relationships among food intake, blood glucose, and serum insulin concentration. Note that glucose concentration is maintained between 80 and 140 mg/dL. Note also the precise release of insulin that has passed through the portal circulation synchronous with and proportional to the food-induced glycemic excursions. Compare and contrast these patterns with the time pattern of insulin action after subcutaneous injection of aspart/glulisine/lispro, regular, NPH, and glargine/detemir insulins. B, pharmacokinetic profiles of human insulin and its analogs. The pharmacokinetic profiles of several currently used insulin preparations are schematically presented. The fast insulin aspart (FiASP) and long-acting analog (Degludec) have been manually inserted consistent with currently available data. These figures are illustrative of and correspond with the time frames in Table 21.11. (B, Modified from Brunton, S.A. (2013). Integrating advances in insulin into clinical practice. *J Fam Pract.* 62(9 Suppl Insulin), S2-S18).

TABLE 21.11 Types of Insulin Preparations and Suggested Action Profiles for Subcutaneous Administration

Insulin Type	Onset of Action (h)	Peak of Action (h)	Duration of Action (h)
Ultrarapid acting analog (faster aspart) ^{a,c}	0.1–0.2	1–3	3–5
Rapid-acting analogs (aspart, glulisine, and lispro)	0.15–0.35	1–3	3–5
Regular/soluble (short acting)	0.5–1	2–4	5–8
NPH	2–4	4–12	12–24 ^a
Basal long-acting analogs			
Glargine ^b	2–4	8–12	22–24 ^a
Detemir	12	4–7	20–24 ^a
Glargine U300	2–6	Minimal peak	30–36
Degludec ^c	0.5–1.5	Minimal peak	>42

NPH, Neutral protamine Hagedorn insulin. All insulins used must be produced under “Good Manufacturing Practice/Good Laboratory Practice” conditions.

^aThe duration of action may be shorter.

^bBiosimilar glargine approved in some countries.

^cNot yet approved worldwide or not for pediatric indication.

(From Danne, T., et al. (2018). ISPAD Clinical Consensus Guidelines 2018: Insulin treatment in children and adolescents with diabetes. *Pediatr Diabetes*, 19(Suppl. 27), 115–135.)

the manufacturer. Diluted insulin is used in very young children and others who are sensitive to insulin and could benefit from the more accurate dosing. Rapid-acting insulin analogs can be given IV, but they have not been shown to be superior to regular insulin.³¹⁷ A newer insulin analog is Fiasp, which has a slightly faster onset of action and peak and shorter duration of action.^{318,319}

Intermediate-Acting Insulin

NPH is now the only available intermediate-acting insulin. Although the delay in peak action of NPH given before breakfast provides a means to cover lunchtime glucose excursions, NPH is less than satisfactory for overnight basal insulin replacement.³²⁰ As with regular insulin, the advent of long-acting insulin analogs has largely replaced NPH in basal-bolus MDI treatment in pediatrics. However, NPH is still an effective, intermediate duration insulin, particularly useful in special circumstances, such as poor adherence and in those who may be less economically affluent.

Long-Acting Insulin Analogs

Glargine (Lantus manufactured by Sanofi) and detemir (Levemir manufactured by Novo Nordisk) were the first two commercially available long-acting insulin analogs developed to meet the body’s basal insulin requirements for regulating hepatic glucose production. Insulin glargine is an analog of human insulin with C-terminal elongation of the beta chain by two arginine residues and replacement of asparagine in position A21 by glycine. This analog is soluble in the acidic solution in which it is packaged but relatively insoluble in the physiological pH of the extracellular fluid. Consequently, microprecipitates of glargine insulin are formed following subcutaneous injection, which markedly delays its absorption into the systemic circulation. The action of insulin detemir is prolonged by the addition of a fatty acid side chain that promotes reversible binding with albumin in the interstitial fluid and in the circulation.

Pharmacokinetic and pharmacodynamic studies have demonstrated that these long-acting insulin analogs have flat and

prolonged time-action profiles. However, compared with glargine, detemir has been shown to have a more consistent pharmacokinetic profile in children and a more consistent pharmacodynamic profile in adults with T1DM.^{321,322} Basal insulin replacement with insulin detemir is more frequently likely to require two daily injections than treatment with insulin glargine.

Insulin degludec is a new, ultralong-acting insulin that is a product of coupling of Des-B30 threonine insulin to fatty acid side chains. After injection, degludec self-associates, forming long chains of insulin that dissociate very slowly. Plasma insulin levels rise reaching peak concentrations 10 to 12 hours after an injection and the half-life is between 17 to 21 hours, roughly double the duration of action of insulin glargine. Degludec has been shown to be safe and effective in children and adolescents with T1DM.³²³

Premixed Insulin

Various premixed insulin formulations, combining either rapid-acting insulin analogs or regular insulin with NPH or intermediate-acting insulin analogs, are currently available. The percentage of rapid acting/regular to intermediate acting varies with the formulation. The practical advantage of premixed insulins to cover both short- and intermediate-term insulin needs in a single shot is also their greatest limitation. There is less flexibility when using premixed insulin for adjusting the individual insulin components, and this can be particularly problematic in young children or those with variable food intake. Use of premixed insulin has been generally limited to patients and families who have difficulties managing more complicated treatment regimens or in resource limited settings.³²⁴

Syringes Versus Pens

Insulin syringes come in a variety of sizes that are particularly useful in young children. In addition to the traditional 100-unit (1-mL) syringe marked in 2-unit increments, 50-unit (0.5-mL) and 30-unit (0.3-mL) syringes are available that are marked in single-unit increments, and 30-unit syringes with 1/2-unit markings can also be ordered. These syringes are available with needle lengths from 8 to 13 mm. Insulin pens are also available, both as disposable pens and reusable pens with disposable cartridges. Half-unit dosing is available and may be useful in smaller children.

Initiating Insulin Therapy

Most newly diagnosed patients who present with symptoms of diabetes or are in DKA require upward of 1 U/kg/day at the start of insulin treatment. For asymptomatic patients who are “incidentally” diagnosed with diabetes, for example, during a routine sports physical examination or a well-child examination, starting doses may only need to be around 0.5 U/kg/day or less. The goal of initial insulin therapy in children with T1D should be to achieve near normoglycemia over the first few weeks of treatment to give the child the opportunity to enter the “honeymoon,” or partial remission phase. The honeymoon period results from a combination of improved function of residual beta cells and reversal of the insulin resistance that accompanies uncontrolled diabetes. Prompt achievement of optimal control rather than the methods used to achieve good control appears to be the most important factor in obtaining these outcomes.³²⁵ The ability to achieve target A1c levels with little or no severe hypoglycemia is greatly enhanced in T1D patients with residual beta-cell function.³²⁶

Some centers admit all newly diagnosed patients to the hospital for initial diabetes education and insulin dose titration, but others have effective outpatient treatment protocols. During the 2 to 3 weeks following discharge, insulin doses are titrated toward target premeal glucose values of 70 to 130 mg/dL during

daily telephone contact; patients are seen in follow-up clinic visits, approximately 2, 6, 13, 26, 39, and 52 weeks following diagnosis.

INSULIN REGIMENS

Plasma insulin levels in nondiabetic individuals are characterized by relatively low basal levels on which are superimposed meal-stimulated spikes in insulin concentrations. Current intensive treatment regimens attempt to simulate this pattern of plasma insulin fluctuations by using a basal-bolus approach to insulin replacement. Because exogenous insulins are injected or infused subcutaneously rather than directly into the portal vein, their rates of absorption may vary. Also the dose that is injected is determined empirically, so it lacks the precision of endogenously secreted insulin. Thus no insulin replacement regimen will precisely duplicate the pattern of normal insulin secretion; there will be periods of increased plasma insulin concentrations that may produce hypoglycemia and periods of low insulin levels that lead to hyperglycemia. Therefore the goal of current insulin regimens is to minimize the frequency and severity of excursions into the hyper- and hypoglycemic range.

The DCCT and its follow-up, the EDIC studies, established basal-bolus therapy using either MDI or CSII as the gold standard of treatment of T1D. However, insulin only works if the youngster receives it, and other factors must be addressed when determining the best insulin regimen for an individual patient. These factors include the availability of an adult parent/guardian to supervise insulin administration, the ability to count carbohydrates and monitor glucose levels with a glucose meter or a continuous glucose monitor (CGM), and the willingness to wear a pump or take four or more injections of insulin daily.

Multiple Daily Injection Regimens

MDI regimens attempt to replicate normal insulin secretion through the use of a long-acting insulin analog to replace basal insulin needs along with bolus injections of rapid-acting insulin analog to cover food intake and to correct elevations in blood glucose levels. Either once or twice daily basal insulin coverage may be used. Typically, basal insulin accounts for approximately 40% to 50% of the insulin total daily dose (TDD). However, children younger than 5 years of age often require basal insulin doses that are 30% to 40% of TDD, and there is considerable variability between patients. Once-daily glargine or detemir may be given either in the morning or in the evening. Patients treated with detemir may be more likely to need twice-daily injections than those treated with glargine. Some pediatric practitioners mix long- and rapid-acting insulin to reduce the number of injections, but studies indicate that such mixing markedly blunts and delays the absorption and action of the rapid-acting insulin component of the mixture.^{313,314}

Any one of the rapid-acting insulin analogs may be used to cover bolus insulin needs. Ideally, the rapid-acting insulin bolus is given 10 to 15 minutes before eating, but this is a difficult goal to achieve in many youths with T1D. For the most precise dosing of bolus insulin, it is necessary to use an insulin-to-carbohydrate ratio (ICR) and insulin sensitivity or correction factor, as well as the rate and direction of change in sensor glucose levels in patients using continuous glucose monitoring devices. The ICR is defined as the grams of carbohydrate that 1 unit of insulin will cover. This ratio may be determined using the 500 rule, namely, dividing 500 by the TDD will give a starting point for the carbohydrate ratio. For example, in a teenager with a TDD of 50 units, 1 unit of insulin will cover 10 grams of carbohydrate ($500/50 = 10$). It should also be noted that the ICR frequently differs by time of day because

more insulin is often needed per gram of carbohydrate before breakfast and less may be given to cover bedtime snacks. These are intended as guides to then individualize insulin dosing based on glucose results. Accuracy in determining the carbohydrate content of meals is of utmost importance but often a problem in adolescents. Teens tend to underestimate the number of carbohydrates in their meal, and refresher meetings with a dietitian can reinforce the importance of maintaining mastery of this skill. Also carbohydrates are not the sole source of nutrients that require insulin for optimum metabolism; formulas exist for adjusting insulin requirements for protein and fat content.³²⁷ In addition to meal coverage, a correction dose of rapid-acting insulin should be given at meal times to “fix” an elevated blood glucose level. Correction doses may also be given at other points in the day for the same reason. Traditional “sliding scale” insulin doses based on blood glucose levels have given way to more sophisticated dosing algorithms based on an insulin sensitivity factor (ISF); namely, how much will blood glucose be lowered by 1 unit of insulin. The ISF may be determined using the 1800 rule: divide 1800 by the TDD. The correction dose can then be calculated according to the following equation:

$$\frac{(\text{Actual blood glucose (BG)} - \text{Target BG})}{\text{ISF}} = \text{Number of units of insulin}$$

No matter how they are initially calculated, ICRs and ISFs are subsequently adjusted based on glucose monitoring results.

Particular advantages of basal-bolus MDI regimens are that they try to mirror the physiological model of insulin secretion, increase flexibility in the timing and size of meals, and provide more opportunities to “course correct” throughout the day in response to abnormal glucose excursions. A particular disadvantage of these regimens is that they require a large number of daily injections. Indeed, because of the flat time-action profile of the long-acting basal insulin analogs, basal-bolus MDI treatment puts a premium on compliance with premeal bolus dosing. Without bolus doses of rapid-acting insulin, long-acting basal insulin alone cannot prevent marked postprandial hyperglycemia. However, strict adherence to premeal bolus dosing is difficult for teenagers with T1D, even when it is made as easy as possible to accomplish with an insulin pump. These are some of the reasons why mean A1c levels in pediatric patients, especially adolescents, with T1DM, remain well above the target levels recommended by the ADA and ISPAD (Box 21.7).³²⁸

Insulin Pump Therapy

CSII delivered by pumps of varying degrees of sophistication have become a standard means of treatment for children with diabetes. Some of the practical benefits of CSII compared with MDI are listed in Box 21.8. A specific risk of pump therapy is that prolonged accidental or purposeful interruption of insulin delivery over several hours can lead to the development of ketones and DKA because the patients are only receiving rapid-acting insulin.³²⁹ This risk can be reduced or eliminated by regular blood glucose testing and to facilitate blood glucose testing, a variety of devices have been developed. Linking these devices with pumps and algorithms led to the emergence of autoregulated insulin delivery systems, which are constantly improving and becoming incorporated into daily practice as detailed later.

A recent ISPAD guidelines chapter focuses on diabetes technology.³³⁰

Ideal candidates for pump therapy include motivated families who are committed to monitoring blood glucose at least 4 times per day and have a working understanding of basic diabetes management, especially carbohydrate counting and using ICR and ISF to calculate insulin doses.

BOX 21.7 Glycemia and Blood Glucose Target Recommendations

Target HbA1c	HbA1c <53 mmol/mol (<7.0%)		
Necessary elements for successful glycemic management	<ul style="list-style-type: none"> This target must be individualized with the goal of achieving a value as close to normal as possible while avoiding severe hypoglycemia, frequent mild to moderate hypoglycemia, and excessive stress/burden for the child with diabetes and their family Factors that must be considered when setting an individualized target include, but are not limited to: <ul style="list-style-type: none"> Access to technology, including pumps and CGM Ability to articulate symptoms of hypoglycemia and hyperglycemia History of severe hypoglycemia/hypoglycemic unawareness History of compliance with therapy Whether child is a high or low glyculator Whether child has continued endogenous insulin production (e.g., in the new onset or “honeymoon” period of diabetes) HbA1c measurements at least quarterly Glucose monitoring using CGM or self-monitored BG measurements up to 6 to 10 times per day Regular review of glucose values with therapy adjustments as necessary 		
Glycemic targets	NICE goal A1c	ISPAD goal A1c	ADA goal A1c
	≤ 48 mmol/mol (< 6.5%)	< 53 mmol/mol (<7%)	< 53 mmol/mol (7%)
Premeal	4.0–7.0 mmol/L (70–126 mg/dL)	4.0–7.0 mmol/L (70–130 mg/dL)	5.0–7.2 mmol/L (90–130 mg/dL)
Postmeal	5.0–9.0 mmol/L (90–162 mg/dL)	5.0–10.0 mmol/L (90–180 mg/dL)	
Prebed	4.0–7.0 mmol/L (70–126 mg/dL)	4.4–7.8 mmol/L (80–140 mg/dL)	5.0–8.3 mmol/L (90–150 mg/dL)

(From DiMeglio, L.A., Acerini, C.L., Codner, E., Craig, M.E., Hofer, S.E., Pillay, K., Maahs, D.M. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: glycemic control targets and glucose monitoring for children, adolescents, and young adults with diabetes. *Pediatr Diabetes*, 19 (Suppl 27), 105–114.)
 ADA, American Diabetes Association; BG, blood glucose; CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; ISPAD, International Society for Pediatric and Adolescent Diabetes; NICE, National Institute for Health and Care Excellence.

BOX 21.8 Practical Benefits of Continuous Subcutaneous Insulin Infusion

- Basal infusion rates adjustable up to every 30 minutes
- Programmable temporary basal rates
- Preprogrammable alternate basal rate profiles
- One site insertion every 2 to 3 days (versus many injections each day)
- Dose calculator
- Pump history functions and ability to upload data to data management systems
- Customizable square wave and dual wave boluses
- Ability to program temporary basal rates for sick days and during and after exercise
- Ability to deliver very small (0.025–0.05 units) doses of insulin

Most currently available insulin pumps are “smart” pumps into which both ICRs and ISFs are programmed to create a bolus calculator. Some pumps have the ability to receive and integrate blood glucose values into their bolus calculator through a wireless link with a specific blood glucose meter. Bolus doses can be administered over a few minutes or as square-wave and dual-wave boluses over a longer period of time. Ideally, bolus doses should be delivered 10 to 15 minutes before the meal to minimize postmeal excursions.³³¹ In young children and picky eaters, a small priming bolus of insulin can be given before the meal to cover glucose levels above the target

and the amount of carbohydrate that will be eaten reliably, followed by additional bolus doses depending on how many carbohydrates are actually consumed.

Basal insulin needs are covered by the rapid-acting insulin, which is delivered through a preprogrammed “basal pattern.” This pattern can be made up of multiple different rates, which allow for a waxing/waning pattern of basal insulin delivery. In addition, most pumps allow for multiple 24-hour basal patterns to be preprogrammed and stored in the memory. For example, some adolescents have a basal pattern for school days and one for weekends to account for their tendency to wake up much later on weekends and holidays. Finally, temporary changes can be made to the basal insulin pattern, which can be an effective tool for dealing with exercise or sick day management.

CSII-Based Treatment Regimens

Because of their increasingly important role in managing T1DM in childhood, a detailed description and discussion of pump therapy follows this section; [Box 21.8](#)¹² summarizes several key elements of pump therapy. Practical suggestions for starting sensor therapy are shown in [Box 21.9](#). Linking CSII therapy with implantable sensors constitutes an important step in establishing a “closed loop system” as detailed below.

The use of insulin pumps (CSII) has increased dramatically in children and adolescents with T1D since the 1990s. The T1D Exchange study group has reported that 33% of children younger than 6 years of age, 47% of children aged 6 to 12 years, 50% of adolescents aged 13 to 17 years, and 52% of patients aged 18 to 25 years used an insulin pump in the cohort of 20,555

BOX 21.9 Practical Suggestions for Starting Sensor Therapy**INITIAL TRAINING FOR INSERTION AND ADHERENCE**

- Sufficient time spent at initiation of sensor therapy
- Ensure adequate insertion technique
- Discuss the use of supplementary adhesive products:
 - Wipes: skin tac IV prep, skin prep
 - Dressings and barriers: tegaderm, IV-3000, hypafix (A US-trained Certified Diabetes Educator (CDE) should verify current brand names in the entire list)
 - External wraps: Coban, PreWrap
- Discuss the use of adhesive removers:
 - unisolve or detachol
 - ointments (baby oil)

CALIBRATION (IF REQUIRED)

- Discuss frequency of and ideal times for calibrations
 - Consider preemptive calibration schedule: encourage calibration three times a day (e.g., before breakfast, dinner and bedtime)
 - Discuss calibrating when glucose is relatively stable (no arrows present, no rapid change on sensor glucose graph)

ALERTS AND ALARMS

- Consider leaving alerts off at the start sensor therapy
- Personalize alerts and use wide thresholds at first (i.e., 70–250 mg/dL [3.9–13.9 mmol/L])
- Adjust over time:
 - with recurrent hypoglycemia, set low alert first
 - with suboptimal control, set high alert first
- Judiciously introduce rate of change or predictive alerts
 - suggest only those that will trigger active intervention by the user

REAL-TIME DATA

- Discuss nonadjunctive use of sensor data
- Discuss recommendations on insulin dose/behavioral adjustments based on sensor glucose values and trends
- Discuss data transfer to the “cloud”

RETROSPECTIVE REVIEW

- Encourage regular downloading and review of data
- Encourage insulin dose/behavioral modifications based on retrospective data reviews

participants (11,641 age <18 years) from the United States.³³² This is matched by the 44.4% of 16,570 children and adolescents from the international SWEET registry,³³³ and with many other countries in the European Union and Israel reporting similar insulin pump use in children and adolescents. Several tertiary pediatric centers report the use of insulin pumps as the predominant treatment modality in their pediatric population.³³⁴ CSII has several practical benefits over MDI, including programmable basal insulin rates with multiple basal rate profiles and the possibility of temporary basal rates, adjustable “dual-wave” boluses, same injection site over 2 to 3 days (versus MDI), dose calculators, and the possibility of data upload, all of which facilitate the day-to-day management of the disease.

The use of insulin pumps is associated with improved metabolic control, lower glycated hemoglobin, or less hypoglycemia in many,^{335,336} but not all,^{337–339} clinical trials. Large cohort studies, including several thousand pediatric patients, have described a significant correlation between better metabolic control and the use of insulin pumps.^{332,333} In addition, the quality of life assessed by the PedsQL-T1DM questionnaire is significantly associated with the use of insulin pumps,³³⁹ and preliminary data suggest better cognition, mood, and behavior.^{340,341}

Selection criteria for insulin pump therapy³⁴² in the pediatric patient population broadened as experience and data on its use accumulated.³³⁶ Commonly, poor glycemic control, frequent hypoglycemia, increased glucose variability regardless of HbA1c, compromised quality of life, and increased risk of chronic complications are listed. Self-blood glucose monitoring (SBGM) is still an important prerequisite, as is parental support. Young age, frequent SBGM, and lower HbA1c at pump initiation are associated with better long-term metabolic control.³⁴³ In many instances, particularly in economically developed countries, SBGM has been replaced by continuous glucose monitoring as detailed below. Conversely, female gender, age older than 10 years in girls, and poor metabolic control at pump initiation are associated with higher risk for attrition from insulin pump therapy.³⁴⁴ Interestingly, children with inadequate metabolic control on MDI may experience the highest decrease in their HbA1c when switched to an insulin pump.³⁴⁵ In preschool children, initiating the insulin pump therapy at the disease onset may help patients to maintain lower HbA1c for up to 8 years.³⁴⁶

Insulin pump therapy initialization is a structured multistep process involving the whole (extended) patient’s family and a diabetes team ideally including a pediatric endocrinologist, a pediatric diabetes nurse educator, a specialized dietitian, a child

psychologist, and a social worker. In addition, adequate training of day-care personnel, a nanny, or school personnel is of crucial importance.³⁴⁷

Basal Insulin Rates

A continuous sequence of small boluses approximately every 10 minutes of a short-acting insulin analog provides basal insulin required for suppressing liver gluconeogenesis and ketogenesis and maintaining basal normoglycemia. Age-specific daily patterns of basal rates differ considerably in dose and distribution.^{348,349} Because insulin resistance increases with age, so does the insulin dose. Although preschool children often need highest basal rates between 10 PM and 2 AM, this pattern changes as puberty approaches when the highest insulin requirement usually starts after 4 AM and lasts until wakeup.

When switching a patient from MDI to CSII, the total basal rate dose usually is calculated based on the previous dose of the long-acting insulin analog and is usually reduced by 10% to 20% if HbA1c was under 8%. Alternatively, the total daily insulin dose is reduced by 20% to 30% and then half is administered as the basal rate. Some clinicians calculate the basal rate dose arbitrarily as approximately 0.4 units of insulin per kilogram body weight, especially when the dose of the long-acting insulin analog or the total daily insulin dose when treated with injections was evidently not appropriate and achieved poor metabolic control.

Programming multiple basal rates in the pediatric population seems prudent, as this was associated with better metabolic control.³⁵⁰ Several initial approaches are used³⁵¹ with likely similar success, as basal rates should be subsequently verified and personalized. In infants and toddlers, a flat initial basal rate may be appropriate and tailored to individual need in the first week of insulin pump use. As modern insulin pumps can deliver as little as 0.025 units of insulin per hour, diluting insulin is rarely needed. Intermittent programming of 0 units of insulin per hour for shorter periods of up to 2 hours is sometimes used for neonates. A higher basal insulin rate will be needed around midnight with a lower rate applied in the afternoon. For older children and adolescents, the day may be divided into five parts: from midnight to 4 AM, covering the period with least basal insulin requirement, from 4 to 7 AM covering the dawn phenomenon period, from 7 AM to 1 PM covering the morning, from 1 to 8 PM covering the afternoon, and from 8 PM to midnight covering the evening.³⁵¹ Sometimes, an

additional time frame for the dusk phenomenon may be needed from 5 to 8 PM.³⁵² Several basal rate patterns may be needed for different situations: a standard pattern for regular weekdays, a weekend or leisure pattern with lower basal insulin rates during the morning (e.g., less stress from school or work or more activity), and a “sick days” basal rate pattern with 30% more insulin throughout the 24 hours.³⁵³

Verifying Basal Insulin Rate Settings

The ideal basal rate will keep the blood glucose levels within the desired range during fasting. The need for basal insulin changes with time and depends on insulin sensitivity that varies with age, lifestyle, growth, and several other fluctuating influences. Basal insulin rates can be best verified when food intake is omitted. The nighttime basal insulin rates may be verified first, as the overnight glucose concentration profile is usually of greatest concern. On an uneventful day after an early dinner (at 5–6 PM) that was meticulously covered by an appropriate bolus, SBGM is performed every 2 to 3 hours from 10 PM until wakeup. If the blood glucose changes for more than ± 40 mg/dL (2 mM), the basal rate approximately 1 hour before the blood glucose change is adjusted for $\pm 10\%$ to 20%. Similarly, a morning basal SBGM profile is performed after an uneventful night, skipping the breakfast and morning snack with SBGM every 2 to 3 hours until lunch. Finally, an afternoon basal SBGM profile is performed after an uneventful morning, skipping lunch, with SBGM every 2 to 3 hours until dinner. Basal insulin rates are adjusted as described for the night basal profile. If the blood glucose level falls below or above the usual range between 70 to 180 mg/dL, the condition is treated, the basal profile testing is discontinued, the basal rate is adjusted accordingly, and the basal rate SBGM profile retested in a couple of days. In preschool children basal rates can be verified in shorter time intervals, as only one meal per day can be omitted. Some verify basal profiles without omitting meals but rather after eating standardized small meals covered by appropriate boluses. CGM can be of obvious assistance and can reduce the frequency of SBGM. Frequent phone contacts with the diabetes team are prudent in the first weeks after insulin pump initialization to individually fine-tune basal insulin rates.³⁵³

Temporary Basal Rates and Basal Insulin Suspend

With little insulin in the body between boluses, reducing or stopping the basal insulin rate is an efficient way to prevent hypoglycemia during physical activity or sport.³⁵⁴ Smart pumps can show the amount of remaining insulin in the body after a bolus (so-called active insulin), which can be of considerable help in preventing exercise-associated hypoglycemia, as “active insulin” can be covered with additional carbohydrates.

Bolus Insulin Dosages

Careful and more frequent dosing of insulin for covering food and correcting high blood glucose levels is associated with better metabolic control when using insulin pumps.³⁵⁵ Modern insulin pumps can assist calculations of bolus insulin reducing errors³⁵⁶ and incorporating physiological variability of insulin sensitivity during the course of the day.

Bolus Insulin for Food Coverage

Principles of calculating boluses for food are same as for MDI, with the additional possibility of more precise dosing down to 0.025 units of insulin in some insulin pumps. Solid practical knowledge of carbohydrate counting is paramount. The arbitrary formula of 500 divided by the total daily insulin dose

for older children and adolescents (300 or 200 for preschool children or toddlers, respectively) can help in determining the initial ICR. The ICR can be higher (one unit of insulin covers fewer carbohydrates) in the morning, sometimes by 30% to 100% compared with midday, and slightly higher again in the evening. Bolus calculators are incorporated into modern pumps and do all required calculations based on preset ICRs, the current blood glucose, the planned amount of carbohydrate ingestion, and the glucose target range, typically between 70 and 120 mg/dL (3.9 and 6.5 mM).

Advanced food-bolus determination incorporates protein counting (15 g of carbohydrates for each additional 100 g of protein after the first 100 g)³²⁷ and the use of prolonged or “dual-wave” bolus administration, which is associated with better postprandial glucose control after ingestion of food with low glycemic index.³⁵⁷

Preferably, the bolus is administered 15 to 20 minutes before the meal as the postprandial peak of blood glucose precedes the peak of rapid-acting insulin analog action.^{358,359} As eating habits in smaller children may be unpredictable, a “split-bolus” approach may be used for optimal postprandial control with only 10% to 20% of the calculated bolus administered 15 to 20 minutes before the meal for covering “certainly” ingested food, and the rest administered after the meal according to the amount actually consumed.

ICRs should be verified on a regular basis with hourly postprandial SBGM or CGM, aiming at a blood glucose under 180 mg/dL (10 mM).

Missed prandial boluses, particularly during the school hours, are the most common cause of suboptimal metabolic control.³⁶⁰ Parental and school-personnel supervision and support is valuable.

Bolus Insulin for Correction of Hyperglycemia

ISF can be estimated by dividing 1800 by the TTD in older children and adolescents (2000 or 2200 in preschool children and toddlers, respectively). Again, bolus calculators incorporated into smart insulin pumps calculate correction boluses based on the preset ISF, current blood glucose level, preset blood glucose target range, and the amount of active insulin from previously administered correction boluses. The amount of active insulin calculated by the insulin pump bolus calculator depends on the setting of the insulin action time, commonly set to 3 hours for boluses used in pediatric population. A shorter insulin action time setting allows for more aggressive correction bolusing.

Also ISFs are regularly verified with SBGM 2 to 4 hours post-correction or with CGM. It is advisable to avoid correction boluses less than 2 hours apart as insulin accumulation (stacking) may occur and provoke hypoglycemia.³⁶¹

Specific Considerations and Acute Complications of Insulin Pump Therapy

Regular physical activity is strongly advised but challenging in youngsters with T1D, particularly in relation to hypoglycemia during and after it.^{362,363} The biggest advantage of CSII may be related to its possibility of reducing physical activity-related hypoglycemia.^{364,365} Basal insulin rate can be stopped or diminished by setting a temporary basal rate immediately prior and during the physical activity, depending on its intensity and duration. The presence of active insulin from previous boluses can be verified and an appropriate carbohydrate snack added before the physical activity, in addition to basal insulin rate modification, effectively preventing hypoglycemia. Finally, strenuous or prolonged exercise often results in delayed hypoglycemia, which can be prevented by a diminished temporary basal insulin rate programmed for several hours post physical

activity. Hypoglycemia can therefore be prevented without an excessive use of additional carbohydrates and thus a negative energy balance can be maintained, which is of particular importance for body weight management.

Reduction of hypoglycemia with sensible use of insulin pump therapy was demonstrated in several studies^{366,367} and has many important practical implications that must be discussed during the training of the family. The possibilities of insulin stacking after too frequent correction bolusing; inappropriate counting of carbohydrates; and insulin modifications related to an acute illness (e.g., vomiting), eating disorder, or physical activity are the most important issues that should be taken into consideration.

DKA was traditionally considered as a risk associated with CSII; however, studies report less DKA with the use of modern insulin pump.^{137,367} As only rapid-acting insulin analogs are used in insulin pumps, significant ketonemia may develop 4 to 6 hours after discontinuation of CSII.³⁶⁸ Partial or complete disconnection of the infusion set and plugging or kinking of the subcutaneous catheter are the most common causes. A DKA rescue plan must be included in the structured education provided to patients and caregivers. Blood or urine ketones must be checked if two consecutive blood glucose levels or the fasting blood glucose levels are over 275 mg/dL (>15 mM), and the possibility of infusion set problems considered. Infusion set must be replaced and an appropriate correction bolus administered, alternatively with an insulin pen injector, if the functioning of the infusion set is not certain. If ketonemia is greater than 1.5 mM, an increased temporary basal rate of 150% to 200% may be needed until blood ketones are normalized. Additional fluid intake is advisable. If ketonemia is associated with an acute illness or remains unexplained, contact with the on-call diabetes team is warranted.

Maintaining normoglycemia during acute severe illness has been demonstrated in adults,³⁶⁹ along with diminishing the danger of hypoglycemia. Despite the lacking published evidence for children, teams in pediatric ICU and neonatal ICU need appropriate training for maintaining stable glycemic control with CSII during acute severe illness.³⁷⁰ Anesthesiologists often suggest a switch to IV insulin during prolonged surgery, which with frequent blood glucose level determination provides a well-established and safe treatment modality. CSII should be restarted just before the termination of IV insulin administration. Discontinuing CSII during intermittent hospitalizations on various pediatric or surgery wards is usually associated with poor metabolic control and should be discouraged. A pediatric endocrinology team should be involved in the appropriate care of hospitalized children with diabetes.

Insulin Pump Therapy in Kindergarten and School

Most schools in developed societies accept chronically ill children and provide necessary care either enforced through law and regulations or voluntarily. A structured plan for school personnel and diabetes-related education similar to the one for caregivers facilitate cooperation among parents, the diabetes team, and the school. A cooperative spirit aimed at the best interests of the child with T1D usually yields optimum long-term results. The diabetes team serves as facilitator and coordinator in the collaboration between parents and the school personnel. Ideally, basic training for school personnel includes but is not limited to SBGM and appropriate recording; management of hypoglycemia including discontinuation of insulin delivery; rescue carbohydrates and administration of glucagon; management of hyperglycemia, including correction boluses and ketones checking, carbohydrate counting and determining boluses; and management of physical activity.^{347,371,372}

Despite significant improvements in metabolic control with the routine use of CSII in pediatric populations, most young people with type 1 diabetes still do not reach target metabolic control,³²⁸ with hypoglycemia and DKA remaining as problems.³⁷³ More emphasis on psychosocial support and successful parenting³⁷⁴ are needed in view of challenging modern environments to which children and adolescents are often exposed.

Blood Glucose Monitoring

The safety and success of any insulin regimen depend on frequent monitoring of blood glucose levels. Intensive diabetes control would have been impossible without the development of accurate and easy-to-use home glucose meters. Current models use glucose-oxidase-based electrochemical methods. The meters are fast and require very small volumes of blood (0.1 mL). The smaller blood volume requirement has allowed alternate site testing (e.g., forearm), which may minimize discomfort and improve adherence to self-monitoring regimens.

Frequent blood glucose monitoring, with a minimum of four tests per day, premeals, and at bedtime, is a minimal goal. The most recent ADA Position Statement¹ and ISPAD Guidelines³⁰⁷ recommend 6 to 10 fingerstick glucose checks daily or use of CGM to safely achieve tight glucose control.

Continuous Glucose Monitoring

It has been known for several years that more frequent SMBG correlates with improved HbA1c levels and reduced frequency of acute complications.^{375,376} Indeed, the introduction of real-time CGM systems has revolutionized management of insulin in the treatment of diabetes. Currently available CGM devices are based on sensors inserted transcutaneously and measure interstitial fluid glucose concentrations using glucose-oxidase-based electrochemical methods. Sensor glucose concentrations can be reported in real time, approximately every 5 minutes, with rate of change indicators and 1- to 24-hour retrospective profiles. This wealth of information allows for adjustments in insulin doses based on a retrospective analysis, as well as immediate, "at the moment" adjustments and predicted adjustments in the rate of insulin delivery or its temporary suspension based on the rate of change. Web-based data managing systems are available to assist patients and healthcare providers (HCPs) in evaluating sensor data alone, or in combination with insulin pump or connected pen data.³⁷⁷

In healthy children aged 2 to 8 years, CGM sensor glucose levels remain mainly within 4 to 7.8 mmol/L (72–140 mg/dL), with 9% under 4 mmol/L (<72 mg/dL), 3% <under 3.5 mmol/L (<63 mg/dL), and 1% over 9.0 mmol/L (>162 mg/dL). Importantly, 0% of sensor glucose values are greater than 11.1 mmol/L (>200 mg/dL) in healthy children. The glycemic pattern is very stable, with mean glucose SD 1.0 ± 0.2 mmol/L and coefficient of variation (CV) of 18.87%. Sensor glucose levels are slightly higher in the evening and lower in the morning.³⁷⁸ These physiological data help to guide glycemic targets in children with T1D.

CGM devices currently include blinded/retrospective CGM, real-time CGM, and intermittently scanned CGM (isCGM). The most common use of retrospective CGM is by HCPs over a short period of time for obtaining information on glucose patterns to assist with diagnosis (e.g., cystic fibrosis related diabetes), with therapy adjustments, and with diabetes education.

Real-time CGMs display (near) current interstitial glucose concentration, provide real-time alarms for predetermined thresholds and predictions of low and high glucose

concentration, as well as rate of change alarms for rapid glycemic variations. Several CGM sensors can transmit signals to the “cloud,” and enable caregivers and/or HCPs to remote-monitor CGM tracings with alerts on their own devices, including smartphones, tablets, or smart watches.³⁷⁹

Current isCGM systems do not constantly display real-time interstitial glucose concentrations, trend arrows, graph, or alarms; glucose levels appear only when the user holds a reader, or a cell phone, close to the sensor. All continuous glucose readings are stored and provided on demand. Most CGMs for blinded and real-time use still require calibration using finger-stick blood glucose; however, novel CGMs and isCGMs are factory calibrated and can completely eliminate the need for calibration via SMBG.³⁸⁰ Sensors are inserted transcutaneously by persons with diabetes or their parents/caregivers, and have a lifetime from 6 to 14 days. A new type of long-term implantable subcutaneous real-time sensors is approved for 3 or 6 months use as an alternative for transcutaneous CGM.³⁸¹

CGM use is constantly increasing in persons with diabetes, including the pediatric population. CGM was used by 4.8% of all young patients and in 2.3% of all pediatric patients in the German-Austrian registry Diabetes Patient Documentation (DPV) in 2012.³⁸² The Type 1 Diabetes Exchange (T1DEx) registry in the United States reported CGM use in 6% of children younger than 13 years, 4% of adolescents, and 6% of young adults.³⁸³ Both registries reported exponential growth of CGM use in 2017, with 18.4% (DPV) and 21.7% (T1DEx) use in the pediatric population.³⁸⁴ Higher use in younger children may reflect parental involvement, parental fear of hypoglycemia, reduced burden of SMBG, better reimbursement schemes for this age group, and improving technology.

Clinical Evidence for Continuous Glucose Monitor

The evidence for CGM efficacy in the pediatric population evolved gradually.^{385–387} The Juvenile Diabetes Research Foundation (JDRF) trial from 2008 provided evidence for the adult population; however, no overall benefit of CGM use was demonstrated in the adolescent and school-age groups, likely because of poor sensor use adherence.³⁸⁸ However, a post hoc analysis of these data limited to those with sensor use of more than 6 days/week also demonstrated significant benefit in the school and adolescent age groups.³⁸⁹ Studies conducted after 2010 demonstrated a more consistent improvement in HbA1c levels and reduced glucose variability in adults and in the pediatric population, particularly with near-continuous CGM use of 6 to 7 days/week.^{390–395} Evidence for benefit among younger and particularly preschool children was initially ambiguous: feasibility was demonstrated in children younger than age 10 years^{396,397}; however, an improvement in glycemic control was not demonstrated in a randomized controlled trial (RCT).³⁹⁸ Particularly in toddlers younger than age 4 years, a reduction in HbA1c after 6 months of use remained elusive, despite a high degree of parental satisfaction and a more sustained use of the devices.³⁹⁹ More recent studies provided additional evidence^{400,401} with clear benefit also in the preschool population when the analysis was strictly focused on periods when CGM was used.⁴⁰² Support for the use of CGM devices in kindergartens and schools is of paramount importance,⁴⁰³ as recently emphasized in international guidelines and position statements.^{404,405}

Fewer studies have focused on the impact of CGM on hypoglycemia. The evidence is rather strong in the adult population, both from retrospective analyses⁴⁰⁶ and RCTs.^{407,408} Initially, a reduction in mild hypoglycemia,⁴⁰⁹ and time spent in hypoglycemia with MDI³⁹⁴ and insulin pump therapy³⁹⁵ was demonstrated, along with a reduction in glucose variability.^{410,411} One RCT in children demonstrated a reduction of severe hypoglycemia⁴¹²; however, most former trials were

either not sufficiently powered for detecting a difference in severe hypoglycemia or focused only on reducing HbA1c.⁴¹³

From the beginning of real-time CGM use, individuals treated with MDI were successfully included.^{394,414–416} However, only recently has stand-alone use of CGM,^{417,418} and particularly isCGM,⁴¹⁹ been used nonadjunctively to SMBG,⁴²⁰ an approach gaining intense attention. Increasing evidence for clinical benefit of isCGM in the pediatric population with T1D,^{421,422} as well as during exercise and sport⁴²³ and used nonadjunctively,³⁸⁰ has brought additional confidence for the successful use of all CGM modalities in this most challenging childhood population.

A growing number of sensors can now reach the proposed mark of mean absolute relative difference of less than 10% (MARD <10%), sufficient to permit self-adjustment of insulin dosage without confirmatory SMBG.⁴²⁴ Real-life data from the T1DEx registry provided evidence of the safety and effectiveness of nonadjunctive sensor use as only 26% of participants verified the sensor glucose by performing SMBG.⁴²⁵ The nonadjunctive use of CGM seems to be most reliable when the person is not hypoglycemic and glucose is not changing rapidly. Serious concerns were raised about the nonadjunctive CGM use⁴²⁶; however, there is clear evidence that similar concerns also apply for many blood glucose meters that the US Food and Drug Administration (FDA) approved for SMBG.⁴²⁷ Practical guidelines for nonadjunctive CGM use address several current concerns.^{428,429}

A long-term implantable sensor was recently approved for up to 6 months of wear in the European Union, and for up to 3 months' wear in the United States. An initial RCT demonstrated efficacy and safety in an adult population with T1D,⁴³⁰ with a subsequent RCT reporting a MARD under 10% in all glucose ranges,⁴³¹ indicating the potential of its nonadjunctive use. The obvious advantages of a long-term implantable sensor may currently still be offset by the implantation and removal procedure, albeit performed in an outpatient diabetology setting. No pediatric data are currently available.

Finally, the reimbursement for CGM largely influences its use.⁴³² Recently, CGM and particularly isCGM received broad approval for reimbursement from private and large public insurance systems. Only with such a broad support for the use of CGM can current guidelines on CGM use,⁴³³ particularly related to time in range (TIR), be implemented. CGM metrics with accepted clinical targets for TIR, time below range (TBR), and time above range (TAR) may become the most common parameters for glucose management.

Practical Deliberations for Continuous Glucose Monitor

Thorough initial education along with setting of realistic expectations is mandatory for a successful CGM use.^{434–436} Direct education and educational materials should also be provided to teachers at kindergartens and schools,⁴³⁷ with written individualized healthcare plans agreed upon between parents, school nurses, professional caregivers, teachers, and the child, when suitable.^{403–405} Remote-monitoring can add to success of diabetes management in kindergartens and schools.⁴³⁸

Alarms and alerts may induce fatigue and an increased psychological burden,⁴³⁹ resulting in higher attrition from CGM. Sensible individualized alarm setting is paramount; these, and other aspects of CGM are extensively discussed in the publication titled: *Role of Continuous Glucose Monitoring in Diabetes Treatment*.⁴⁴⁰

Inadequate sensor adhesiveness and skin irritation, particularly in young children where body surface is limited, might be underreported, and represent important barriers to long-term CGM use.⁴⁴¹ Supplementary adhesive products (e.g., liquid adhesives, adhesive wipes, transparent dressings, special tapes)

and external wraps are recommended to help secure the sensor to the skin.⁴⁴² Skin issues might become more prevalent with longtime use of sensors. Regular site rotation to prevent skin irritation and trauma because of frequent application and removal of sensor adhesives is recommended. Adhesive removers also help to prevent skin trauma.

Sensor-Augmented Pump Therapy

Sensor-augmented pump (SAP) therapy comprises a CGM linked to an insulin pump through an algorithm. After the initial RCT in the adult population where SAP did not show an advantage over insulin pump therapy alone, and even increased hypoglycemia, likely because of inadequate sensor wear and use,⁴⁴³ SAP was demonstrated superior to MDI with SMBG in a 1-year RCT, including a pediatric population 7 to 18 years of age, resulting in a significant decrease in HbA1c and neutral effect on hypoglycemia.⁴⁴⁴ School-children used sensors more frequently compared with adolescents; more sensor use was associated with lower HbA1c, decreased hyperglycemic exposure, and decreased glycemic variability independently from HbA1c.^{391,410,444} Similarly, in the 6-month continuation phase, participants in the initial SAP intervention arm retained the improvement in HbA1c at 18 months, with those who crossed over to SAP from the initial MDI group also achieving significantly decreased HbA1c.^{392,444}

The second level of SAP therapy came with an automated low sensor glucose threshold insulin suspend (LGS) modality,⁴⁴⁵ which demonstrated less time spent in hypoglycemia compared with SAP without LGS.⁴⁴⁶ An RCT conducted in the home setting demonstrated that the use of LGS modality reduced the area under the curve (AUC) for a glucose of under 3.9 mmol/L (<70 mg/dL) for nocturnal hypoglycemia by more than one-third, with significantly lower percentage of sensor readings in hypoglycemic ranges, without any elevation of HbA1c.⁴⁴⁷ In another RCT focused on young individuals with proven impaired hypoglycemia awareness, a reduction in combined severe (seizures/coma) and moderate hypoglycemia was associated with the use of LGS compared with pump with SMGB.⁴⁴⁸ Additional evidence from pediatric observational trials corroborates the results from RCTs,⁴⁴⁹ along with an analysis of uploaded data from 935 SAP users providing 49,867 user-days with LGS used for 82%.⁴⁵⁰ A moderate delayed rise in glucose 2 hours after resumption of insulin was described in all studies; however, no significant difference in BOHB was observed, even after LGS was activated during the night at or above normoglycemia.⁴⁵¹

The third level of SAP therapy came with a predictive low sensor glucose insulin suspend (PLGS) modality, further decreasing the risk of hypoglycemia with suspending insulin delivery before predicted hypoglycemia. Home use of an investigational PLGS in adolescents and adults reduced hypoglycemia exposure by 81% and time spent less than 60 mg/dL (<3.3 mmol/L) by 70%,⁴⁵² with comparable results in a pediatric cohort⁴⁵³ and in cohorts with different risk factors for hypoglycemia,⁴⁵⁴ without a significant elevation in BOHB levels.⁴⁵³

Currently, the commercially available PLGS modality interrupts insulin delivery when the sensor glucose is predicted to reach 20 mg/dL (1.1 mmol/L) above the preset low glucose limit within 30 minutes, and automatically resumes basal insulin delivery at normoglycemia. In an initial in-clinic assessment hypoglycemia was avoided in 60% of the 69 experiments.⁴⁵⁵ An RCT with 100 children and adolescents demonstrated a decrease in the number of hypoglycemic events by 40% when the PLGS was used at home, with a modest concomitant rise in the time spent in the mild hyperglycemic range.⁴⁵⁶ A 6-month multicenter RCT demonstrated reduced time spent with sensor glucose less than 63 mg/dL (<3.5 mmol/L), without a

difference in HbA1c level at the end of the study.⁴⁵⁷ Data from real-life assessment support results from RCTs, emphasizing the importance of proper education and training.⁴⁵⁸

More recently, an RCT tested the second commercially available PLGS system in 102 adults, adolescents, and children, and demonstrated a 31% relative overall reduction in sensor time less than 70 mg/dL (<3.9 mmol/L), without a difference in mean glucose concentration, but with a significant increase in AUC greater than 180 mg/dL and time over 250 mg/dL.⁴⁵⁹ However, BOHB levels were assessed on 1954 mornings, half of which occurred after a PLGS activation overnight, and the frequency of BOHB over 0.6 mmol/L was not different.³³⁰ Consensus recommendations on the use of SAP with PLGS do not recommend additional blood BOHB testing.^{460,461}

Practical Deliberations for Sensor-Augmented Pump

Success with SAP, as with CGM, anchors on understanding of the system functionality and realistic expectations. Suggested topics at SAP initialization may include required frequency of sensor use, and possible alarm fatigue.⁴⁶² Alarms for PLGS may be set to off, particularly during the night, possibly with the low threshold alarm on. Should a hypoglycemic event occur despite the insulin suspension with PLGS, rescue carbohydrate intake should be judicious to prevent rebound hyperglycemia. Regular analysis of downloads assists decisions related to all SAP settings, and particularly to those related to PLGS settings.⁴⁶²

AUTOMATED INSULIN DELIVERY—CLOSED-LOOP (ARTIFICIAL PANCREAS)

Devices consisting of pumps, sensors, and algorithms have been recently developed as commercially available products for patients with T1D. These devices are still undergoing refinements with the aim of producing a complete closed-loop system for delivering insulin, a so-called *artificial pancreas*. Automated insulin delivery systems are based usually on CGM, an insulin pump and a computed algorithm that controls the amount and the pace of the insulin delivered to the patients.

The first step toward automated control of insulin delivery was the low-glucose-suspend feature. This feature is an add-on algorithm for patients who are using a sensor-augmented pump approach that allows the basal rate of the patient's pump to be suspended for up to 2 hours if the patient fails to respond to the sensor's low-glucose alarm (see Continuous Glucose Monitoring section earlier). These systems have been tested in adults and children with T1D and have been found to significantly decrease the extent and duration of nocturnal hypoglycemia in patients with T1DM.

The "low glucose suspend" feature was followed by the development of the commercial PLGS, which has the ability to suspend the pump's basal level even before the patient's glucose levels reach the predetermined hypoglycemic range. In this PLGS system, the basal insulin delivery is suspended when the algorithm predicts, based on the sensor glucose trends showing that the patient's blood glucose levels will soon reach the hypoglycemic zone, even though the blood glucose level at that time is still within the normal range (see Continuous Glucose Monitoring section earlier).

Several groups of investigators, as well as pump and sensor manufacturers, are currently developing closed-loop systems also known as *artificial pancreas*. These systems use controller algorithms that automatically regulate insulin infusion rates delivered by the pump, based on the sensor glucose readings every few (generally every 5) minutes yielding 288 readings per 24 hours. The main three basic algorithmic approaches in practice today are the proportional integrative derivative

(PID)⁴⁶³ model predictive control (MPC),⁴⁶⁴ and fuzzy logic.^{465,466} Initially, proof of concept studies with a small number of patients in clinical research centers were conducted^{467,468} and demonstrated their safety and effectiveness. In 2011 the first multicenter, multinational, prospective, randomized crossover-controlled study, involving 54 children and adolescents, using automated insulin delivery in a resort area outside of a hospital's protected environment, was successfully conducted and reported.⁴⁶⁹ That study was followed by a series of studies using closed-loop systems in diabetes camps,^{470–473} as well as out patient environments,^{474–477} and were proven to be safe and effective and within the capabilities of the subjects. These results eventually led to the first European regulatory approval—the CE-Mark—for the algorithm of the automated insulin delivery in 2015. The automated insulin delivery “system” composed of pump, sensor, and meter (Minimed Medtronic 670G), was approved for those aged 14 years and older in the FDA in 2016. By 2018 the system received FDA clearance for age 7 years and older, as well as approval by the European CE-Mark. This automated insulin infusion system is user friendly, and capable of changing the insulin infusion rate according to the blood glucose levels. However, attempts to develop a fully closed-loop system failed to show desired results, mainly because of significant postprandial glycaemic excursions compared with results with premeal boluses.⁴⁷⁸ Therefore the automated insulin delivery systems available today are actually each a “hybrid” system, where basal insulin delivery is controlled by the algorithm, but the patient needs to manually announce meals together with assessment of the carbohydrate content and individual insulin sensitivity to deliver a premeal insulin bolus. Advanced hybrid closed-loop algorithms, currently in clinical studies, are able to inject boluses to correct high glucose levels in between meals or inject a postmeal bolus if patients forget to inject a premeal bolus. Several thousand patients with T1D are already successfully using a commercially available “hybrid” system (670G Medtronic) in the United States, and it is anticipated to become available commercially also in Europe and other parts of the world during 2019. The automated insulin delivery systems available commercially today are based on delivery of insulin only, and therefore are able to increase basal insulin delivery, as well as to inject boluses, if required, and to decrease or even suspend insulin delivery, when needed. However, there are also continuous efforts by several groups in the world to develop a dual-hormone system that will automatically inject insulin and glucagon with the aim to achieve as near as normal metabolic control^{479–483} without hypoglycemia, and better than a system based on insulin only achieves. Yet, there are still several efficacy and safety concerns that have been raised regarding continuous or repeated glucagon injections, so that clinical studies to overcome these concerns of dual-hormone closed-loop systems are ongoing.

Studies of out patients in “free living” conditions demonstrated improved TIR of sensor glucose levels between 70 and 180 mg/dL (3.9–10 mmol/L) associated with reduction in time spent in the hypoglycemia range via use of automated insulin delivery compared with the use of pump alone or with SAP therapy.^{484–502} Automated insulin delivery systems were also shown to reduce nocturnal hypoglycemia following physical activity in the afternoon,⁵⁰³ as well as increased treatment satisfaction.^{504–508}

With the increasing number of patients using closed-loop systems and their growing accessibility, it is important to prepare both patients and HCPs for the new and still evolving technology and to adjust expectations with reality. The ability of the current systems to mitigate hypoglycemia is not absolutely perfect; patients are still required to be alert and respond to the systems' hypoglycemia alarms by ingestion of carbohydrate. Patients should be prepared to face and respond appropriately

to some technical difficulties and alarms that might occur, to be taught when to stop automated insulin delivery and revert to their previous pump settings, to be alert to situations when the system might automatically revert the patients from auto mode to manual pump mode, and what to do when and if sensor glucose levels remain elevated over time.

Future closed-loop systems might include incorporation of additional data derived from other sensors, such as heart rate monitoring, degrees of physical activity, body temperature, sleep pattern, mood, geographic location, and automated carbohydrate counting to be integrated with new artificial intelligence (AI) and algorithms that will replace the hybrid closed-loop systems with a fully automated one, an “artificial endocrine pancreas.”

THE EMERGING DIGITAL WORLD OF DIABETES

Diabetes is a chronic disease that affects all aspects of the patient's daily routine. Patients with diabetes are required to make many decisions each day regarding their activity, diet, and insulin regimen. Patients' interactions with HCPs usually occur once every few months in most diabetes centers in the world. In between these clinical visits, patients are required to manage their diabetes on their own, or with the help of parents, other relatives, or other caregivers in their surroundings.⁵⁰⁹ Moreover, the worldwide increase in the number of patients with diabetes, both type 2 and type 1,⁵¹⁰ the inadequate number of HCPs who specialize in diabetes,^{511,512} and the limited time available for each patient's visit⁵¹³ create the need for new ways to cope with the care of diabetes. Over recent years, digital technology has made huge progress, and the number of people who have access to digital technology via Internet and smartphones has increased dramatically.⁵¹⁴ Therefore attempts emerged to use digital solutions to help patients cope with their diabetes in-between clinical visits, and to help the HCPs with the increasing amount of data during the clinical visit. With the increased use of electronic health records, CSII, CGM, isCGM, and recently the information derived from connected pens (insulin pens that transmit to the cloud information on the dose and the timing of insulin administration), the HCPs are dealing with “big data” derived from different sources, which requires integration, analysis, and eventually a personalized tailored recommendation to each of their patients. Tools based on AI have been developed to help physicians during and in-between patients' visits. Automated diagnosis of diabetic retinopathy (DR) based on fundus photography using AI and deep learning algorithms^{515,516} have been developed and clinically tested. Recently, a study was conducted aiming to evaluate physicians' adjustments of insulin pump settings based on CSII downloads, CGM, and SMBG glucose data gathered over 3 weeks of patients with T1D.⁵¹⁷ In that study, 26 physicians from 16 centers in Europe, Israel, and South America were asked to adjust insulin dosing of 15 patients with T1D. The study showed that the rate of full agreement between physicians on: (1) the trend in insulin adjustments of the basal insulin infusion rate, (2) the insulin: carbohydrate ratio (CR), and (3) the correction factor (CF) were $41 \pm 9\%$, $45 \pm 11\%$, and $45.5\% \pm 13\%$; for complete disagreement they were $12 \pm 7\%$, $9.5 \pm 7\%$, and $10 \pm 8\%$, respectively. Significantly similar results were found between the physicians and an automated decision support algorithm developed for HCPs.⁵¹⁸ Other AI algorithms are being tested with the aim to provide personalized prevention and intervention programs to help HCPs to choose the optimal personalized treatment using deep and machine learning algorithms based on large datasets.^{519–521} The use of AI allows real-time processing of big data coming in from different sources, such as health record datasets, personal genetic information, geographic areas, downloads from devices, and sensors, and turn this information into an

actionable personalized plan for use by the HCPs or by the patients themselves.^{522,523} It appears that patients will soon be able to use such AI tools on a daily basis to adjust their therapeutic regimens to achieve better glucose control without the need to wait for their scheduled meetings with their HCPs.

The Open-Source Movement

With the notion of many patients with T1D that the technology is advancing faster than the regulatory bodies in the world can evaluate and approve, an initiative has emerged from several individuals and groups to form an open-source consortium.⁵²⁴ Tools that use open-source need not be FDA approved and are not for sale; these tools are free to be used by anyone as an open-source system that permits people with diabetes to build their own artificial pancreas for themselves, their spouses, or their children. Some of the open-source algorithms use industry proprietary products, such as pumps and CGMs, with the aim to accelerate the pace by which these tools become available for patients' use to improve their own diabetes management. Another interesting initiative is the nonprofit open source Tidepool project.⁵²⁵ This organization developed a hub for diabetes data that collects data from pumps, CGMs, blood glucose monitors, and other mobile devices and integrates them and allows patients with diabetes to visualize and use it. The Tidepool platform encourages patients to donate their data with the aim to create a huge data source to be available to patients and researchers for developing better algorithms for patients' use.

Technology Challenges

Hundreds of Internet smartphone apps and algorithms for data capturing, as well as glucose management, social support, diet advice, teaching tools, and other different advisors, have been developed and are available for use.^{526,527} However, most tools available today are not FDA approved and their safety and effectiveness were not tested in well-designed prospective RCTs. Real-life studies that test their benefits compared with standard of care are scarce. Privacy issues related to the question of who owns the data derived from the different sensors and devices and the potential of hacking, which might compromise the privacy of the patients and safety of the device' users, is continuously discussed.⁵²⁸ The influx of data derived from different devices and sensors like CSII and CGM raised the need to define new digital biomarkers beyond the traditional HbA1c values, such as time spent in range, hypoglycemia, and hyperglycemia and glucose variability.^{529,530} With more and more sensors and connected devices and the use of AI, which will provide new tools for both patients and HCPs, the interaction and relationships between patients and HCPs during clinic visit and in-between visits will ultimately change. These changes might also change the economic basis that is currently used to manage diabetes in different places in the world.

Digital tools have the potential to empower patients and to provide them with the tools that will be available for their use 24/7; this is so necessary to cope with a chronic disease like T1D from which there is not a single day of respite.

This succinct overview of the computerized digital revolution related to diabetes, particularly in children, is meant to alert the reader to this fast-evolving field with its promise and perils. Promise, because the application of these technologies is at the core of advances in the management of diabetes. This is demonstrated by the development of closed-loop systems for insulin delivery coupled with glucose sensing, the so-called *artificial pancreas*, as well as AI systems to mine, interpret, and apply the huge data emanating from these technologies. Perils, because much of the technology is still under development, lacking stringent safety features, which are so

crucial for the self-treatment of the patients and their families. Above all, our comments as presented here intend to educate the reader, but they should not be viewed as an endorsement or critique of any specific system or product.

LIVING WITH DIABETES

Medical Nutrition Therapy

Proper nutritional management is critical to the short- and long-term health of children with diabetes. In general, terms, such as diet, should be avoided in favor of meal plan or healthy food choices—both for the negative connotation associated with the former and for the simple fact that nutritional requirements for normal growth and development are the same in diabetic and nondiabetic children. Moreover, reasonably accurate estimations of the carbohydrate content of meals are important to optimal glycemic control with current basal-bolus treatment regimens. Indeed, the popularity of basal-bolus therapies and the use of carbohydrate counting to adjust the dose of rapid-acting insulin taken with each meal have fundamentally changed the treatment paradigm.

The traditional approach of adjusting the patient's lifestyle around fixed insulin doses and fixed amounts of carbohydrate intake with each meal has been replaced by a more flexible approach that attempts to adjust the insulin regimen to the patient's lifestyle. The day-to-day variations in appetite in children and adolescents make the latter approach more likely to be successful. It is important to note that in some patients and some families, the traditional approach of trying to consume consistent carbohydrate servings per meal/snack may be more successful because it fits their personalities and lifestyle better.

The currently favored model of nutritional therapy is carbohydrate counting, based on the conceptual model of matching carbohydrate "doses" to insulin doses.^{531,532} Because the total carbohydrate content of foods (rather than the type of carbohydrate) has the greatest impact on blood glucose, the amount of carbohydrates ingested per meal or snack needs to be estimated as accurately as possible. Although the protein and fat contents of meals do affect the pattern of postmeal glucose excursions,^{533,534} they are not usually counted to simplify the procedure. Accounting for fat and protein is often included in more advanced education and for use of insulin pump bolus adjustments (dual or square wave boluses).³²⁷ Food labeling requirements have simplified the process because most foods are clearly labeled with the number of carbohydrate grams per serving and the serving size. Foods less easily quantified may be weighed or estimated, and eating out may be a particular problem. Although the prohibition of "sweets" has been done away with, we still recommend that patients avoid sugar-containing beverages.

In the flexible approach to nutrition counseling, there is no set intake. Rather, the child and parents decide the meal content. The carbohydrates are counted and an insulin dose is calculated based on a ratio of the number of insulin units per grams of carbohydrate determined by the empirical trial-and-error method. Actual insulin-carbohydrate ratios vary from child to child and in the same child according to the time of day. Breakfast often requires relatively more insulin than lunch or dinner. Carbohydrate counting can also be used in the traditional approach to dietary treatment to provide consistent carbohydrate servings per meal/snack. Indeed, a simple approach that stresses consistency in the timing and size of meals may be effective at the time of diagnosis of diabetes—when parents and patients are too overwhelmed to be able to learn more advanced nutritional concepts.⁵³⁵

The ADA's recommendations regarding general nutritional principles in diabetes also take into consideration the long-term goal of preventing the macrovascular and microvascular

complications of diabetes.⁵³⁵ Consequently, heart-healthy diets low in cholesterol and saturated fats are encouraged. Long-term goals of nutritional management of diabetes include maintenance of nutrient intake balance of about 50% carbohydrate, 20% protein, and 30% fat (of which no more than 10% should be saturated). Most important, growth and weight gain should be monitored—and regular follow-up with a dietitian trained in diabetes management should be encouraged to individualize a meal plan for each child based on his or her needs and food preferences. We face an epidemic of childhood obesity in developed countries, and one of the adverse consequences of intensive insulin treatment is that 30% to 35% of pediatric patients with T1D in the United States are overweight or obese.⁵³⁶ Thus any tendency for body mass index (BMI) Z scores to increase excessively needs to be dealt with promptly.⁵³⁷

Exercise

Establishment and maintenance of an active lifestyle should be a goal for all children, but especially children with diabetes, to enhance cardiovascular health. Exercise and increased physical fitness are associated with improved insulin sensitivity and glucose utilization, the clinical correlates of which are lower insulin requirements, lower blood pressures, and better lipid profiles. Improvements in physical fitness are also frequently associated with greater self-esteem and increased motivation to participate in diabetes care. Recent guidelines and consensus statements have reviewed this topic in detail.^{538,539}

Despite its benefits, acute bouts of exercise in children with T1DM actually make regulation of blood glucose levels more difficult. Hypoglycemia is a common complication during exercise,⁵⁴⁰ and excessive snacking to prevent hypoglycemia can result in hyperglycemia and negate some of the metabolic and cardiovascular benefits of exercise. These difficulties are compounded by the irregular pattern of physical activity that characterizes most youth who are not participating in organized sports or regimented training programs and by conventional methods of diabetes management that feature fixed basal insulin replacement doses. The Diabetes Research in Children Network demonstrated that the risk of nocturnal hypoglycemia nearly doubled in youth with T1D following antecedent afternoon exercise versus sedentary days.⁵⁴¹

The effects of exercise must be carefully considered in the context of the entire diabetes care plan. Children participating in school sports or other programs should be counseled to monitor blood glucose before, during, and after exercise to optimize glycemic management. In addition, patients and parents should be aware that late hypoglycemic effects of afternoon exercise are often observed 7 to 11 hours after the bout of exercise in the afternoon,⁵⁴² a phenomenon that appears to be caused by an increase in nonoxidative glucose disposal during sleep, which may serve to support repletion of muscle glycogen stores.⁵⁴²

In patients on pump therapy, simply suspending the basal infusion rate can markedly reduce the risk of hypoglycemia during exercise⁵⁴⁰—and similar benefits may accrue from reducing the overnight basal rates after very active days. Furthermore, real-time CGM can be extremely valuable to provide alerts of impending hypoglycemia. Integrated systems with CGM and pumps that include predicted low glucose suspend reduce hypoglycemia in pediatric studies.^{457,543}

Studies that have examined methods of managing glycemia during exercise illustrate that there is an almost infinite number of combinations of conditions that need to be considered. Because of this complexity, trial and error remains the principal method of managing glucose levels during and after exercise in children and adolescents with T1DM,⁵⁴⁴ although guiding principles exist in numerous guidelines and reviews.^{538,539}

Residual β -Cell Function (the Honeymoon Period)

After the initiation of insulin treatment in newly diagnosed patients, secretory function of residual beta cells improves and the insulin resistance and glucotoxicity of decompensated diabetes are reversed—heralding the onset of the partial remission (honeymoon period) of T1DM.⁵⁴⁵ Most patients require a progressive reduction in their daily dose to avoid excess hypoglycemia. A minority of children (<5% of patients) can even maintain normoglycemia for a time without any administered insulin. Although opinion varies, we do not discontinue insulin treatment unless a daily dose of 0.1 U/kg still causes hypoglycemia.

The duration of the honeymoon phase in children with T1DM is variable, but the lowest HbA1c levels and TDD are usually observed between 3 and 6 months postdiagnosis and both often rise between 9 and 15 months.⁵⁴⁶ With current MDI and CSII treatment methods, the large majority of youth with T1D retain substantial residual insulin secretion, as measured by C-peptide responses to mixed meal tolerance tests, during the first year of their disease, and this can be extended for several years thereafter with maintenance of optimal glycemic control.⁵⁴⁷ In comparison to patients who are C-peptide negative, patients with residual beta-cell function have lower HbA1c levels and total daily insulin doses—as well as a reduced risk of hypoglycemia.⁵⁴⁸ These clinical and metabolic benefits serve as a strong rationale for current research involving immune interventions directed at preserving beta-cell function in newly diagnosed patients.⁵⁴⁹ On the other hand, earlier and more aggressive application of sensor-augmented pump therapy around the time of diagnosis does not appear to confer any advantages over standard MDI and CSII with respect to HbA1c levels or C-peptide responsiveness in youth with T1DM.⁵⁴⁶

Hypoglycemia

Historically, the dark side of intensive treatment regimens that are effective in lowering HbA1c levels is that they also increase the risk of hypoglycemia. In the DCCT, intensive therapy was associated with an approximate threefold increased risk for severe hypoglycemia versus conventional treatment and, irrespective of treatment group, the rate of severe hypoglycemia was about 50% higher for adolescents than for adults.⁵⁴⁸ Hypoglycemia has become the most significant barrier to the pursuit and maintenance of tight glycemic control among people with T1DM, and effectively managing the risk for hypoglycemia is especially important in the treatment of children and adolescents with this disease. However, more recent data from multiple registries worldwide indicate a flattening of the DCCT report, which had demonstrated a curvilinear inverse relationship of A1c with severe hypoglycemia.⁵⁵⁰ Contemporary data indicate that A1c goals can be met without an increase in severe hypoglycemia.⁵⁵¹

The ADA and the ISPAD harmonized their definitions with that of the International Hypoglycemia Study Group^{552–554} as: (1) clinical hypoglycemia alert (a glucose value ≤ 70 mg/dL [3.9 mmol/L]) that requires attention to prevent hypoglycemia; (2) clinically important or serious hypoglycemia (a glucose value < 54 mg/dL [3.0 mmol/L]) indicates serious, clinically important hypoglycemia; and (3) severe hypoglycemia defined as an event associated with severe cognitive impairment (including coma and convulsions) requiring external assistance by another person. In nondiabetic adults, less than 70 mg/dL is the plasma glucose level at which counterregulatory hormone responses engage and at which awareness of symptoms normally occurs. It should be noted, however, that such responses may be triggered at higher glucose levels in healthy 8 to 16 year

olds and in children and adolescents with T1DM who have poor glycemic control.⁵⁵⁵

The two main mechanisms that cause symptoms and signs of hypoglycemia are an outpouring of catecholamines (which results in pallor, sweating, apprehension, trembling, and tachycardia) and the effects of cerebral glucopenia, which include hunger, drowsiness, mental confusion, seizures, and coma. Mood and personality changes may be more subtle cerebral glucopenic effects that provide an early clue that plasma glucose has fallen to a dangerous level. Symptomatic episodes in which patients are able to treat themselves without the assistance of others are considered minor or mild hypoglycemia, whereas episodes in which there is sufficient cognitive impairment that treatment requires the assistance of another person are considered major or severe hypoglycemic events. Hypoglycemic events that cause seizures or coma are usually classified as a subset of severe hypoglycemia. In severe hypoglycemia, ingestion of carbohydrates may be precluded because of loss of consciousness, seizures, or coma—and treatment may require administration of a glucagon injection or IV glucose infusion.

In nondiabetic children, the initial response to falling plasma glucose levels is a prompt suppression of insulin secretion. If plasma glucose continues to fall and threshold values for release of antiinsulin counterregulatory hormones are reached, there are abrupt increases in circulating concentrations of glucagon and epinephrine. Plasma growth hormone and cortisol levels also increase, but these hormones are less important in acutely counteracting the effects of insulin. Defective counterregulation occurs in patients with T1DM because exogenously supplied insulin levels do not decrease in response to low blood glucose levels and the ability to secrete glucagon in response to hypoglycemia is lost early in the course of the disease.^{545,556} Consequently, patients with T1DM depend on sympathetic nervous system responses—especially increases in plasma epinephrine levels—to prevent hypoglycemia.

The episodes of mild hypoglycemia that frequently accompany intensive treatment blunt catecholamine responses and symptom awareness to subsequent hypoglycemic challenges. This phenomenon has been called *hypoglycemia-associated autonomic failure (HAAF)*.^{557,558} Studies have shown that such defects in counterregulatory hormone responses are common in both young children and adolescents with T1DM who are well controlled.⁵⁵⁹ Catecholamine responses to hypoglycemia are also impaired during sleep, which is an important reason why most of the severe hypoglycemia events occur during the night.⁵⁶⁰ As noted earlier, bouts of exercise in the afternoon sharply increase the risk of hypoglycemia on the following night.⁵⁴⁴

The use of basal-bolus therapy with insulin analogs and insulin pumps has reduced the frequency of severe hypoglycemia but by no means has eliminated this problem. HbA1c levels can be reduced in patients who use CGM with or without integration in a sensor-augmented pump system without increasing the risk of hypoglycemia, but reducing severe hypoglycemia remains a clinical priority. In the T1D Exchange Clinic Registry that includes more than 10,000 children with T1D, 1 in 20 patients reported having at least one seizure or coma event related to hypoglycemia in the prior 12 months.⁵³⁶ It is also noteworthy that the risk of severe hypoglycemia in patients with HbA1c levels over 9.5% in this cohort was similar to that in patients with HbA1c levels under 8%. These data indicate that poor control, per se, is not an effective strategy for reducing the risk of hypoglycemia.⁵³⁶

It is important that the patient and family recognize early symptoms and signs of hypoglycemia and known precipitating factors. Mild episodes should be immediately treated with 10–15g (or ~0.3 g/kg) of carbohydrate (e.g., glucose tablets,

juice, or glucose gel). Parents and school nurses also need to be instructed on how to perform injections of glucagon (0.5–1.0 mg) when the patient has lost consciousness and is unable to swallow exogenous carbohydrate. If exercise has been the precipitating factor, the patient should be instructed about preventive measures. Development of orally active glucagon preparations is ongoing and shows promise as an efficient, effective, and self-administered method for avoiding hypoglycemia without the need for cumbersome preparation of the current injectable forms.

Sick Day Management

Children with intercurrent illnesses, such as infections or vomiting, should be closely monitored for elevations in blood glucose levels and ketonuria. On sick days, blood glucose levels should be checked every 2 hours—and the urine should be checked for ketones with every void. Supplemental doses of rapid-acting insulin (0.1–0.3 U/kg) should be given every 2 to 4 hours for elevations in glucose or ketones. Even in the absence of marked hyperglycemia, the presence of ketones indicates insulin deficiency and therefore the need for supplemental insulin.

Adequate fluid intake is essential to prevent dehydration and hasten the excretion of ketones. Fluids, such as flat soda, clear soups, popsicles, and gelatin water are recommended to provide some electrolyte and carbohydrate replacement. In the child tolerating oral rehydration, a fluid “dose” of 1 ounce (30 mL) per year of age per hour serves as a rough guideline, the sugar content of which depends on the serum glucose. For blood glucose values greater than 200 mg/dL, sugar-free fluids should be given. For those levels between 140 and 200 mg/dL, a mixture of sugar-free and sugar-containing fluids should be given. For blood sugar under 140 mg/dL, only sugar-containing fluids should be given.

If emesis precludes normal oral intake, the intermediate or long-acting insulin should be discontinued and small frequent doses of short- or rapid-acting insulin should be given. In pump-treated patients, if emesis precludes normal oral intake, basal insulin should be continued, but higher or lower temporary basal rates may also be used, to avoid hypoglycemia or ketosis depending on changes in glucose and ketone levels. Once the ketones have cleared and the child is tolerating an oral diet, the family may resume the normal routine. If vomiting is persistent and ketones remain moderate or large after several supplemental insulin doses, arrangements should be made for potential hydration and evaluation in a hospital emergency department.

Special attention should be paid to persistent hyperglycemia in patients using pump therapy, as absence of long-acting basal insulin can result in development of ketones and DKA if the rapid acting insulin is not delivered. Because only rapid-acting insulin is administered, an interruption of insulin delivery will result in increases in blood and urine ketone levels in 4 to 6 hours.³²⁹ These interruptions are most commonly caused by problems with the infusion set, such as kinking or occlusion of the subcutaneous catheter. Thus assessment of hyperglycemia and any symptoms of nausea and vomiting must include an evaluation of the integrity of the infusion set and infusion site.

Associated Autoimmune Diseases

Patients with T1DM are at increased risk for other autoimmune diseases. These are detailed in Chapter 22, in the discussion of multiple endocrine deficiency syndromes. Chronic lymphocytic thyroiditis is frequently associated with T1DM in children, and as many as one in five may have thyroid antibodies in their

serum. Only a small proportion of these patients develop clinical hypothyroidism, however. The interval between the diagnosis of diabetes and that of thyroid disease averages about 5 years. Physicians should anticipate the possibility of hypothyroidism in patients with T1DM by periodic examination of the thyroid gland and measurement of serum thyroid-stimulating hormone (TSH) concentration.

When diabetes and thyroid disease coexist, the possibility of adrenal insufficiency should be considered. This may be heralded by decreased insulin requirements, increased pigmentation of the skin and buccal mucosa, salt craving, weakness, and postural hypotension. Rarely, frank Addisonian crisis is the first evidence of adrenal failure. This syndrome generally occurs in the second decade of life or later.

Celiac disease affects from 2% to 8% of children with T1DM,⁵⁶¹ and most of them are not aware of any gastrointestinal symptoms. Early diagnosis requires screening of tissue transglutaminase antibodies, which have high degrees of reproducibility, specificity, and sensitivity if total circulating IgA concentrations are not abnormally low. Antibody-positive children should be referred to a pediatric gastroenterologist for confirmation by small bowel biopsy, as well as for counseling and disease management. When typical signs and symptoms of malabsorption are present or the patient has frequent hypoglycemia or unexplained behavioral mood swings, dietary intervention is indicated. When symptoms are minimal or absent, the decision to introduce gluten-free diets with their additional restrictive and inconvenient burdens on the patient and family are less clear-cut.⁵⁶² To guide their decision, the parents and older children need to be made aware of the risk of bowel malignancies in untreated celiac disease in later life and of other long-term complications of this disease.

Associated Psychosocial Problems

Diabetes in a child affects the lifestyle and interpersonal relationships of the entire family. Guidelines for psychosocial management and support of families with childhood diabetes have been published. Feelings of anxiety and guilt are common in parents. Similar feelings, coupled with denial and rejection, are equally common in children—particularly during the rebellious teenage years. These issues are not unique to T1DM but are observed in families with children who have other chronic disorders difficult to treat. These stresses are often exaggerated in single-parent low-income families and impair their ability to effectively carry out needed self-management tasks, resulting in poor metabolic control.⁵⁶³ Language and cultural barriers are additional obstacles in immigrant families.⁵⁶⁴

Psychosocial difficulties and conflict between patients and parents may result in nonadherence to instructions regarding healthy food choices, insulin therapy, and frequency of blood glucose monitoring. In this era of basal-bolus insulin therapy, missing premeal bolus doses is the most common cause of elevated HbA1c levels, especially in adolescents. Consequently, one of the major advantages of CSII over MDI in pediatric patients is that the bolus history function of current insulin pumps provides a record of the number of daily bolus doses that were actually administered.⁵⁶⁵ Deliberate overdosage with insulin resulting in hypoglycemia or omission of insulin to limit weight gain (also referred to as *diabulimia*) may be pleas for psychological help. Occasionally, they may be manifestations of suicidal intent. Frequent admissions to the hospital for ketoacidosis or hypoglycemia should arouse suspicion of underlying emotional and family conflict. Feelings of being different or of being alone are also common.

The clinician managing a child or adolescent with diabetes should be aware of his or her pivotal role as counselor and

advisor and should anticipate the common emotional problems of the patient. When emotional problems are clearly responsible for poor compliance with the medical regimen, referral for psychological help is indicated. In pediatric centers, psychologists form part of the management team for children with diabetes. Although clinical depression is one of the most common comorbidities in youth with T1DM, problems in this area may not be evident during routine clinic visits, especially in preteens who may present as somewhat withdrawn rather than rebellious.⁵⁶⁶ As a result, treatment guidelines suggest that screening questionnaires for depressive symptoms should be administered to youth with T1DM on a regular basis.⁵⁶⁷ However, it has been difficult to implement this recommendation in most clinical practices.

Outpatient Care

The importance of frequent follow-up by the diabetes health-care team cannot be overemphasized. Children and adolescents with T1DM should be routinely cared for at a diabetes center that uses a multidisciplinary team knowledgeable about and experienced in the management of young patients. This team should ideally consist of pediatric diabetologists, diabetes nurse specialists, nutritionists, and social workers or psychologists.

The ADA and the ISPAD have published guidelines for care of the child and adolescent with diabetes.^{1,568} Regular follow-up visits with the physician or diabetes nurse specialist/practitioner every 2 to 3 months are recommended for most patients. The main purpose of these visits is to ensure that the patient is achieving primary treatment goals. Outpatient visits provide an opportunity to review glucose monitoring (and CGM profiles), to adjust the treatment regimen, and to assess child and family adjustment. At each visit, glucose records should be reviewed and appropriate dosage or schedule changes should be instituted. The nutritionist and psychologist or social worker also gives follow-up advice and support.

A detailed interim history should include questions relating to general health, energy, fatigue, polyuria or nocturia, intercurrent illnesses, hypoglycemic episodes, and the presence of symptoms, such as abdominal pain, bloating, or diarrhea. It is important to remember the comorbidities of other autoimmune disorders that occur with an increased frequency in children with T1DM: thyroiditis, adrenal insufficiency, and celiac disease. The child should be weighed and measured at each visit, and blood pressure documented. Physical examination of the child should focus on the general organ systems and on examination of the skin and insulin injection/pump insertion sites for signs of lipohypertrophy or pigmentary changes, palpation of the thyroid, and determination of the stage of sexual development stage.

Lipohypertrophy was commonly observed in the past but is now a rare complication of insulin therapy.⁵⁶⁹ A deceleration in growth, delay in sexual development, or finding of goiter may herald hypothyroidism. The astute clinician should also consider that frequent unexplained hypoglycemia or reduction in insulin requirements in the absence of exercise or activity might be subtle indicators of hypothyroidism or adrenal insufficiency. Similarly, although a history of having frequent foul-smelling greasy stools is a more obvious indicator of celiac disease, the majority of cases are asymptomatic.

Measurement of glycosylated hemoglobin (HbA1c) provides the gold standard by which to judge the adequacy of the insulin regimen and use of point-of-service methods that can be performed in the office in a few minutes offers the opportunity to make immediate changes in the insulin regimen while the patient is being seen. Results of point-of-care measurements compare favorably with laboratory methods.⁵⁷⁰

Even more important, the results of this test delivered during face-to-face encounters with the clinician serve as the quarterly “report cards” for the child and the parents. Teenagers may not be able to identify with the concept of working hard on their diabetes to be healthier many years in the future, but most are able to understand good grades. Thus the HbA1c level provides a tangible outcome with which they can identify. The goal of treatment is to achieve HbA1c levels as close to normal as possible. Based on DCCT results and ADA recommendations,¹ our general goal of therapy is to try to keep all patients under 7.5%, although ISPAD has recently lowered the goal to 7.0%.³⁰⁷ HbA1c levels are determined at least every 3 months.

Routine screening for associated autoimmune diseases with T4, TSH, and tissue transglutaminase IgA every 1 to 2 years is recommended. Monitoring of the diabetic child for potential complications and cardiovascular risk factors is another important function of the clinic visit. The normal urinary albumin to creatinine ratio is less than 30 mg albumin to grams creatinine, microalbuminuria is 30 to 300 mg/g, and macroalbuminuria is greater than 300 mg/g. Elevated spot samples should be confirmed at least a second positive. Preferably this would be either a first morning void or a timed overnight collection to rule out benign orthostatic proteinuria. If the confirmatory test also demonstrates microalbuminuria, treatment with angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy or referral to pediatric nephrologist is indicated. All diabetic children with hypertension, regardless of albumin excretion status, should be considered candidates for these medications, as well. Current recommendations for nondiabetic children are that blood pressures above the 90th percentile for age warrant lifestyle intervention and those about the 95th merit the use of pharmacological agents with further details available in ADA and ISPAD guidelines.^{1,571}

Other screening studies for complications of diabetes include measurement of serum lipid concentrations. The current standards of care for adults with diabetes indicate that low-density lipoprotein (LDL) concentrations should be maintained below 100 mg/dL, whereas pharmacological therapy for dyslipidemia in children with diabetes is not recommended unless dietary interventions fail to lower LDL concentrations below 130 mg/dL. The ADA recommends that annual dilated retinal examinations be obtained in patients who are over 10 years or age and have had T1DM for 3 or more years. On the other hand, the yield from such examinations is very low in children and adolescents who have normal blood pressure, HbA1c levels that meet current targets, and who are without microalbuminuria.⁵⁷² A detailed discussion of complications, their diagnosis, prognosis and treatments in children with diabetes is found below.

Management During Surgery

Management objectives during surgery are the prevention of hypoglycemia, excessive loss of fluids, and ketosis during anesthesia. The regimens described here are generally applicable, but vigilance and individual adjustments for each patient are necessary to achieve these goals. Evidence-based, controlled studies of perioperative care have not been carried out in children, but detailed expert reviews of management have been published in the anesthesia and pediatric diabetes literature⁵⁷³ and recent ISPAD Guidelines.^{574,575}

The most reliable and straightforward approach to achieving management objectives during major elective or emergency surgery is to use IV infusions of glucose and insulin during the perioperative period. For surgical emergencies that can be briefly delayed, such as acute appendicitis, rehydration and metabolic balance should be restored before the operation. Elective major operations should be performed first thing in the morning, and the glucose and insulin infusions should be started 2 hours or

more before proceeding to the operating room. For elective surgeries, an infusion of 5% glucose in 0.45% or 0.9% saline solution is begun on the morning of surgery—and 1 unit of regular insulin is infused intravenously for each 4 to 6 g of administered glucose. One unit of regular insulin for every 2 to 4 g of exogenous glucose may be required in surgical emergencies because of elevated circulating concentrations of stress hormones or in insulin-resistant obese diabetic patients.

The rate at which IV fluids are administered should be sufficient to provide maintenance fluid requirements plus estimated losses during surgery and other fluid deficits. The blood glucose concentration should be monitored at periodic intervals before, during, and after surgery. Concentrations of 120 to 150 mg/dL should be the goal. This can be achieved by varying the rate of infusion of the glucose and electrolyte mixture or the rate of insulin administration. The IV insulin and glucose infusions can be continued until the patient is awake and capable of taking regular meals, at which time their usual injection or insulin pump regimen can be reinstated. Use of continuous glucose monitoring devices during surgery in children and adolescents has not been adequately studied.

In patients who receive long-acting insulin in the morning and are undergoing surgery of short duration, a standard and effective approach is as follows: on the morning of surgery, half of the usual morning dose of long-acting insulin is administered subcutaneously, the usual dose of rapid-acting insulin is omitted unless needed to correct hyperglycemia, and a maintenance IV infusion of the electrolyte and glucose solution is initiated if needed. Similarly, in patients on insulin pump therapy who are undergoing short procedures, the CSII can be continued at the usual or slightly reduced overnight basal rate. Insulin pump-treated patients can also be maintained on CSII for major procedures, as long as the integrity of the infusion and infusion site is ensured.

The nighttime dose of glargine or detemir insulin may provide sufficient basal insulin coverage for surgery in patients who receive these long-acting insulins before dinner or bedtime. A reduction in the glargine or detemir dose by 20% to 30% on the night before surgery should be considered in patients who have had a tendency to low prebreakfast plasma glucose levels. With all three regimens, a correction dose of rapid-acting insulin can be given subcutaneously immediately after the procedure if needed for hyperglycemia—and repeated as needed to balance initial oral intake (e.g., carbohydrate-containing clear liquids). When the IV infusion is discontinued and the patient is ready to resume regular meals, the usual treatment regimen is reinstated.

Nonautoimmune Type 1 Diabetes

Not all forms of apparently classic T1DM have associated markers of autoimmunity.⁵⁷⁶ In one report, among children presenting with newly diagnosed DM, fewer of those younger than 5 years of age at diagnosis had positive titers of islet cell and GAD antibodies—and fewer had a honeymoon phase within 6 months—compared with a group with onset at a mean age of 10 years.⁵² Similarly, Japanese investigators have reported that some patients with idiopathic T1DM have a nonautoimmune fulminant disorder with abrupt onset characterized by absence of circulating antibodies, evidence of insulinitis in pancreatic biopsies, and high concentrations of pancreatic enzymes—suggesting an acute inflammatory process in the pancreas.^{53,54}

MODY syndromes may also be initially considered to be T1DM.^{13,14} However, a strong family history of vertically transmitted DM in two to three generations, absence of autoimmune markers, and relatively milder features of diabetes should alert the physician to the possibility of a monogenic form of diabetes, such as MODY.

COMPLICATIONS OF DIABETES

The complications of DM can be acute or chronic. Acute complications are primarily those related to morbidity associated with DKA, such as cerebral edema, stroke, pancreatitis, deep vein thrombosis, rhabdomyolysis, pneumomediastinum, pulmonary edema, and mucormycosis. These complications have been described in the section on DKA. Chronic or long-term complications are either metabolic or vascular.

Chronic Metabolic Complications

Mauriac Syndrome

Mauriac syndrome is a metabolic complication of poor glycemic control unique to children. Mauriac syndrome is characterized by growth failure, short stature, delayed puberty, and marked hepatomegaly from massive deposition of glycogen.⁵⁷⁷ This syndrome is seen in children who have been under very poor control (HbA1c usually >10%) for several years. The etiology of the growth retardation is not entirely clear, but the effect of chronic insulinization, which is central to this syndrome, on the growth hormone-IGF-1 (GH-IGF1) axis has been implicated in the pathogenesis of the growth failure.⁵⁷⁸ A recent report of a loss of function mutation in the catalytic subunit of liver glycogen phosphorylase kinase in a patient with Mauriac syndrome implicates this enzyme in the pathogenesis of the hepatomegaly.⁵⁷⁹ Glycogen phosphorylase kinase activates glycogen phosphorylase, the enzyme that catalyzes the first step in breakdown of glycogen. It is posited that in this subject, hyperglycemia because of poor glycemic control combined with the loss of function mutation, inhibited glycogen phosphorylase thereby blocking glycogenolysis and resulting in the massive hepatomegaly that is characteristic of Mauriac disease. Although the more severe forms of this syndrome are rare in the developed countries, the presence of hepatomegaly or subtle degrees of growth failure, especially the blunting of the pubertal growth spurt, is not an uncommon observation in clinical practice in the United States.⁵⁸⁰

Limited Joint Mobility

Limited joint mobility (LJM) is an early clinically evident complication of T1D in children and adolescents.⁵⁸¹ LJM is caused by periarticular connective tissue thickening and stiffness and can manifest as single joint involvement, multiple joint involvement with hand deformity, and even limitation of movement of the spine. The pathology is thickening of the dermis and epidermis with accumulation of collagen and loss of skin appendages.⁵⁸² LJM is associated with poor glycemic control, substantial growth impairment, and increase risk of microvascular disease. The marked decrease in the frequency of LJM and statural deficit in children and youth with diabetes in the 20 years between the late 1970s and 1990s can be attributed to improved metabolic control of children and adolescents.⁵⁸¹ The prevalence of LJM in a population may serve as a measure of quality of diabetes control.

Chronic Vascular Complications

The long-term vascular complications of diabetes include microvascular and macrovascular disease. Microvascular diseases include nephropathy, retinopathy, and neuropathy. Macrovascular disease manifestations include cardiac disease, peripheral vascular disease, and stroke.

Risk Factors for Complications

Until the early 1990s, the dogma was that chronic complications of T1D complications were unrelated to metabolic

control and were inherent to the disease and hence could not be delayed or prevented. A paradigm shift in our understanding of the risk factors for chronic complications ensued from the findings of the DCCT. The DCCT was a multicenter, randomized controlled clinical trial involving 1441 patients with T1D conducted in North America from 1983 to 1993.⁵⁸³ Study participants included 195 pubertal adolescents (aged 13–17 years). Patients were randomized to either intensive or conventional treatment; intensive via pump or MDI of insulin and conventional via two injections of combinations of regular insulin plus NPH or a similar long-acting product. The DCCT provided unequivocal evidence that intensive diabetes treatment and improved glycemic control confer a significant risk reduction for microvascular complications compared with conventional treatment. In the adolescent cohort, the intensive treatment (mean HbA1c 8.1%) compared with conventional treatment (mean HbA1c 9.8%) for a median duration of 7.4 years, reduced the risk and progression of DR by 53%, clinical neuropathy by 60%, and microalbuminuria by 54%. A follow-up study, which continued to track the participants of the DCCT, namely the EDIC study, demonstrated that this positive effect continued after the end of the intervention: that is, that there was a memory effect of improved glycemic control.⁵⁸⁴ The findings of the DCCT and EDIC studies revolutionized our understanding of the risk factors for microvascular complications and made achievement of better glycemic control the cornerstone of current diabetes regimens. Long-term vascular complications are a function of the degree and duration of poor metabolic control. Hence it is not surprising that clinically evident chronic vascular complications are less common in childhood and adolescence compared with the adult. However, studies have shown that early functional and structural abnormalities may be present even within the first few years after the onset of the disease. Thus the importance of understanding and recognizing the pathophysiology and risk factors in childhood and adolescence is to enable the adoption of management strategies and regimens during this period that could prevent or delay the appearance of overt complications later in adult life.

Other Risk Factors for the Development of Complications

Longer duration of diabetes, older age, and puberty are risk factors for complications. A lower prevalence of microvascular complications has been reported for adolescent boys compared with girls.^{585,586} Longitudinal studies have also reported that younger age of T1D onset, particularly before puberty, is associated with a longer time free of complications, such as nephropathy and retinopathy.⁵⁸⁷ Smoking is associated with an increased risk of developing persistent albuminuria, excessive cardiovascular morbidity, and mortality.⁵⁸⁸ High blood pressure and alterations in the circadian blood pressure rhythm have been associated with the risk of developing nephropathy and retinopathy in youth with T1D.⁵⁸⁹ Dyslipidemia was associated with microalbuminuria and retinopathy development in the DCCT/EDIC and other studies.⁵⁹⁰ Higher BMI is a risk factor for microalbuminuria, retinopathy, neuropathy, and cardiovascular disease (Table 21.12).⁵⁹¹ Studies have revealed that there is clustering of complications. For example, complications that co-occur more frequently than expected are retinopathy and diabetic kidney disease, retinopathy and arterial stiffness, and arterial stiffness and cardiovascular autonomic neuropathy.⁵⁹²

Diabetic Nephropathy

Diabetic nephropathy (DN) is one of the most frequent and serious chronic complications of DM. Overt DN is the most

TABLE 21.12 Screening Recommendations and Risk Factors for Vascular Complications in Children and Adolescents With Type 1 Diabetes Mellitus

	Initiation of Screening	Screening Methods	Risk Factors
Nephropathy	11 years with 2–5 years of diabetes	Urinary albumin/creatinine ratio	Hyperglycemia High BP Lipid abnormalities Smoking
Retinopathy	11 years with 2–5 years of diabetes	Fundal photography or mydriatic ophthalmoscopy	Hyperglycemia High BP Lipid abnormalities Higher BMI
Neuropathy	11 years with 2–5 years of diabetes	History Physical examination Clinical tests	Hyperglycemia Higher BMI Age Diabetes duration Genetics
Macrovascular disease	11 years with 2–5 years of diabetes	Lipid profile every 2 years, BP annually	Hyperglycemia High BP Lipid abnormalities Higher BMI Smoking

BMI, Body mass index; *BP*, blood pressure.
(Modified from Donaghue, K.C., et al. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes*, 19 (Suppl 27), 262–274.)

common cause of excess mortality associated with DM. Overt DN is exceptionally rare before puberty and is uncommon even in teens with long-standing diabetes. However, the processes that lead to overt DN are initiated early in the course of the diabetes. Studies have yielded histological evidence for early stages of DN within 2 to 5 years after diagnosis of T1D.⁵⁹³

DN was originally described as a glomerulopathy associated with diffuse or nodular glomerulosclerosis (Kimmelsteil-Wilson lesion).⁵⁹⁴ However, less than one-third of patients with T1D and microalbuminuria have the typical glomerulopathy described by Kimmelsteil and Wilson. The pathophysiological changes in DN include hyperplasia or hypertrophy of various cell types of the glomerulus and tubules, thickening of glomerular and tubular basement membranes, and expansion of tubulointerstitial and mesangial compartments.⁵⁹⁵ Recent studies have focused on the role of podocytes in the development of DN. Podocytes are unique, highly specialized, terminally differentiated visceral epithelial cells in the renal glomerulus that wrap around the capillaries of the glomerulus.⁵⁹⁶ Podocytes play a key role in maintaining glomerular selectivity of permeability. The development of proteinuria is associated with morphological changes in podocytes causing their dysfunction and subsequent apoptosis, ultimately leading to depletion of these cells within the glomerulus, including foot process effacement. Foot process effacement and decreased podocyte number and/or density per glomerulus have been reported in both T1D and T2D.⁵⁹⁷ Although most of the studies have focused on glomerular changes in the diabetic kidney, more recently, important changes in other sites of the renal tissue have been reported, including the tubules, interstitium, medulla, and papilla.⁵⁹⁸

The identity, evolution, and the temporal sequence of events and processes that play a role in the initiation and progression of DN is incompletely understood. Hyperglycemia is necessary for the initiation of renal structural injury, as subjects without diabetes do not develop the same type of nephropathy. Early in the course of T1D, hyperglycemia disturbs the homeostasis of blood flow and vascular permeability in the glomerulus. In vitro studies have demonstrated that hyperglycemia induces mesangial cell matrix production and mesangial cell apoptosis. Increased concentration of mitochondrial reactive oxygen species is implicated in the pathogenesis of DN. Hyperglycemia-induced overproduction of mitochondrial superoxide is posited to activate pathways of vascular tissue damage, leading to cellular redox imbalance and oxidative stress.⁵⁹⁹ Another process believed to play a significant role in DN is accumulation of advanced glycation end products (AGEs). Many of the proteins with a long life, such as collagen, are extensively glycosylated in patients with diabetes. Furthermore, various AGE receptors, such as RAGE, have been described in the kidney and implicated in mediating some of the deleterious effects of AGEs by inducing the expression of transforming growth factor (TGF)- β and other cytokines that are proposed to mediate the transdifferentiation of epithelial cells to form myofibroblasts, a key step in the development of tubulointerstitial fibrosis.⁵⁹⁹ Activation of intracellular signaling molecules, such as protein kinase C (PKC), has also been shown to result in glomerular basement membrane (GBM) hyperplasia and signal transduction for growth factors.⁶⁰⁰ Increased activity of the GH-IGF1 axis and increased prorenin activity have been suggested as contributing to the development of DN.^{601,602} Low-grade inflammation could play a significant role in the pathogenesis of DN via activation of cytokines, such as TGF- β . Downregulation of the renal expression of nephrin (a transmembrane protein localized to the slit membrane between adjacent podocytes of the glomerulus and expressed by podocytes) is impaired in T1D and been implicated in the malfunctioning of the renal filtration barrier in DN and development of albuminuria.⁶⁰³

The natural history of DN in T1D is traditionally described in the following stages.

Stage 1 (hyperfiltration stage) is the initial phase associated with an increase in glomerular filtration rate (GFR) and increased capillary glomerular pressure. The exact pathophysiology of this stage remains unclear.⁶⁰⁴ Studies have demonstrated a correlation between elevated GFR and later development of proteinuria.⁶⁰⁵ Stage 2 (silent stage) is characterized by absence of overt evidence of renal dysfunction with maintenance of GFR and lack of albuminuria. However, renal morphological studies demonstrate significant structural changes, including basement membrane thickening and mesangial expansion in these nonalbuminuric subjects.⁶⁰⁶ In Stage 3 (microalbuminuric stage) the urinary albumin excretion rate is increased to the microalbuminuric range of 20 to 200 mcg/min or 30 to 300 mg/24 hours. Persistent microalbuminuria is defined when microalbuminuria is confirmed on at least two occasions 3 to 6 months apart. Microalbuminuria is considered as the earliest marker of the development of DN. Microalbuminuria at the higher end of the normal range at the age of 10 to 16 years is associated with an increased risk of progression to microalbuminuria and future cardiovascular disease risk, independently of HbA1c.⁶⁰⁷ However, recent studies showed that microalbuminuria may be temporary and does not necessarily reflect permanent renal impairment.⁶⁰⁸ The reversal of microalbuminuria is more likely when it is of short duration; glycemic control is appropriate; and systolic blood pressure, serum cholesterol, and triglyceride levels are in the normal range.⁶⁰⁹ Conversely, early lesions in both glomerular

and tubular structures may occasionally be present in nonalbuminuric subjects.⁶¹⁰ Stage 4 (macroalbuminuria stage) is defined by the development of urinary albumin excretion rate greater than 300 mg/24 hours (200 mcg/min). This stage is strongly predictive of subsequent progress to renal failure, if left untreated. Stage 5 (renal impairment) characterized by uremia and end-stage renal disease can occur in up to 40% of T1D patients. These patients require either dialysis or renal transplantation.

Screening for Microalbuminuria

The earliest clinical marker of DN is microalbuminuria, and current guidelines recommend that annual screening for microalbuminuria should start from age 11 years with 2 to 5 years diabetes duration.⁵⁹¹ Microalbuminuria is defined as:

- Albumin/creatinine ratio (ACR) 2.5 to 25 mg/mmol or 30 to 300 mg/g (spot urine) in males and 3.5 to 25 mg/mmol or 42 to 300 mg/g in females (because of lower creatinine excretion)
- or
- Albumin excretion rate (AER) between 20 and 200 mcg/min or between 30 and 300 mg/24 h in 24 hours or timed urine collections.

Microalbuminuria is confirmed by finding two or all of three samples abnormal over a 3 to 6 month period. Assessing ACR in a spot urinary sample is the easiest method to carry out in an office setting and it generally provides accurate information. First-voided urine in the morning is preferable because of the diurnal variation in albumin excretion and postural effects. A random sample can be used but is associated with an increased risk of false positive results, and therefore abnormal results should be confirmed with first morning urine collections. Confounding factors that could result in false positive (increased ACR/AER) tests include strenuous exercise, infections, nondiabetic kidney disease (i.e., IgA or other types of nephritis), marked hyperglycemia, fever, and menstrual bleeding. Timed overnight or 24-hour collections are more burdensome and generally do not enhance accuracy or prediction of DN.^{609,610}

Effective antihypertensive therapy in patients with nephropathy prolongs the time to end-stage renal disease. Hypertension in children is defined as blood pressure equal to or above the 95th percentile for age, sex, and height, whereas in adolescents (age ≥ 13 years), it is defined as systolic blood pressure ≥ 130 and/or diastolic blood pressure ≥ 80 . Elevated blood pressure (previously known as *prehypertension*) is defined as blood pressure over the 90th percentile for age, sex, and height, or from the age of 13 years, as blood pressure between 120 to 129/80 mmHg. Similar to overt hypertension, elevated blood pressure is associated with adult hypertension and potentially amenable to treatment. Children and adolescents found to have elevated blood pressure or hypertension should have elevated blood pressure confirmed on 3 separate days. Confirmation of hypertension may be assisted by 24-hour ambulatory blood pressure measurements. In children and adolescents with elevated blood pressure, initial treatment includes lifestyle interventions encompassing dietary modifications and moderate to vigorous physical activity at least 3 to 5 days per week (30–60 minutes per session). If target BP is not reached within 3 to 6 months of initiating lifestyle intervention, pharmacological treatment should be considered (Table 21.13). Pharmacological treatment of hypertension in children and adolescents should be initiated with an angiotensin-converting enzyme (ACE) inhibitor, angiotensin II receptor blocker (ARB), long-acting calcium channel blocker, or a thiazide diuretic.⁶¹¹ ACE inhibitors are recommended for use in children and

TABLE 21.13 Recommended Threshold Values for Intervention and Primary Prevention of Microvascular and Cardiovascular Disease in Children and Adolescents With Type 1 Diabetes

Threshold Value	Intervention
BP >90th percentile for age, gender and height	Lifestyle intervention: exercise, less screen time and diet
BP >90th percentile despite lifestyle intervention	ACE inhibitor or other BP lowering agent If microalbuminuria is present: ACE inhibitor or ARB
BP >95th percentile for age, gender and height	Lifestyle intervention and ACE inhibitor or other BP lowering agent If microalbuminuria is present: ACE inhibitor or ARB
LDL cholesterol >2.6 mmol/L (100 mg/dL)	Dietary and lifestyle intervention
LDL cholesterol >3.4 mmol/L (130 mg/dL) and one or more CVD risk factors	Statins

ACE, Angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BP, blood pressure; CVD, cardiovascular disease; LDL, low-density lipoprotein.

(Modified from Donaghue, K.C., et al. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: microvascular and macrovascular complications in children and adolescents. *Pediatr. Diabetes*, 19 (Suppl 27), 262–274.

adolescents with hypertension and albuminuria, but an ARB can be used if the ACE inhibitor is not tolerated because of side effects. The goal of treatment is blood pressure consistently under the 90th percentile for age, sex, and height. In adults, ACE inhibitors and ARBs reduce progression from microalbuminuria to macroalbuminuria and increase the regression rate to normoalbuminuria. However, a metaanalysis revealed that the beneficial effects were more modest in those with the lowest levels of microalbuminuria.⁶¹² In addition, there are still concerns regarding the use of ACE inhibitors in protecting long-term renal function in young people without hypertension. Young people with albuminuria would potentially be taking ACE inhibitors for decades. Side effects include cough, hyperkalemia, headache, and impotence. A key safety issue related to the use of ACE inhibitors, as well as to ARBs, is the potential risk of congenital malformation when used during pregnancy. These risks should be discussed with the family before starting treatment and appropriate birth control measures recommended.

Diabetic Retinopathy

Worldwide and in the United States, DR is a leading cause of new cases of blindness.⁶¹³ The significance of DR cannot be overstated in the pediatric age group. Untreated, this complication has disastrous consequences as it typically leads to blindness. In the landmark study of DR carried out in Wisconsin starting in 1980 to 1982, after 15 years of diabetes, 98% had background retinopathy and after 35 years' duration 62% had proliferative retinopathy.⁶¹⁴ The DCCT study demonstrated the value of tight glycemic control in reducing the risk of blindness.⁵⁸³ Subsequent changes in diabetes management have been associated with a reduction in proliferative retinopathy resulting in decreased rates of DR. More recent data from a cohort in midadolescence (median age 16.4 years) with median diabetes duration of 8.6 years revealed that DR had declined from 53% (in 1990–1994) to 23% (in 2000–2004) and to 12% (in 2005–2009).⁶¹⁵ In a younger group aged 11

to 17 years (median age 14.5 years, duration 2–5 years), the prevalence of mild background retinopathy declined from 16% in 1990 to 1994 to 7% in 2003 to 2006.⁶¹⁴ Furthermore, those with shorter duration had considerably less retinopathy, and retinopathy was present in only 6% of the youngest group (aged 11–13 years).

The retina is comprised of neural, glial, and vascular components. Whereas all three components are adversely affected in DM, from a clinical point of view, it is the changes in retinal vasculature that are most obviously affected by diabetes. In diabetes, presumably because of changes in retinal metabolism, there is a demand for increased retinal blood flow. It is posited that increased retinal blood flow is the earliest finding in DR and damage from chronic vasodilation of the retinal vessels leads to the formation of the characteristic retinal lesions seen in DR.⁶¹⁶ Other mechanisms have been postulated to explain retinovascular damage, including oxidative stress, increased glucose metabolism by the polyol pathway, advanced glycosylation end products that may give rise to basement membrane thickening, and activation of protein kinase C.⁶¹⁷

DR is classified into the following types⁵⁹¹: nonproliferative retinopathy is characterized by microaneurysms, retinal hemorrhages both pre- and intraretinal, cotton wool spots related to ischemia and microinfarction, hard exudates because of protein and lipid leakage, intraretinal microvascular abnormalities (IRMAs), and venular dilatation and tortuosity. Mild and moderate stages of nonproliferative retinopathy are not vision-threatening and do not invariably progress to more severe stages of retinopathy. Severe nonproliferative retinopathy (previously known as *preproliferative*) is characterized by vascular obstruction, increase in number of retinal hemorrhages and microaneurysms, IRMAs, marked venous abnormalities, and ischemia and infarctions of the retinal nerve fibers causing cotton wool spots. Proliferative diabetic retinopathy (PDR) is characterized by neovascularization in the retina and/or vitreous posterior surface. The vessels can rupture or bleed into the vitreoretinal space, which is vision-threatening. Diabetic macular edema/maculopathy is characterized by decreased vascular competence and microaneurysm formation, which produce exudation and swelling in the central retina.

Assessment of Retinopathy

Presently, the most sensitive detection methods for retinopathy screening are a clinical biomicroscopic fundus slit examination through dilated pupils by an ophthalmologist or optometrist and mydriatic seven-field stereoscopic retinal photography.⁵⁹¹ The latter is optimal for research but is not widely available in the clinical setting. Other methods are mydriatic and nonmydriatic two-field fundal photography, direct ophthalmoscopy, indirect ophthalmoscopy, fundus fluorescein angiography, and optical coherence tomography. Rapid advancements in photography and optics combined with AI and machine learning have opened up new, innovative, and more efficient techniques for screening for DR that could be adopted for routine use in the clinical arena in the near future.⁶¹⁸

Current recommendations are that screening for DR should start from age 11 years with 2 to 5 years diabetes duration.⁵⁹¹ A comprehensive initial eye examination should also be considered to detect cataracts, major refractive errors, or other ocular disorders. For those with diabetes duration less than 10 years, mild nonproliferative retinopathy (microaneurysms only) and good glycemic control, biennial screening assessment by biomicroscopic examination or fundal photography may be sufficient. The frequency of retinopathy screening should be more frequent if there are high-risk features for visual loss. Initial worsening of DR can occur with the initiation of improvement in glycemic control especially in patients with long-term uncontrolled

diabetes and severe underinsulinization.^{619,620} Careful retinal examination should be performed in all such patients before initiating intensive insulin therapy. To date, there is no evidence that controlling the speed or magnitude of HbA1C decreases will reduce the risk of DR in patients with diabetes.⁶¹⁹ Pregnancy is a recognized risk for acceleration and progression of retinopathy⁶²¹; hence screening for retinopathy should be undertaken preconception, every trimester and 1 year postpartum.

Treatment for Retinopathy

Treatment options for sight-threatening retinopathy include laser photocoagulation and/or antivascular endothelial growth factor (VEGF) therapy. Pan-retinal laser photocoagulation (PRP or commonly known as *laser therapy*) has been proven to reduce the progression of visual loss by more than 50% in patients with PDR.⁶²² However, photocoagulation is not indicated for eyes with mild or moderate nonproliferative retinopathy. Complications of laser therapy are vitreal and choroidal hemorrhages or visual sequelae of misplaced burns. For PDR, intravitreal injection of anti-VEGF monoclonal antibody (e.g., aflibercept) is now increasingly used and shows better 12-month results for visual acuity than PRP.⁶²³ This treatment is not destructive but does require repeated visits and injections for efficacy (e.g., monthly injections for the first 5 months with up to nine injections in the first year), and carries the rare risk of ocular infection. For diabetic macular edema involving vision loss, anti-VEGF monoclonal antibody (e.g., bevacizumab) is now considered standard of care and has shown superior outcomes over 5 years compared with laser treatment.⁶²⁴ Surgical treatment, such as vitrectomy, may be indicated for persistent vitreous hemorrhage, tractional retinal detachment, or extensive fibrosis.⁶²⁵

Diabetic Neuropathy

Diabetic neuropathy is a major complication of diabetes. In contrast to adults, children and adolescents with diabetic neuropathy, because they are early on in the disease process, are asymptomatic.⁶²⁶ The importance of recognizing and diagnosing diabetic neuropathy in children and adolescents is that early detection of subclinical neuropathy during childhood will enable earlier intervention, with the possibility of reducing or delaying the incidence of clinical neuropathy later in life.

Classification of Diabetic Neuropathies (Modified from Pop-Busui et al.⁶²⁷)

A. Diffuse neuropathy

- Distal symmetric polyneuropathy (DSPN)
 - Primarily small-fiber neuropathy
 - Primarily large-fiber neuropathy
 - Mixed small- and large-fiber neuropathy (most common)
- Autonomic
 - Cardiovascular
 - Gastrointestinal (including gastroparesis, enteropathy, colonic hypomotility)
 - Urogenital (including diabetic cystopathy, erectile dysfunction, female sexual dysfunction)
 - Sudomotor dysfunction (including hypohydrosis/anhidrosis, gustatory sweating)
 - Hypoglycemia unawareness
 - Abnormal pupillary function

B. Mononeuropathy—Isolated cranial or peripheral nerve (e.g., cranial nerve [CN] III, ulnar, median, femoral, peroneal)

C. Radiculopathy or polyradiculopathy—lumbosacral polyradiculopathy, proximal motor amyotrophy

The most common type of childhood diabetic neuropathy is DSPN. Large nerve fiber dysfunction manifests as loss of distal tendon reflexes and altered perception of vibration, proprioception, and/or tactile sensation. Small fiber dysfunction manifests as hyperesthesia and decreased pain and temperature sensation. More than half of the children with early diabetic DSPN may be asymptomatic.⁶²⁸ The reported prevalence rates for diabetic neuropathy in children vary from under 10% to 30%.^{628,629} This wide variance possibly reflects differences in patient selection, clinical status, the type of diagnostic tests used, and the criteria used to define diabetic neuropathy. Abnormal nerve conduction studies can be detected as early as within 6 months after diagnosis of T1D, with improvement after correction of hyperglycemia.⁶³⁰ The presence of diabetic autonomic neuropathy is likely underestimated owing to suboptimal screening. Subclinical diabetic autonomic neuropathy has been reported to be between 8% and 43% in children with T1D.⁶³¹ Diabetic neuropathy is also an early complication of T2D. Recent studies have reported that prevalence of DSPN was significantly higher in youth with type 2 compared with those with T1D (25.7% vs. 8.2%).⁶²⁹

All patients should be assessed for DSPN 5 years after the diagnosis of T1D and at diagnosis of T2D, and at least annually thereafter.⁶²⁷ Assessment should include a history and physical examination, and either temperature or pinprick sensation (small-fiber function) and vibration sensation using a 128-Hz tuning fork (large-fiber function).⁶²⁷ All patients should have an annual 10-g monofilament testing to assess for feet at risk for ulceration and amputation. Electrophysiological testing or referral to a neurologist is rarely needed for screening, except in situations where the clinical features are atypical or the diagnosis is unclear. Atypical features include motor greater than sensory neuropathy, rapid onset, or asymmetrical presentation. Tight glucose control is the cornerstone of prevention of diabetic neuropathy. Despite the advances in elucidating the pathogenesis of diabetic neuropathy, there is a paucity of treatment options that alter the natural history of DSPN or reverse DSPN once established.⁶³² Pharmacological agents, such as pregabalin and duloxetine, have been proven to be of benefit in the treatment of neuropathic pain.⁶²⁷

A final point regarding neural changes in children with diabetes is the accumulating evidence that acute hyperglycemia impairs brain function manifest as lower spatial working memory along with changes in relevant areas of functional MRI in adolescents with T1DM.⁶³³ Also, chronic hyperglycemia in younger children is associated with altered white matter development, which might contribute to their documented mild cognitive deficits.⁶³⁴

Macrovascular Disease

Cardiovascular disease is a leading cause of mortality in patients with T1D. Whereas overt diabetes-related cardiovascular disease complications are rare until adulthood, the early markers and the pathophysiological foundations of these complications are manifest in adolescents and young adults with T1D.⁶³⁵ In general, progress in reducing macrovascular complications has lagged behind similar efforts targeted toward the management of microvascular complications of T1D. The reasons for this disparity may relate to differences in the pathogenesis and the longer time needed to develop macrovascular complications.⁶³⁵ Because cardiovascular events (such as stroke, myocardial infarction) are absent or rare in childhood and adolescence, studies have had to rely on surrogate parameters and indices (such as carotid intima thickness). Traditional risk factors for cardiovascular disease are hyperglycemia, hypertension, dyslipidemia, and diabetic kidney disease. However, the precise relationship of these risk factors to macrovascular disease in the pediatric age group is unclear at the present time. Hence in contrast to studies in the adult, pediatric studies have

failed to demonstrate a clear relation between HbA1c concentrations and measures of vascular health.⁶³⁶ Obesity is a risk factor for early death because of cardiovascular event and the rates of obesity are increasing significantly in children and adolescents with T1DM. The role of lifestyle and behavioral factors, such as exercise, diet, smoking, stress, sleep, and depression is also important in the pathogenesis of macrovascular disease in patients with T1DM.

Hyperglycemia, hypertension, and dyslipidemia are major contributory factors for cardiovascular disease. However, achieving reduction of these risk factors in children and adolescents remains a challenge for a variety of reasons.⁶³⁶ The insidious nature of the effect of these risks factors, compounded with the long time needed for the development of cardiovascular disease masks the importance of these risk factors. Lack of rigorous studies in the pediatric age group is another factor that detracts from assigning a high priority to these risk factors in children and adolescents. The majority of the evidence available in the pediatric age group are inferred from adult studies.

In T2D, comorbidities may already be present at the time of diagnosis of T2D in youth. Therefore blood pressure measurement, a fasting lipid panel, assessment of random urine albumin to creatinine ratio, and a dilated eye examination should be performed at diagnosis. Thereafter, screening guidelines and treatment recommendations for hypertension, dyslipidemia, urine albumin excretion, and retinopathy are similar to those for youth with T1D. Additional problems that may need to be addressed include polycystic ovary disease and other comorbidities associated with pediatric obesity, such as hepatic steatosis, sleep apnea, orthopedic complications, and psychosocial concerns.

TYPE 2 DIABETES MELLITUS

Typical

T2DM, formerly known as *NIDDM*, is a heterogeneous disorder characterized by defective insulin secretion that progressively fails to compensate for insulin resistance.⁶³⁷ The cause of insulin resistance is usually obesity,^{637–642} although agents such as growth hormone and cortisol also antagonize insulin action and may unmask inadequately compensated insulin secretion. The high concentrations of placental growth hormones during mid to late gestation likewise may unmask inadequate insulin secretion, resulting in gestational diabetes—a harbinger of permanent diabetes later in life.

The mechanism of insulin resistance caused by obesity may in part relate to changes in fatty acid metabolism that interfere with normal glucose metabolism, and in part to factors synthesized within fat cells that antagonize insulin action.⁶⁴³ Most notable are the hormones adiponectin, leptin, and resistin,⁶⁴³ which are produced by fat cells and are a likely major link between obesity and diabetes. Genetic regulation of the ability to compensate for insulin resistance may be another mechanism that predisposes or protects from T2DM.^{644,645} Thus in addition to the genetic components responsible for impaired insulin secretion (which are being investigated, progressively identified, and presently cannot be changed),^{646–650} the key modifiable factor responsible for the epidemic of T2D in children is the epidemic of obesity increasingly recognized throughout the world.^{650–654}

T2DM is being increasingly recognized in children, particularly obese adolescents and especially but not exclusively in certain ethnic groups, such as Native American Indians, non-Hispanic black Americans, Hispanic Americans, Pacific Islanders, and Southeast Asians in the developing world.^{650–654} Here, too, a genetic predisposition for insulin resistance together with the epidemic of obesity is responsible for a rapid increase in the proportion of patients with T2D—representing up to half of the newly presenting cases in one major medical center in the

United States.^{650,651} In these vulnerable groups, rates of T2DM have almost doubled from 2002 to 2012.⁴⁷

In addition to obesity, these patients often have a family history of T2D, indicative of genetic predisposition. They also may have acanthosis nigricans, are more commonly female, and often have poor metabolic control that predisposes them to the earlier appearance of microvascular and macrovascular complications.⁶⁵¹ There is an accelerated tempo in the development of complications associated with DM; impaired renal function manifest as albuminuria occurs in ~6% within 5 years of diagnosis and about 2.3% have end-stage renal failure by 10 years after diagnosis. Microvascular changes, for example, retinopathy, also appear earlier, and reduction of brain volume with microstructural changes in white matter and decreased cognitive function have been reported.⁶⁵⁵ In the TODAY study, those with a BMI 35 kg/m² or more demonstrated rapid progression of cardiovascular risk factors despite treatment; hypertension tripled over 4 years from 11% to 34%; microalbuminuria increased from 6% to 17% within 3 years; and high-risk LDL (LDL >130 mg/dL or taking medication) increased from 4.5% to 11% after 3 years.⁶⁵⁶ As previously noted, lifestyle intervention and metformin failed and the introduction of insulin promotes further weight gain.^{657,658} For the carefully chosen patient and in the appropriate facility, bariatric surgery offers promising results with amelioration in all manifestations of disturbed metabolism and associated vascular abnormalities, although vitamin and iron deficiencies, as well as surgical complications remain.⁶⁵⁹

Patients with T2DM may present initially in DKA suggesting T1D, but after recovery they may manifest a prolonged honeymoon phase—the so-called *atypical DM*, a form of T2D, as documented by significant insulin or C-peptide levels not consistent with T1D. They also lack markers of islet autoimmunity and the classic HLA associations.⁶⁵¹

In those with a family history of T2DM, insulin resistance (as shown by impaired insulin-stimulated glucose disposal and higher insulin values during oral glucose tolerance or so-called hyperglycemic clamps) is demonstrable in the first decade of life before clinically demonstrable changes in glucose tolerance.^{650,651} The sites of this presumably genetic impairment in insulin sensitivity have not yet been identified but clearly precede clinical DM brought about by obesity-induced insulin resistance.^{650,651} There may be other factors that induce insulin resistance.

There is also an emerging number of newly identified genetic factors associated with impaired insulin secretion and action. Genome-wide scans have consistently identified three major genetic linkages: the K_{ATP}, especially the Kir 6.2 subunit encoded by the *KCNJ11* gene; the peroxisome proliferator-activated receptor γ (*PPARG*); and transcription factor 7 like 2 (*TCF7L2*).^{653,654,660} The development of sophisticated arrays that permit genotyping of literally hundreds of thousands of polymorphisms has revealed additional loci of genetic markers in T2DM, including a polymorphism in the zinc transporter SLC30A8 (expressed only on pancreatic β cells) and genes potentially involved in pancreatic development (*IDE*, *KIF11*, *HHEX*) or function (*EXT2-ALX4*).^{647–654} These sophisticated genetic screening approaches are likely to identify more of the complex genetic traits that underlie T2DM. However, the complexity of the genetic contributions to T2DM remain challenging.^{661,662}

From a practical point of view, T2DM in children and adolescents should be viewed as a major public health issue—and without effective lifestyle interventions (such as weight reduction combined with regular exercise), treatment options are limited and only modestly successful.^{650,651} Bariatric surgery is successful in adults and in small population samples has been a durable and effective means to reverse metabolic disturbances, including hyperlipidemia, diabetes, hypertension

albuminuria.⁶⁵⁹ Exogenous insulin may be necessary to control blood glucose initially, but its appetite-stimulating effects challenge attempts at weight reduction. Sulfonylureas may be temporarily helpful, as may meglitinide—which acutely increase insulin secretion.

Metformin, which sensitizes tissue to insulin action and diminishes hepatic glucose production, is the most frequently used agent for treating T2D in children. The FDA has approved it for use for those over 10 years of age. The FDA has not approved for use in children thiazolidinediones (glitazones) that sensitize tissue to insulin, but nevertheless they have reportedly been used.⁶⁶⁰ These agents are potentially hepatotoxic, and the original product was withdrawn for this reason. Newer glitazones (such as rosiglitazone) are under investigation in adolescents with T2DM.⁶⁶⁰ Alpha-glucosidase inhibitors that slow carbohydrate absorption and lipase inhibitors to diminish fat absorption are available and are approved, but clinical trials in children/adolescents for efficacy and compliance have not yet been published. In addition, agents such as GLP-1 receptor agonists, dipeptidyl-peptidase (DPP)4 inhibitors, and SGLT2 antagonists used in adults with T2DM are undergoing trials in adolescents but are not yet approved for use in this age group. This represents a deficiency in our ability to treat T2DM in adolescents and youth.^{650,651} Compared with placebo, the addition of liraglutide, a GLP-1 analog, to metformin and insulin in adolescents with T2DM succeeded in lowering HbA_{1c}, and liraglutide is now approved for use in youth with T2DM.⁶⁶³ It is anticipated that similar studies examining the efficacy and safety of the variety of agents now approved for use in adults with T2DM will become available for use in adolescents and youth in the near future.

GENETIC DEFECTS OF BETA CELL FUNCTION

MODY Syndromes

The term MODY was originally conceptualized as a form of maturity-onset (i.e., type 2) diabetes. However, the MODY syndromes are best considered a group of disorders of monogenic defects in beta-cell function. Affected patients may have only modest elevation of glucose, may remain asymptomatic for many years, and may become clinically apparent during intercurrent illness or pregnancy that unmasks the limitation in insulin secretion.^{13,14} Clinical criteria used to establish the diagnosis include the following:

- Dominant inheritance with at least two (and preferably three) consecutive affected generations
- Onset before age 25 to 30 years
- Evidence of significant but impaired residual insulin secretion reflected in C-peptide levels whether or not the patient is being treated with insulin

Some 14 specific genetic defects have been identified (see also Chapter 10) but of these, the most common are MODY2, MODY3, MODY1, and MODY5 (Table 21.14), which together account for over 85% of all forms. Moreover, milder defects in the K_{ATP} genes *KCNJ11* and *ABCC8*, as well as defects in the insulin gene itself (*INS*), have been found in patients who fulfill the criteria for a diagnosis of MODY. Of these genetic defects that together account for no more than 2% to 5% of diabetes, about two-thirds (65%) are MODY 3 (*HNF1A*), together with MODY 2 (glucokinase defect), and the remainder constitute the other defects. MODY2 has a prevalence of approximately 1:1000 births and may remain entirely asymptomatic until discovered during routine laboratory evaluations, as for example, during pregnancy.^{25,119} Mild stable hyperglycemia may be present from birth and not require treatment except during stress, such as infections in an infant or child, or pregnancy in a young adult.

TABLE 21.14 Classification of Maturity-Onset Diabetes of Youth

Type	Gene	Chromosome	Treatment	Relative Frequency (%)
MODY 1	<i>HNF4A</i>	20q12–q13.1	Insulin/SU	5
MODY 2	Glucokinase	7p15–p13	Exercise/ diet	22
MODY 3	<i>HNF1A</i>	12q24.2	Insulin/SU	58
MODY 4	<i>IPF1/PDX1</i>	13q12.2	Insulin	<1
MODY 5	<i>HNF1β</i>	17q12	Insulin/SU	2
MODY 6	<i>NEUROD1/ Beta-2</i>	2q32	Insulin	<1
MODY 7	<i>KLF11</i>	2p25	Insulin	<1
MODY 8	<i>CEL</i>	9q34.3	Insulin	<1
MODY 9	<i>PAX4</i>	7q32	Insulin	<1
MODY 10	Insulin	11p15.5	Insulin	<1
MODY 11	<i>BLK</i>	8p23.1	Insulin	<1
MODY 12	<i>ABCC8</i>	11p15.1	SU	<1
MODY 13	<i>KCNJ11</i>	11p15.1	SU	<1
MODY 14	<i>APPL1</i>	3p14.3	Insulin	<1

ABCC8, ATP binding cassette subfamily C member 8; *APPL1*, adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1; *BLK*, BLK proto-oncogene, Src family tyrosine kinase; *CEL*, carboxyl-ester lipase; *HNF*, hepatic nuclear factor; *IPF/PDX*, insulin promoter factor/pancreas-duodenum homeobox; *KCNJ11*, potassium channel family J number 11; *KLF*, Kruppel-like factor; *NEUROD1/Beta-2*, neurogenic differentiation factor 1/beta-cell E-box transactivator 2; *PAX*, paired homeobox; *SU*, sulfonylurea drugs.

- Maturity-onset diabetes of youth (MODY)1, 2, & 3 together constitute 85% of all known MODY syndromes; if MODY5 is included, almost 90% of all MODY syndromes are defined
- MODY2 is stable, rarely requires insulin treatment except during pregnancy to protect the fetus from hyperglycemia, and has an excellent prognosis for avoidance of vascular complications
- MODY1 & 3 frequently respond to sulfonylurea drugs initially but may progress to insulin dependence and risk for development of vascular complications dependent on metabolic control
- MODY constitutes about 1.5%–3.0% of new-onset childhood diabetes and should be suspected in those with a family history of multiple affected members in two to three generations, with onset before age 35 years, absence of markers of autoimmunity, “mild diabetes” requiring <0.5 U/kg insulin from the outset or unusually prolonged “honeymoon” phase

With glucokinase deficiency, microvascular complications of diabetes are rare.²⁵ In the other common forms of MODY, such as MODY3 and MODY1, however, onset is usually in the early teens to 20s, glucose intolerance may become progressively worse and hence require treatment, and microvascular complications may develop later in life. Renal cysts or other renopelvic anomalies may occur in MODY 5 (*HNF1β*). Notably, if the genetic defect in insulin promoter factor-1 (*IPF1*, MODY 4) is homozygous, pancreatic agenesis results and this is a cause for permanent neonatal diabetes associated with exocrine, as well as endocrine, insufficiency (see Chapter 10). Likewise, homozygous mutations in glucokinase have been associated with congenital diabetes. In contrast, gain-of-function mutations in glucokinase cause persistent hyperinsulinemic hypoglycemia of infancy (see Chapter 7). Thus the MODY syndromes are monogenic defects of islet cell formation (MODY 4) or of transcription factors (MODY 1, 3, 5, and 6)—or a defect in the functional glucose sensor glucokinase (MODY 2).^{13,14,664,665}

Table 21.15 compares and contrasts the four most common types of diabetes found in adolescents: type 1, type 2, atypical DM, and MODY. The monogenic MODY syndromes have been extensively reviewed in terms of chemical, biochemical, and molecular analyses,^{13,14,664,665} and are also discussed in Chapter 10. Other monogenic defects reportedly associated with a type 2 clinical picture include mutation in *GLUT2* and the glycogen synthase genes.^{660,664}

Other Forms of Monogenic Diabetes

Mitochondrial Diabetes

Mitochondrial genetic defects that are associated with diabetes are commonly but not invariably associated with neuromuscular disorders, including deafness, migraine, seizures, and mental retardation. For example, the MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) syndrome may initially present in childhood with short stature, go on to deafness in teen years, and develop diabetes and encephalopathy in midlife. DM may be the only manifestation of a mitochondrial disorder encoded by a gene defect within the mitochondria (all of which are maternally inherited) or a nuclear DNA-encoded gene necessary to the oxidative phosphorylation sequence within mitochondria.

This defective energy pathway leads to progressive impairment of insulin secretion, and thus an initially mild hyperglycemia may progressively worsen. This is the case with the most common form of diabetes caused by a mitochondrial gene mutation at nucleotide pair (np) 3243 of the mitochondrial genome, often associated with deafness. Remarkably, this same genetic defect may be associated with the MELAS syndrome. Initially, patients with np 3243 mutations can be controlled by diet alone but later may require insulin. DM presenting in infancy and severe from the outset and requiring insulin may be associated with mitochondrial DNA deletions, as seen in the Kearns-Sayre syndrome and Pearson syndrome.^{666,667} Fig. 21.7 identified the critical role of energy production, including the mitochondrial oxidative phosphorylation pathway for normal insulin secretion.

Defects in this pathway may be responsible for transient or permanent neonatal DM, especially those that involve activating mutations in the K_{ATP} channel subunits Kir 6.2 (*KCNJ11*) and its regulatory subunit sulfonylurea receptor SUR1 (*ABCC8*) as discussed in Chapter 10. Inactivating mutations in the K_{ATP} subunits cause neonatal hyperinsulinemic hypoglycemia of variable severity, as described in detail in Chapter 7. Diabetes in the mitochondrial syndromes is generally well controlled by exogenous insulin.⁶⁶⁷

Wolfram Syndrome

Wolfram syndrome is characterized by diabetes insipidus, DM, optic atrophy, and deafness (DIDMOAD).^{668,669} There is a selective loss of beta cells, which is responsible for the DM. Genetic linkage studies in consanguineous families with autosomal-recessive inheritance led to the positional cloning of a gene on the short arm of chromosome 4 (termed *WFS1*, and now identified as Wolframin).

Although the function of this gene is not entirely understood, it is expressed in many tissues (most abundantly in beta cells, compared with the exocrine pancreas). Mutations in Wolframin have been identified in many families with Wolfram syndrome. Affected individuals are usually compound heterozygotes. The Wolframin gene may have a role in beta-cell and neural tissue survival, and there does not appear to be a correlation between the observed mutation and severity of disease. Defects in Wolframin have been implicated in the idiopathic common nonimmune form of T1DM.⁶⁶⁸ A second locus of Wolfram syndrome has been mapped to the long arm of chromosome 4 in several consanguineous Jordanian families.⁶⁶⁸ In these patients, diabetes insipidus was not a feature—but upper gastrointestinal bleeding and ulceration were prominent.

Although a mitochondrial form of Wolfram syndrome has been proposed, a defect in mitochondrial DNA could not be confirmed in one large cohort.⁶⁶⁹ It has been suggested that DM (age <15 years) and progressive optic atrophy are highly predictive of Wolfram syndrome. The sequence of appearance

TABLE 21.15 Comparison of the Common Forms of Youth-Onset Diabetes

Characteristic	Type 1 Diabetes	Type 2 Diabetes	Classic MODY	Atypical Diabetes Mellitus
Age at onset	Peaks at 5 and 15 years of age	Teenage years, young adults	<25 years of age	>40 years of age
Predominant ethnic groups affected	White	Hispanic, African American, Native American	Occurs in all ethnic groups	African American
Male-to-female ratio	1.1:1	1:1.5	1:1	1:3
Severity at onset	Acute, severe, insulin required	Subtle, insulin not required	Subtle, insulin not required	Acute, severe, insulin required
Islet autoimmunity	Present	Absent	Absent	Absent
HLA-DR3, -DR4	Very common	No increased frequency	No increased frequency	No increased frequency
Ketosis, DKA	Common	Uncommon	Rare	Common at onset
Long-term course	Insulin-dependent	Noninsulin-dependent	Noninsulin-dependent	Noninsulin-dependent
Prevalence of obesity	Uncommon	≥90%	Uncommon	40%
Proportion of cases of 100% youth-onset diabetes	Most common form of youth-onset diabetes	Rising in frequency; ± as common as type 1 diabetes in specific populations	≤5% of youth-onset diabetes in whites	≤10% of cases of youth-onset diabetes in African Americans
Percentage of probands with an affected first-degree relative	≤15%	Variable but common	100%	>75%
Mode of inheritance	Nonmendelian, generally sporadic	Nonmendelian but strongly familial	Autosomal dominant	Autosomal dominant
Number of genes controlling inheritance	Polygenic	Polygenic	Monogenic	Monogenic
Pathogenesis	Autoimmune beta cell destruction: insulinopenia	Insulin resistance plus relative insulinopenia	Insulinopenia	Insulinopenia

DKA, Diabetic ketoacidosis; MODY, maturity-onset diabetes of the young.

(Modified from Winter, W E., Nakamura, M., & Hause, D. (1999). Monogenic diabetes mellitus in youth. *Endocrinol Metab Clin North Am*, 28, 765–785.)

of the stigmata is nonautoimmune T1D in the first decade of life; central diabetes insipidus and sensorineural deafness in two-thirds to three-fourths of the patients in the second decade; renal tract anomalies in approximately half in the third decade; and neurological complications, such as cerebellar ataxia and myoclonus, in half to two-thirds in the fourth decade. Other features include primary gonadal atrophy in the majority of males and a progressive neurodegenerative course with neurorespiratory death at a median age of 30 years. Depression has been reported as a frequent feature of relatives of patients with Wolfram syndrome.⁶⁶⁸

Thiamine-Responsive Diabetes Mellitus (Roger Syndrome)

This syndrome is characterized by megaloblastic anemia, DM, and sensorineural deafness—all of which may respond to vitamin B₁ (thiamine). DM is mild to moderate, insulin secretion may improve with thiamine therapy, and there are no associated autoimmune markers. It is caused by a defect in the thiamine transporter,^{670,671} which has now been identified as being caused by mutations in the *SLC19A2* gene. This gene encodes the membrane-bound thiamine transporter THTR-1.

Uptake of thiamine by the combined pathways of an active high-affinity carrier and a passive low-affinity carrier leads to accumulation of intracellular thiamine, which is then converted to its active form, thiamine pyrophosphate. This cofactor enables proper function of transketolase, which is important in the pentose phosphate shunt, the key to ribose synthesis and hence nucleic acid production and of pyruvate dehydrogenase, α ketoglutarate dehydrogenase, and branched chain acid dehydrogenase—all of which are key to oxidative decarboxylation. Mutations in the high-affinity transporter THTR-1 lead to cell death in those cells that have a high rate of nucleic acid turnover (such as bone marrow cells) and activity (such as pancreatic

beta cells), thereby explaining the association of thiamine-responsive anemia with diabetes in those affected by this mutation.

Drug or Chemical Induced

A number of drugs and chemical agents may be toxic to the beta cell. Best known for the diabetogenic effects are the immunosuppressive drugs cyclosporine, sirolimus, and tacrolimus, which are toxic to beta cells—causing insulin-dependent diabetes in a significant proportion of patients treated with these agents for organ transplantation. Their toxicity to pancreatic beta cells is compounded by the use of immunosuppressive glucocorticoids after transplantation, which antagonize insulin action and together with impaired β -cell number or function lead to diabetes.

Streptozotocin and the rodenticide Vacor are also beta-cell toxic, causing diabetes. Box 21.1 listed other agents that may induce diabetes. Among these, L-asparaginase (used in chemotherapy for leukemia) and diazoxide (used to treat persistent hyperinsulinemic hypoglycemia in infancy) may cause diabetes. All of these agents, especially glucocorticoids, may combine to unmask clinical DM.^{672–674}

Diseases of the Exocrine Pancreas

Cystic fibrosis (CF) is one of the most common inborn errors of metabolism, involving the chloride channel encoded on chromosome 7 and affecting approximately one in 2500 live-born white infants. The increasing survival of patients with CF and the increased use of glucocorticoids to suppress bronchopulmonary inflammation have brought to the fore an entity termed *cystic fibrosis-related diabetes* (CFRD), characterized by variable impairment of carbohydrate intolerance.

Some have mild DM clinically apparent only while receiving steroids, whereas others require insulin for a nonimmune form of insulinopenic DM. Up to 75% of adults with CF have CFRD. Islet amyloid is prominent, both insulin secretion and insulin sensitivity may be impaired, and DKA may occur—as may microvascular complications. When fasting hyperglycemia (7 mM = 126 mg/dL or higher) is documented, therapy with insulin is indicated. Insulin also facilitates optimal nutrition and growth and hence promotes a sense of wellbeing. The diagnostic and management criteria for CFRD have been extensively reviewed.^{16,675}

Ionizing Radiation to the Abdomen

Ionizing radiation to the abdomen during childhood for a condition, such as nephroblastoma, has been associated with the development of DM some 20 years later in 5% to 10% of children so treated.⁶⁷⁶

Pancreatectomy

Extensive pancreatectomy performed for the management of severe hyperinsulinemic hypoglycemia of infancy is associated with diabetes in approximately 50% or more of long-term survivors.²²

Virus Infections

Several viruses have been implicated in the cause of T1DM in children. Coxsackievirus B₄ has been shown in one case report as likely fulfilling Koch's postulates for a direct beta-cell toxic effect in causing acute fulminant DM. In other cases of coxsackievirus infections (as well as with the established association among rubella, cytomegaloviruses, and enteroviruses), molecular mimicry between antigenic determinants in the virus and certain islet cell antigens has been implicated as the mechanism leading to an autoimmune form of T1DM. Finally, a superantigen-triggered immune response has been suggested for some viral infections and may be the mechanism related to the acute onset of DM with the hemolytic-uremic syndrome.^{21,61,677,678} Patients with new-onset diabetes frequently have evidence of enteroviral infection in their islets.⁶⁷⁹ Peptides derived from a particular viral genus possess insulin-like properties, including binding and stimulation of insulin and IGF-1 receptors providing a further potential link between viral infection and the onset of DM via the mechanism of molecular mimicry; antibodies to the virus might also affect endogenous cells producing insulin.⁶⁸⁰

GENETIC DEFECTS IN INSULIN ACTION

Type A Insulin Resistance With Acanthosis Nigricans

This syndrome is characterized by severe insulin resistance, acanthosis nigricans in the absence of obesity, or lipotrophy. Affected females also have hyperandrogenism, possibly as a secondary manifestation of the hyperinsulinemia with stimulation of androgen synthesis by ovarian theca cells. Glucose intolerance is variable and includes symptomatic diabetes. The hyperandrogenism presents as clinical and biochemical findings suggestive of polycystic ovary syndrome (PCOS).

Some patients (predominantly black females with obesity, acanthosis nigricans, and accelerated growth suggestive of gigantism) may represent insulin resistance owing to obesity, with downregulation of the insulin receptor. The gigantism may represent a "spillover" effect of insulin acting through the IGF-1 receptor rather than the insulin receptor.^{681,682}

Type B Insulin Resistance

Type B insulin resistance is a rare syndrome associated with evidence of immune dysfunction (such as the defined autoimmune disease rheumatoid arthritis) or of nonspecific features of autoimmunity (such as elevated sedimentation rate or high levels of antinuclear antibodies). As with other autoimmune disorders, females are predominantly affected. An insulin-resistant diabetes develops together with acanthosis nigricans and features of PCOS (such as hirsutism).

The syndrome is caused by serum autoantibodies against the insulin receptor resulting in impaired function. However, the receptor may be activated by the presumed conformational changes induced by the antibody and cause severe hypoglycemia rather than diabetes. Treatment may require high doses of insulin to try to control the hyperglycemia, along with immunosuppressive drugs to suppress antibody production.⁶⁸²

Leprechaunism (Donohue Syndrome)

Leprechaunism is a syndrome characterized by intrauterine growth retardation, fasting hypoglycemia, and postprandial hyperglycemia in association with profound resistance to insulin in a patient whose serum concentrations of insulin may be 100-fold that of comparable age-matched infants during an oral glucose tolerance test. Various defects of the insulin receptor have been described, thereby attesting to the important role of insulin and its receptor in fetal growth and possibly in morphogenesis. Even a probable complete absence of functional insulin receptors caused by homozygous inheritance of missense mutation in the insulin receptor, however, resulted in normal organogenesis and a live-born infant who had a severe form of leprechaunism. Most of these patients die during the first years of life.^{683,684}

Rabson-Mendenhall Syndrome

The Rabson-Mendenhall syndrome is defined by clinical features that appear to be intermediate between those of acanthosis nigricans with insulin resistance type A and leprechaunism. Features include extreme insulin resistance, acanthosis nigricans, abnormalities of the teeth and nails, and pineal hyperplasia. It is not clear whether this syndrome is entirely distinct from leprechaunism. However, patients with Rabson-Mendenhall syndrome tend to live beyond the first year of life. Defects in the insulin receptor gene have been described in this syndrome.^{684,685}

Lipotrophic Diabetes

Lipotrophic diabetes presents an interesting paradox. Whereas classic T2DM is generally associated with an excess of fat and its metabolic consequences (as described previously), a paucity of fat also causes severe insulin resistance and marked metabolic disturbances. Table 21.16 lists the genetic syndromes associated with lipotrophic diabetes. A primary cause has been identified in the form called *familial partial lipotrophy* (also known as *Dunnigan syndrome*). This is a gene defect localized to chromosome 1q21-22 and its product lamin A/C.

This autosomal disease usually manifests peripubertally as subcutaneous fat in the extremities and trunk but with progressively more fat in the face and neck as puberty progresses. Visceral and interfascicular fat also increases. Females seem to develop DM and dyslipidemia earlier and more severely than males. It is unclear why and how the lamin A/C mutations cause this lipotrophy syndrome, especially as mutations in this gene also are associated with a progressive form of muscular dystrophy (Emery-Dreifuss syndrome), cardiomyopathy, and cardiac conduction defects.^{686,687} The gene altered

TABLE 21.16 Genetic Syndromes of Lipoatrophy

Syndrome	Lipoatrophy	Gene/Locus	Inheritance	OMIM
PRIMARY LIPOATROPHY SYNDROMES				
Congenital generalized lipoatrophy (Seip-Berardinelli)	Generalized	9q34	AR	269700
Dunnigan syndrome	See text for details	Gmg3lg; 11q13p		
	Familial partial	1q21–22	AD	151660
	See text for details	Lamin A/C		
Others	Numerous distributions	Unknown	AD/AR	N/A
COMPLEX SYNDROMES ASSOCIATED WITH LIPOATROPHY				
Mandibuloacral dysplasia	Congenital, partial	Unknown	AR	248370
	Involves extremities			
Werner syndrome	Congenital, partial	8p12	AR	277700
	Involves extremities	Werner helicase		
Cockayne syndrome	Congenital, partial	5	AR	216400
	Involves extremities	CSA		
Carbohydrate-deficient glycoprotein syndrome	Transient, partial	16p13.3	AR	212065
SHORT syndrome ^a	Buttocks	PMM1 and 2		
AREDYLD syndrome ^b	Generalized, congenital	Unknown	AR	269880
	Generalized, congenital	Unknown	Unknown	207780

AD, Autosomal dominant; AR, autosomal recessive; OMIM, Online Mendelian Inheritance in Man, database providing information about genetic syndromes; PMM1, PMM2, phosphomannomutase 1 and 2.

There is evidence for genetic heterogeneity because similar clinical features may be caused by different genetic mutations.

^aShort stature, hyperextensibility, hernia, ocular depression, Rieger anomaly, and teething delay.

^bAcrorenal field defect, ectodermal dysplasia, and lipoatrophic diabetes; not clear if this is a variation of Seip-Berardinelli syndrome.

(From Arioglu, E., Rother, K.I., Reitman, M.L., et al. (2000). Lipoatrophy syndromes. *Pediatr Diabetes* 1:155; Brown, R.J., Araujo-Vilar, D., Cheung, P.T., et al. (2016). The diagnosis and management of lipodystrophy syndromes: a Multi Society Practice Guideline. *J Clin Endocrinol Metab*, 101 (12), 4500–4511.)

in Berardinelli-Seip congenital lipodystrophy has been localized to chromosome 11q13.274.⁶⁸⁷ In addition, defects in genes encoding the enzyme AGPAT2, the endoprotease ZMPSTE24, the kinase AKT2, the nuclear receptor PPAR γ , and the protein BSCL 2 have been found in patients with lipodystrophies.^{686,687}

ACQUIRED DEFECTS IN INSULIN ACTION

These defects range from hormonal disorders, such as pheochromocytoma and Cushing syndrome that antagonize insulin action to disease to drug-acquired forms of lipodystrophy. Antiinsulin receptor antibodies may be found in some collagen vascular disorders and can cause a T2DM syndrome characterized by acanthosis nigricans and severe insulin resistance. This is generally referred to as *acanthosis nigricans with insulin resistance type B*. The type A syndrome consists of a variety of insulin receptor mutations, some of which were described earlier. The type B syndrome is rarely described in childhood.⁶⁸²

GENETIC SYNDROMES WITH DIABETES AND INSULIN RESISTANCE OR INSULIN DEFICIENCY

A number of genetic syndromes are associated with DM. In children, four relatively common genetic syndromes may be associated with diabetes. In trisomy 21 (Down syndrome) and Turner syndrome (a single normal X chromosome), there is an increased incidence of autoimmune disorders, especially of the thyroid. T1DM also has a higher prevalence in patients with Down syndrome than in the general population.

In Turner syndrome, insulin secretory reserve may be limited such that treatment with growth hormone (now common) can result in impaired glucose tolerance or T2D. In Klinefelter syndrome (XXY), insulin resistance is a major feature—but autoimmune associations have been described. In Prader-Willi

syndrome, the reported high frequency of DM may not be caused simply by the insulin resistance as part of the obesity of this syndrome but possibly by a primary defect in insulin secretion.^{688–692}

Alström syndrome consists of retinal dystrophy, sensorineural deafness, obesity and associated diabetes, cardiomyopathy, hypertriglyceridemia, liver disease, and urological abnormalities. Severe insulin resistance may lead to acanthosis and diabetes. Mutations in the gene *ALMS1* have been identified in these patients.⁶⁹³ Bardet-Biedl syndrome also has atypical retinitis pigmentosa as a key feature, along with central obesity, polydactyly, mental retardation, hypogonadism, and renal dysfunction. Eleven loci have been linked to this syndrome, with abnormalities in cilia-like structures a central theme.⁶⁹⁴ The association of these and other syndromes with diabetes (listed in Box 21.1) can be ascertained by searching the Online Mendelian Inheritance in Man database (OMIM, available at www.ncbi.nlm.nih.gov/omim).

Gestational Diabetes

Gestational diabetes is a disease of the second and third trimesters of pregnancy that is caused by congenital or acquired defects in insulin secretion that result in the inability to compensate for the increased demands of insulin as a result of the counterinsulin effects of placental growth hormones.⁶⁹⁵

Neonatal Diabetes

Discoveries in the molecular basis for pancreas formation and regulation of insulin secretion have propelled the syndromes of neonatal diabetes from the backwater of rarity to the forefront of research. These syndromes probably occur more frequently than previously considered, and they may have an overall incidence of about 1:100,000 births^{696–703} in European populations. Moreover, some of those with activating mutations in

K_{ATP} channel subunits Kir 6.2 or SUR1 lend themselves to treatment with oral sulfonylurea hypoglycemic drugs, such as glibenclamide—which results in endogenous insulin secretion with near-normal glycemic control and at least partial reversal of neuromuscular manifestations.^{13,664,665,696–712} These entities are described in detail in Chapter 10.

Impaired Glucose Tolerance

The term impaired glucose tolerance is used to characterize individuals who have a plasma glucose concentration in excess of 140 mg/dL but less than 200 mg/dL at 2 hours after initiation of the standard oral glucose tolerance test, but do not have symptoms of diabetes or fasting hyperglycemia. Such a constellation may represent the earliest phase of a gene defect in insulin secretion or action or be one step in evolving DM type 1 or 2. The hyperglycemia may be a chance discovery during an intercurrent illness, during therapy with corticosteroids, or as part of a screening of close relatives of patients with defined genetic syndromes.

In those who have impaired glucose tolerance but do not have fasting hyperglycemia, repeated oral glucose tolerance tests (OGTTs) are not recommended. Investigations in such children indicate that the degree of impaired glucose tolerance tends to remain stable except in those who have markedly subnormal insulin response.^{713,714} The arbitrarily designated response that identifies impaired glucose tolerance is defined as a fasting plasma glucose value of less than 100 mg/dL and a value at 2 hours of more than 140 mg/dL. The determination of serum insulin responses during the glucose tolerance test is not a prerequisite for reaching a diagnosis. Because the magnitude of the insulin response may have prognostic value, however, some investigators request measurements of insulin during the OGTT tests.⁷¹⁴

Pancreas and Islet Transplantation

In an attempt to cure insulin-dependent diabetes, transplantation of a segment of the pancreas or of isolated islets has been increasingly performed in humans.^{715–719} These procedures are technically demanding and associated with the risks and complications of rejection and its treatment by immunosuppression. Therefore segmental pancreas transplantation is generally performed in association with transplantation of a kidney for a patient with end-stage renal disease owing to DN in whom the immunosuppressive regimen is indicated for the renal transplant.

Several thousand such transplants have been performed worldwide since the late 1980s. With experience and newer immunosuppressive agents, functional survival of the pancreatic graft may be achieved for as long as several years—during which patients may be in metabolic control with no or minimal exogenous insulin and reversal of some of the microvascular complications. Because children and adolescents with DM are not likely to have end-stage renal disease as a result of diabetes, however, pancreas transplantation as a primary treatment cannot be recommended (nor its risks justified) in children. An exception may be reimplantation of autologous islets after pancreatectomy for recurrent pancreatitis.⁷¹⁹

Attempts to transplant isolated islets have been equally challenging because of techniques to harvest sufficient islets and the issue of rejection.⁷¹⁷ Some of the newer antirejection drugs, notably cyclosporine and tacrolimus, are toxic to the islets of Langerhans—impairing insulin secretion and even causing diabetes.⁷¹⁷ The Edmonton protocol for islet transplantation avoids steroid and uses an anti-IL-2 receptor antibody and tacrolimus instead of other immunosuppressants.⁷¹⁷ Initial

success had been promising, but long-term survival of the graft beyond 3 to 5 years is unusual—and there have been serious side effects of the procedure, as well as failure to restore hypoglycemia unawareness.⁷¹⁷ Hence this islet transplantation protocol cannot be recommended in children.

Research continues to improve techniques for the yield, viability, and loss of immunogenicity of islets of Langerhans for transplantation. Transplantation has been investigated of islets coated or microencapsulated with a film of protective chemicals that permit diffusion of insulin and nutrients but prevent T-cell contact and therefore avoid rejection.⁷¹⁸ These novel approaches have been frustrated in the long term by overgrowth of fibroblasts that progressively impair glucose sensing by the islets and insulin diffusion from them. Should these technical problems be overcome, or methods to avoid rejection become established, transplantation of pancreas or islets as primary treatment for diabetes may be entertained after their risks are carefully compared with and weighed against potential benefits—especially in children.⁷¹⁹ Islet regeneration by modulating the immune response is also under scrutiny.⁷²⁰

CONCLUDING REMARKS

Progress continues to be spectacular in understanding and treating DM. We have moved from a glimmer of understanding that diabetes is a syndrome of broad categories (insulin dependent and noninsulin dependent) to an understanding of disease susceptibility and disease causality at a molecular level. T1D is acknowledged to be an autoimmune disease with major susceptibility loci in various immune regulatory genes, especially the HLA complex, although the trigger(s) for this autoimmune process remains elusive; various viruses head the list but yet to be identified environmental factors also may be involved. Recent studies suggest immunization against rotavirus as protective; this remains to be proven. Evidence for autoimmunity via the presence of circulating antibodies to islet cell components precedes clinical manifestation by months to years; treatment with anti-CD3 antibodies appears to delay clinical onset of disease in those with two antibodies but no impairment of glucose tolerance. Positional cloning and genome-wide association techniques have identified other gene markers, and we now recognize the importance of mitochondrial gene defects in some types of insulin-dependent diabetes that may not be autoimmune. The predisposition of the beta cell to autoimmune destruction (homicide) versus a predisposition to apoptosis (suicide) is the topic of intensive debate and investigators raise questions about our accepted paradigms of this disease.^{1,44,45} More spectacular has been the unraveling of the molecular basis of monogenic defects, such as MODY and neonatal diabetes and their treatment, including the use of sulfonylureas. Not surprisingly, this knowledge is being rapidly applied to predict the likelihood of disease appearance in individuals whose susceptibility can be quantified by the presence of certain antibodies and by limitations in first-phase insulin response. Population surveys in individuals not known to be at risk for diabetes have just begun. We are in the early stages of attempts to prevent the disease, reminiscent of the early trials examining the relationship between control and microvascular complications.

The monumental DCCT and European studies have irrefutably established the link between control and complications, requiring new standards of care. Progress and understanding of insulin secretion and insulin action have been equally spectacular for the insights provided in defining non-insulin-dependent forms of diabetes at physiologic, biochemical, and molecular levels. Therapy with human insulin is now standard and a plethora of long-acting, as well as fast-acting

preparations are now available. Trials with improved insulin-delivery systems, including computerized artificial pancreas, as well as pancreas and islet transplantation, are in progress and have entered the realm of clinical practice. The FDA in the United States has approved two artificial pancreas systems for use, and improvements in these devices are bound to change the course and treatment of T1DM. The beneficiaries of these advances are our patients, whose interests will continue to be best served by bidirectional scientific inquiry from bench to bedside.

REFERENCES

- Chiang JL, Maahs DM, Garvey KC, et al. Type 1 diabetes in children and adolescents. A position statement by the American Diabetes Association. *Diabetes Care*. 2018;41(9):2026–2044.
- Ize-Ludlow D, Sperling MA. The classification of diabetes mellitus: a conceptual framework. *Pediatr Clin North Am*. 2005;52(6):1533–1552.
- Pambianco G, Costacou T, Ellis D, Becker DJ, Klein R, Orchard TJ. The 30-year natural history of type 1 diabetes complications: the Pittsburgh Epidemiology of Diabetes Complications Study experience. *Diabetes*. 2006;55(5):1463–1469.
- Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*. 2001;414(6865):799–806.
- Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet (London, England)*. 2014;383(9911):69–82.
- Couper JJ, Harrison LC. Controversies in medicine: redefining the diagnosis of type 1 diabetes. *Med J Aust*. 2019;211(4):157–159.
- Hattersley A, Greeley SAW, Polak M, Rubio-Cabezas O, Njolstad PR, Mlynarski W, et al. The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. 2018;10(Suppl 27):47–63.
- American Diabetes Association. Classification and diagnosis of diabetes mellitus. *Diabetes Care*. 2018;36(Suppl 1):S13–S27.
- Wang J, Miao D, Babu S, Yu J, Barker J, Klingensmith G, et al. Prevalence of autoantibody-negative diabetes is not rare at all ages and increases with older age and obesity. *J Clin Endocrinol Metab*. 2007;92(1):88–92.
- Trucco M, Giannoukakis N. Immunoregulatory dendritic cells to prevent and reverse new-onset Type 1 diabetes mellitus. *Exp Opin Biol Ther*. 2007;7(7):951–963.
- Stark R, Roden M, ESCI Award 2006. Mitochondrial function and endocrine diseases. *Eur J Clin Invest*. 2007;37(4):236–248.
- Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell*. 2012;148(6):1145–1159.
- Flannick J, Johansson S, Njolstad PR. Common and rare forms of diabetes mellitus: towards a continuum of diabetes subtypes. *Nat Rev Endocrinol*. 2016;12(7):394–406.
- Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*. 2001;345(13):971–980.
- Kelly A, Moran A. Update on cystic fibrosis-related diabetes. *J Cystic Fibrosis*. 2013;12(4):318–331.
- Moran A, Becker D, Casella SJ, Gottlieb PA, Kirkman MS, Marshall BC, et al. Epidemiology, pathophysiology, and prognostic implications of cystic fibrosis-related diabetes: a technical review. *Diabetes Care*. 2010;33(12):2677–2683.
- Goodyer WR, Gu X, Liu Y, Bottino R, Crabtree GR, Kim SK. Neonatal beta cell development in mice and humans is regulated by calcineurin/NFAT. *Develop Cell*. 2012;23(1):21–34.
- Barlow AD, Nicholson ML, Herbert TP. Evidence for rapamycin toxicity in pancreatic beta-cells and a review of the underlying molecular mechanisms. *Diabetes*. 2013;62(8):2674–2682.
- Esposti MD, Ngo A, Myers MA. Inhibition of mitochondrial complex I may account for IDDM induced by intoxication with the rodenticide Vacor. *Diabetes*. 1996;45(11):1531–1534.
- Strosberg J, Hoffe S, Gardner N, Choi J, Kvols L. Effective treatment of locally advanced endocrine tumors of the pancreas with chemoradiotherapy. *Neuroendocrinology*. 2007;85(4):216–220.
- Nesmith JD, Ellis E. Childhood hemolytic uremic syndrome is associated with adolescent-onset diabetes mellitus. *Pediatr Nephrol (Berlin Germany)*. 2007;22(2):294–297.
- Adzick NS, DeLeon DD, States LJ, Lord K, Bhatti TR, Becker SA, Stanley C. Surgical treatment of congenital hyperinsulinism: results from 500 pancreatectomies in neonates and children. *J Pediatr Surg*. 2019;54(1):27–32.
- Bacha F, Gungor N, Lee S, Arslanian SA. Progressive deterioration of beta-cell function in obese youth with type 2 diabetes. *Pediatr Diabetes*. 2013;14(2):106–111.
- Imperatore G, Boyle JP, Thompson TJ, Case D, Dabelea D, Hamman RF, et al. Projections of type 1 and type 2 diabetes burden in the U.S. population aged <20 years through 2050: dynamic modeling of incidence, mortality, and population growth. *Diabetes Care*. 2012;35(12):2515–2520.
- Steele AM, Shields BM, Wensley KJ, Collough K, Ellard S, Hattersley AJ. Prevalence of vascular complications among patients with GCK mutations and prolonged mild hyperglycemia. *JAMA*. 2014;311(31):279–286.
- Kadowaki T, Kadowaki H, Yazaki Y. Polymorphism of the glycogen synthase gene and non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;328(21):1568–1569.
- Henriksen EJ, Dokken BB. Role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. *Curr Drug Targets*. 2006;7(11):1435–1441.
- Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M, et al. Mutations in the insulin receptor gene. *Endocr Rev*. 1992;13(3):566–595.
- Reynet C, Kahn CR. Rad: a member of the Ras family overexpressed in muscle of type II diabetic humans. *Science (New York, NY)*. 1993;262(5138):1441–1444.
- Polonsky KS, Sturis J, Bell GI. Seminars in Medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus – a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med*. 1996;334(12):777–783.
- Udler MS, Kim J, von Grothuss M, Bonas-Guarch S, Cole JB, Chio J, et al. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: a soft clustering analysis. *PLoS Med*. 2018;15(9). e1002654.
- Kahn BB, McGraw TE. Rosiglitazone. PPARgamma, and type 2 diabetes. *N Engl J Med*. 2010;363(27):2667–2669.
- Saltiel AR. Insulin resistance in the defense against obesity. *Cell Metab*. 2012;15(6):798–804.
- Owen KR, McCarthy MI. Genetics of type 2 diabetes. *Curr Opin Genet Dev*. 2007;17(3):239–244.
- Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2000;11(9):351–356.
- Duggirala R, Blangero J, Almasy L, Arya R, Dyer TD, Williams KL, et al. A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. *Am J Hum Genet*. 2001;68(5):1149–1164.
- Robertson RP, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes*. 2004;53(Suppl 1):S119–S124.
- Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007;445(7130):881–885.
- Schranz DB, Bekris L, Landin-Olsson M, Torn C, Nilang A, Toll A, et al. Newly diagnosed latent autoimmune diabetes in adults (LADA) is associated with low level glutamate decarboxylase (GAD65) and IA-2 autoantibodies. Diabetes Incidence Study in Sweden (DISS). *Horm Metab Res*. 2000;32(4):133–138.
- Libman IM, Becker DJ. Coexistence of type 1 and type 2 diabetes mellitus: “double” diabetes? *Pediatr Diabetes*. 2003;4(2):110–113.
- Florez JC. Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. *J Clin Endocrinol Metab*. 2008;93(12):4633–4642.
- Harjutsalo V, Sund R, Knip M, Groop PH. Incidence of type 1 diabetes in Finland. *JAMA*. 2013;310(4):427–428.
- Forlenza GP, Rewers M. The epidemic of type 1 diabetes: what is it telling us? *Curr Opin Endocrinol Diabetes Obes*. 2011;18(4):248–251.
- Soleimanpour SA, Stoffers DA. The pancreatic beta cell and type 1 diabetes: innocent bystander or active participant? *Trends Endocrinol Metab*. 2013;24(7):324–331.
- Tuomilehto J. The emerging global epidemic of type 1 diabetes. *Curr Diabetes Rep*. 2013;13(6):795–804.

46. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA*. 2013;309(23):2473–2479.
47. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. SEARCH for Diabetes in Youth Study. Incidence trends of type 1 and type 2 diabetes among youths, 2002–2012. *N Engl J Med*. 2017;376(15):1419–1429.
48. Haynes A, Bulsara MK, Bower C, Jones TW, Davis EA. Regular peaks and troughs in the Australian incidence of childhood type 1 diabetes mellitus (2000–2011). *Diabetologia*. 2015;58:2513–2516.
49. Skrivvarhaug T, Stene LC, Drivvoll AK, Strøm H, Joner G. Incidence of type 1 diabetes in Norway among children aged 0–14 years between 1989 and 2012: has the incidence stopped rising? Results from the Norwegian Childhood Diabetes Registry. *Diabetologia*. 2014;57:57–62.
50. Libman IM, LaPorte RE, Becker D, Dorman JS, Drash AL, Kuller L. Was there an epidemic of diabetes in nonwhite adolescents in Allegheny County, Pennsylvania? *Diabetes Care*. 1998;21(8):1278–1281.
51. Pinhas-Hamiel O, Zeitler P. Acute and chronic complications of type 2 diabetes mellitus in children and adolescents. *Lancet (London, England)*. 2007;369(9575):1823–1831.
52. Hathout EH, Sharkey J, Racine M, Thomas W, Nahab F, El-Shahawy M, et al. Diabetic autoimmunity in infants and pre-schoolers with type 1 diabetes. *Pediatr Diabetes*. 2000;1(3):131–134.
53. Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y. A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. Osaka IDDM Study Group. *N Engl J Med*. 2000;342(5):301–307.
54. Urakami T, Inami I, Morimoto S, Kubota S, Owada M. Clinical characteristics of non-immune-mediated, idiopathic type 1 (type 1B) diabetes mellitus in Japanese children and adolescents. *J Pediatr Endocrinol Metab*. 2002;15(3):283–288.
55. Knip M, Veijola R, Virtanen SM, Hyoty H, Vaarala O, Akerblom HK. Environmental triggers and determinants of type 1 diabetes. *Diabetes*. 2005;54(Suppl 2):S125–S136.
56. Menser MA, Forrest JM, Bransby RD. Rubella infection and diabetes mellitus. *Lancet (London, England)*. 1978;1(8055):57–60.
57. Ou D, Mitchell LA, Metzger DL, Gillam S, Tingle AJ. Cross-reactive rubella virus and glutamic acid decarboxylase (65 and 67) protein determinants recognised by T cells of patients with Type 1 diabetes mellitus. *Diabetologia*. 2000;43(6):750–762.
58. Laitinen OH, Honkanen H, Pakkanen O, Oikarinen S, Hankaniemi MM, Huhtala H, et al. Coxsackievirus B1 is associated with induction of beta-cell autoimmunity that portends type 1 diabetes. *Diabetes*. 2014;63(2):446–455.
59. Elding Larsson H, Vehik K, Gesualdo P, Akolkar B, Hagopian W, Krischer J, et al. Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. *Pediatr Diabetes*. 2014;15(2):118–126.
60. Hiemstra HS, Schloot NC, van Veelen PA, Willemens SJ, Franken KL, van Rood JJ, et al. Cytomegalovirus in autoimmunity: T cell cross-reactivity to viral antigen and autoantigen glutamic acid decarboxylase. *Proc Natl Acad Sci USA*. 2001;98(7):3988–3991.
61. Craig ME, Nair S, Stein H, Rawlinson WD. Viruses and type 1 diabetes: a new look at an old story. *Pediatr Diabetes*. 2013;14(3):149–158.
62. Krogvold L, Edwin B, Buanes T, et al. A low grade enteroviral infection in the islets of Langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes*. 2015;64(5):1682–1687.
63. Richardson HJ, Rodriguez-Calvo T, Gerling IC, et al. Islet cell hyper expression of HLA Class I antigens: a defining feature in Type 1 diabetes. *Diabetologia*. 2016;59(11):2448–2458.
64. Eisenbarth GS. Banting Lecture 2009: An unfinished journey: molecular pathogenesis to prevention of type 1A diabetes. *Diabetes*. 2010;59(4):759–774.
65. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet*. 2009;41(6):703–707.
66. Pietropaolo M, Towns R, Eisenbarth GS. Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes. *Cold Spring Harbor Perspect Med*. 2012;2(10).
67. Sosenko JM, Skyler JS, Palmer JP, Krischer JP, Yu L, Mahon J, et al. The prediction of type 1 diabetes by multiple autoantibody levels and their incorporation into an autoantibody risk score in relatives of type 1 diabetic patients. *Diabetes Care*. 2013;36(9):2615–2620.
68. Daneman D. Type 1 diabetes. *Lancet (London, England)*. 2006;367(9513):847–858.
69. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*. 2010;464(7293):1293–1300.
70. Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, et al. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes*. 2005;54(10):2995–3001.
71. Nejentsev S, Smink LJ, Smyth D, Bailey R, Lowe CE, Payne F, et al. Sequencing and association analysis of the type 1 diabetes-linked region on chromosome 10p12–q11. *BMC Genet*. 2007;8:24.
72. Payne F, Cooper JD, Walker NM, Lam AC, Smink LJ, Nutland S, et al. Interaction analysis of the CBLB and CTLA4 genes in type 1 diabetes. *J Leukocyte Biol*. 2007;81(3):581–583.
73. Taniguchi H, Lowe CE, Cooper JD, Smyth DJ, Bailey R, Nutland S, et al. Discovery, linkage disequilibrium and association analyses of polymorphisms of the immune complement inhibitor, decay-accelerating factor gene (DAF/CD55) in type 1 diabetes. *BMC Genet*. 2006;7:22.
74. Todd JA. Statistical false positive or true disease pathway? *Nat Genet*. 2006;38(7):731–733.
75. Dorman JS, LaPorte RE, Stone RA, Trucco M. Worldwide differences in the incidence of type 1 diabetes are associated with amino acid variation at position 57 of the HLA-DQ beta chain. *Proc Natl Acad Sci USA*. 1990;87(19):7370–7374.
76. Allen C, Palta M, D'Alessio DJ. Risk of diabetes in siblings and other relatives of IDDM subjects. *Diabetes*. 1991;40(7):831–836.
77. Hu X, Deutsch AJ, Lenz T, et al. Additive and interaction effects of three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. *Nat Genet*. 2015;47(8):898–905.
78. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes*. 2018;19(3):346–353.
79. Redondo M, Geyer S, Steck AK, et al. A type 1 diabetes genetic risk score predicts progression of islet autoimmunity and development of type 1 diabetes in individuals at risk. *Diabetes Care*. 2018;41(9):1887–1894.
80. Paul DS, Teschendorff AE, Dang MA, et al. Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. *Nat Comm*. 2016;7:13555.
81. Conrad B, Trucco M. Superantigens as etiopathogenetic factors in the development of insulin-dependent diabetes mellitus. *Diabetes Metab Rev*. 1994;10(4):309–338.
82. Symon DN, Hennessy ER, Smail PJ. Smoked foods in the diets of mothers of diabetic children. *Lancet (London, England)*. 1984;2(8401):514.
83. Eisenbarth GS. Prevention of type 1A diabetes mellitus. *Endocr Pract*. 2012;18(5):745–749.
84. Skyler JS, Ricordi C. Stopping type 1 diabetes: attempts to prevent or cure type 1 diabetes in man. *Diabetes*. 2011;60(1):1–8.
85. Thomas HR, Gitelman SE. Altering the course of type 1 diabetes: an update on prevention and new-onset clinical trials. *Pediatr Diabetes*. 2013;14(5):311–321.
86. Atkinson MA, Bowman MA, Kao KJ, Campbell L, Dush PJ, Shah SC, et al. Lack of immune responsiveness to bovine serum albumin in insulin-dependent diabetes. *N Engl J Med*. 1993;329(25):1853–1858.
87. Lamb MM, Simpson MD, Seifert J, Scott FW, Rewers M, Norris JM. The association between IgG4 antibodies to dietary factors, islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young. *PLoS One*. 2013;8(2):e57936.
88. Knip M, Virtanen S, Becker D, Dupre J, Krischer J, Akerblom H, et al. (2011). Early feeding and risk of type 1 diabetes: experiences from the Trial to Reduce Insulin-dependent diabetes mellitus in the Genetically at Risk (TRIGR). *Am J Clin Nutr*, 94 (6 Suppl):1814s–1820s.
89. Frederiksen B, Kroehl M, Lamb MM, Seifert J, Barriga K, Eisenbarth GS, et al. Infant exposures and development of type 1 diabetes mellitus: The Diabetes Autoimmunity Study in the Young (DAISY). *JAMA Pediatr*. 2013;167(9):808–815.

90. Writing group for the TRIGR Study Group, K, Akerblom HK, Al Taji E, et al. Effect of hydrolyzed infant formula vs conventional formula on risk of type 1 diabetes: the TRIGR randomized clinical trial. *JAMA*. 2018;319(1):38–48.
91. Hathout EH, Beeson WL, Ischander M, Rao R, Mace JW. Air pollution and type 1 diabetes in children. *Pediatr Diabetes*. 2006;7(2):81–87.
92. Skyler JS. Primary and secondary prevention of Type 1 diabetes. *Diabetic Med*. 2013;30(2):161–169.
93. Campbell-Thompson M, Wasserfall C, Kaddis J, Albanese-O'Neill A, Staeva T, Nierras C, et al. Network for Pancreatic Organ Donors with Diabetes (nPOD): developing a tissue biobank for type 1 diabetes. *Diabetes Metab Res Rev*. 2012;28(7):608–617.
94. Verge CF, Gianani R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, et al. Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes*. 1996;45(7):926–933.
95. Diabetes Prevention Trial—Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med*. 2002;346(22):1685–1691.
96. Gale EA. Can we change the course of beta-cell destruction in type 1 diabetes? *N Engl J Med*. 2002;346(22):1740–1742.
97. Gale EA, Bingley PJ, Emmett CL, Collier T. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet (London, England)*. 2004;363(9413):925–931.
98. Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, Teplizumab, in relatives at risk for type 1 diabetes. *N Engl J Med*. 2019;381(7):603–613.
99. Haller MJ, Long SA, Blanchfield JL, et al. Low-dose anti-thymocyte globulin preserves C-peptide, reduces HbA1c, and increases regulatory to conventional T-cell ratios in new-onset Type 1 diabetes. *Diabetes*. 2019;68(6):1267–1276.
100. Michels A, Zhang L, Khadra A, et al. Prediction and prevention of type 1 diabetes: update on success of prediction and struggles at prevention. *Pediatr Diabetes*. 2015;16(7):465–484.
101. Barker JM, McFann KK, Orban T. Effect of oral insulin on insulin autoantibody levels in the Diabetes Prevention Trial Type 1 oral insulin study. *Diabetologia*. 2007;50(8):1603–1606.
102. Rewers M, Hyöty H, Lernmark A, et al. The Environmental Determinants of Diabetes in the Young (TEDDY) Study: 2018 Update. *Curr Diabetes Rep*. 2018;18(12):136.
103. Jacobsen LM, Newby BN, Perry DJ, et al. Immune mechanisms and pathways targeted in type 1 diabetes. *Curr Diabetes Rep*. 2018;18(10):90.
104. Perrett KB, Jachno K, Nolan TM, et al. Association of ROTA virus vaccination with the incidence of Type 1 diabetes in children. *JAMA Pediatr*. 2019;173(3):280–282.
105. Rogers MAM, Basu T, Kim C. Lower incidence rate of type 1 diabetes after receipt of the rotavirus vaccine in the United States 2001–2017. *Sci Rep*. 2019;9:7727.
106. Tager HS. Lilly lecture 1983. Abnormal products of the human insulin gene. *Diabetes*. 1984;33(7):693–699s.
107. O'Rahilly S, Gray H, Humphreys PJ, Krook A, Polonsky KS, White A, et al. Brief report: impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. *N Engl J Med*. 1995;333(21):1386–1390.
108. Gabbay KH. The insulinopathies. *N Engl J Med*. 1980;302(3):165–167.
109. Steiner DF, Tager HS, Chan SJ, Nanjo K, Sanke T, Rubenstein AH. Lessons learned from molecular biology of insulin-gene mutations. *Diabetes Care*. 1990;13(6):600–609.
110. Steiner DF. The proinsulin C-peptide—a multirole model. *Exp Diabetes Res*. 2004;5(1):7–14.
111. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. 1997;16(3):303–306.
112. Wardlaw SL. Clinical review 127: Obesity as a neuroendocrine disease: lessons to be learned from proopiomelanocortin and melanocortin receptor mutations in mice and men. *J Clin Endocrinol Metab*. 2001;86(4):1442–1446.
113. Frank GR, Fox J, Candela N, et al. Severe obesity and diabetes insipidus in a patient with PCSK1 deficiency. *Mol Genet Metab*. 2013;110(1–2):191–194.
114. Nanjo K, Miyano M, Kondo M, Sanke T, Nishimura S, Miyamura K, et al. Insulin Wakayama: familial mutant insulin syndrome in Japan. *Diabetologia*. 1987;30(2):87–92.
115. Felig P, Wahren J, Sherwin R, Hendler R. Insulin, glucagon, and somatostatin in normal physiology and diabetes mellitus. *Diabetes*. 1976;25(12):1091–1099.
116. Unger RH, Orci L. Glucagon and the A cell: physiology and pathophysiology (second of two parts). *N Engl J Med*. 1981;304(26):1575–1580.
117. Woods SC, Porte Jr D. Neural control of the endocrine pancreas. *Physiol Rev*. 1974;54(3):596–619.
118. Ashcroft, F.M., Rorsman, P. K(ATP) channels and islet hormone secretion: new insights and controversies. *Nat Rev Endocrinol*. 9(11):660–669.
119. Chakera AJ, Steele AM, Gloyn AL, et al. Recognition and management of individuals with hyperglycemia because of a heterozygous glucokinase mutation. *Diabetes Care*. 2015;38(7):1383–1392.
120. Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JPt, Boyd 3rd AE, Gonzalez G, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science (New York, NY)*. 1995;268(5209):423–426.
121. Philipson LH, Steiner DF. Pas de deux or more: the sulfonylurea receptor and K⁺ channels. *Science (New York, NY)*. 1995;268(5209):372–373.
122. Thomas PM, Cote GJ, Wohlk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science (New York, NY)*. 1995;268(5209):426–429.
123. Liang Y, Matschinsky FM. Mechanisms of action of nonglucose insulin secretagogues. *Ann Rev Nutr*. 1994;14:59–81.
124. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab*. 2013;17(6):819–837.
125. Nauck MA, Meier JJ. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. *Lancet Diabetes Endocrinol*. 2016;4(6):525–536.
126. Porte Jr D, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes*. 2005;54(5):1264–1276.
127. Cheatham B, Kahn CR. Insulin action and the insulin signaling network. *Endocr Rev*. 1995;16(2):117–142.
128. Kido Y, Nakae J, Accili D. Clinical review 125: the insulin receptor and its cellular targets. *J Clin Endocrinol Metab*. 2001;86(3):972–979.
129. Bevan P. Insulin signalling. *J Cell Sci*. 2001;114(Pt 8):1429–1430.
130. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*. 2006;7(2):85–96.
131. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science (New York, NY)*. 2000;289(5487):2122–2125.
132. Belfiore A, Malaguarnera R, Vella V, et al. Insulin receptor isoforms in physiology and disease: an updated view. *Endo Rev*. 2017;38(5):379–431.
133. Haeusler RA, McGraw TE, Accili D. Biochemical and Cellular properties of Insulin Receptor Signaling. *Nat Rev Mol Cell Biol*. 2018;19:31–44.
134. Biddinger SB, Kahn CR. From mice to men: insights into the insulin resistance syndromes. *Annu Rev Physiol*. 2006;68:123–158.
135. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol*. 1981;240(6):E630–E639.
136. Schade DS, Eaton RP. The temporal relationship between endogenously secreted stress hormones and metabolic decompensation in diabetic man. *J Clin Endocrinol Metab*. 1980;50(1):131–136.
137. Wolfsdorf J, Glaser N, Sperling MA. Diabetic ketoacidosis in infants, children, and adolescents: A consensus statement from the American Diabetes Association. *Diabetes Care*. 2006;29(5):1150–1159.
138. Wolfsdorf J, Craig ME, Daneman D, Dunger D, Edge J, Lee W, et al. Diabetic ketoacidosis in children and adolescents with diabetes. *Pediatr Diabetes*. 2009;10(Suppl 12):118–133.

139. Foster DW, McGarry JD. The metabolic derangements and treatment of diabetic ketoacidosis. *N Engl J Med.* 1983;309(3):159–169.
140. Glaser N, Barnett P, McCaslin I, Nelson D, Trainor J, Louie J, et al. Risk factors for cerebral edema in children with diabetic ketoacidosis. The Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. *N Engl J Med.* 2001;344(4):264–269.
141. Glaser N. New perspectives on the pathogenesis of cerebral edema complicating diabetic ketoacidosis in children. *Pediatr Endocrinol Rev.* 2006;3(4):379–386.
142. Sperling MA. Cerebral edema in diabetic ketoacidosis: an underestimated complication? *Pediatr Diabetes.* 2006;7(2):73–74.
143. White PC, Dickson BA. Low morbidity and mortality in children with diabetic ketoacidosis treated with isotonic fluids. *J Pediatr.* 2013;163(3):761–766.
144. Decourcey DD, Steil GM, Wypij D, Agus MS. Increasing use of hypertonic saline over mannitol in the treatment of symptomatic cerebral edema in pediatric diabetic ketoacidosis: an 11-year retrospective analysis of mortality*. *Pediatr Crit Care Med.* 2013;14(7):694–700.
145. Zeitler P, Haqq A, Rosenbloom A, Glaser N. Hyperglycemic hyperosmolar syndrome in children: pathophysiological considerations and suggested guidelines for treatment. *J Pediatr.* 2011;158(1):9–14. e1–2.
146. Kitabchi AE, Umpierrez GE, Murphy MB, et al. Management of hyperglycemic crises in patients with diabetes. *Diabetes Care.* 2001;24(1):131–153.
147. Wolfsdorf JL, Glaser N, Agus M, Fritsch M, Hanas R, Rewers A, et al. ISPAD Clinical Consensus Guidelines 2018: diabetic ketoacidosis and the hyperglycemic hyperosmolar state. *Pediatr Diabetes.* 2018;19(Suppl 27):155–177.
148. Palmer BF, Clegg DJ. Electrolyte and Acid-Base Disturbances in Patients with Diabetes Mellitus. *N Engl J Med.* 2015;373(6):548–559.
149. Kamel KS, Halperin ML. Acid-base problems in diabetic ketoacidosis. *N Engl J Med.* 2015;372(6):546–554.
150. Hanas R, Lindgren F, Lindblad B. A 2-yr national population study of pediatric ketoacidosis in Sweden: predisposing conditions and insulin pump use. *Pediatr Diabetes.* 2009;10(1):33–37.
151. Dabelea D, Mayer-Davis EJ, Saydah S, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA.* 2014;311(17):1778–1786.
152. Dunger DB, Sperling MA, Acerini CL, et al. ESPE/LWPES consensus statement on diabetic ketoacidosis in children and adolescents. *Arch Dis Child.* 2004;89(2):188–194.
153. Sheikh-Ali M, Karon BS, Basu A, et al. Can serum beta-hydroxybutyrate be used to diagnose diabetic ketoacidosis? *Diabetes Care.* 2008;31(4):643–647.
154. Burge MR, Hardy KJ, Schade DS. Short-term fasting is a mechanism for the development of euglycemic ketoacidosis during periods of insulin deficiency. *J Clin Endocrinol Metab.* 1993;76(5):1192–1198.
155. Bas VN, Uytun S, Torun YA. Diabetic euglycemic ketoacidosis in newly diagnosed type 1 diabetes mellitus during Ramadan fasting. *J Pediatr Endocrinol Metab.* 2015;28(3-4):333–335.
156. Peters AL, Buschur EO, Buse JB, Cohan P, Diner JC, Hirsch IB. Euglycemic diabetic ketoacidosis: a potential complication of treatment with sodium-glucose cotransporter 2 inhibition. *Diabetes Care.* 2015;38(9):1687–1693.
157. Goldenberg RM, Berard LD, Cheng AY, et al. SGLT2 Inhibitor-associated diabetic ketoacidosis: clinical review and recommendations for prevention and diagnosis. *Clin Ther.* 2016;38(12):2654–2664. e2651.
158. Misaghian-Xanthos N, Shariff AI, Mekala K, et al. Sodium-glucose cotransporter 2 inhibitors and diabetic ketoacidosis: a case series from three academic institutions. *Diabetes Care.* 2017;40(6):e65–e66.
159. Chase HP, Garg SK, Jelley DH. Diabetic ketoacidosis in children and the role of outpatient management. *Pediatr Rev.* 1990;11(10):297–304.
160. Levy-Marchal C, Papoz L, de Beaufort C, et al. Clinical and laboratory features of type 1 diabetic children at the time of diagnosis. *Diabet Med.* 1992;9(3):279–284.
161. Komulainen J, Lounamaa R, Knip M, Kaprio EA, Akerblom HK. Ketoacidosis at the diagnosis of type 1 (insulin dependent) diabetes mellitus is related to poor residual beta cell function. Childhood Diabetes in Finland Study Group. *Arch Dis Child.* 1996;75(5):410–415.
162. Levy-Marchal C, Patterson CC, Green A. Geographical variation of presentation at diagnosis of type 1 diabetes in children: the EURO-DIAB study. European and Diabetes. *Diabetologia.* 2001;44(Suppl 3):B75–80.
163. Hanas R, Lindgren F, Lindblad B. Diabetic ketoacidosis and cerebral oedema in Sweden—a 2-year paediatric population study. *Diabetes Med.* 2007;24(10):1080–1085.
164. Rodacki M, Pereira JR, Nabuco de Oliveira AM, et al. Ethnicity and young age influence the frequency of diabetic ketoacidosis at the onset of type 1 diabetes. *Diabetes Res Clin Pract.* 2007;78(2):259–262.
165. Roche EF, Menon A, Gill D, Hoey H. Clinical presentation of type 1 diabetes. *Pediatr Diabetes.* 2005;6(2):75–78.
166. Usher-Smith JA, Thompson M, Ercole A, Walter FM. Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in children: a systematic review. *Diabetologia.* 2012;55(11):2878–2894.
167. Fritsch M, Schober E, Rami-Merhar B, Hofer S, Frohlich-Reiterer E, Waldhoer T. Diabetic ketoacidosis at diagnosis in Austrian children: a population-based analysis, 1989–2011. *J Pediatr.* 2013;163(5):1484–1488.
168. Zucchini S, Scaramuzza AE, Bonfanti R, et al. A multicenter retrospective survey regarding diabetic ketoacidosis management in Italian children with type 1 diabetes. *J Diabetes Res.* 2016;2016:5719470.
169. Neu A, Hofer SE, Karges B, Oeverink R, Rosenbauer J, Holl RW. Ketoacidosis at diabetes onset is still frequent in children and adolescents: a multicenter analysis of 14,664 patients from 106 institutions. *Diabetes Care.* 2009;32(9):1647–1648.
170. King BR, Howard NJ, Verge CF, et al. A diabetes awareness campaign prevents diabetic ketoacidosis in children at their initial presentation with type 1 diabetes. *Pediatr Diabetes.* 2012;13(8):647–651.
171. Gibb FW, Teoh WL, Graham J, Lockman KA. Risk of death following admission to a UK hospital with diabetic ketoacidosis. *Diabetologia.* 2016;59(10):2082–2087.
172. Quinn M, Fleischman A, Rosner B, Nigrin DJ, Wolfsdorf JL. Characteristics at diagnosis of type 1 diabetes in children younger than 6 years. *J Pediatr.* 2006;148(3):366–371.
173. Letourneau LR, Carmody D, Wroblewski K, et al. Diabetes presentation in infancy: high risk of diabetic ketoacidosis. *Diabetes Care.* 2017;40(10):e147–e148.
174. Bui H, To T, Stein R, Fung K, Daneman D. Is diabetic ketoacidosis at disease onset a result of missed diagnosis? *J Pediatr.* 2010;156(3):472–477.
175. Szypowska A, Skorka A. The risk factors of ketoacidosis in children with newly diagnosed type 1 diabetes mellitus. *Pediatr Diabetes.* 2011;12(4 Pt 1):302–306.
176. Pinkney JH, Bingley PJ, Sawtell PA, Dunger DB, Gale EA. Presentation and progress of childhood diabetes mellitus: a prospective population-based study. The Bart's-Oxford Study Group. *Diabetologia.* 1994;37(1):70–74.
177. Rewers A, Klingensmith G, Davis C, et al. Presence of diabetic ketoacidosis at diagnosis of diabetes mellitus in youth: the Search for Diabetes in Youth Study. *Pediatrics.* 2008;121(5):e1258–e1266.
178. Usher-Smith JA, Thompson MJ, Sharp SJ, Walter FM. Factors associated with the presence of diabetic ketoacidosis at diagnosis of diabetes in children and young adults: a systematic review. *BMJ.* 2011;343:d4092.
179. Rosilio M, Cotton JB, Wieliczko MC, et al. Factors associated with glycemic control. A cross-sectional nationwide study in 2,579 French children with type 1 diabetes. The French Pediatric Diabetes Group [see comments]. *Diabetes Care.* 1998;21(7):1146–1153.
180. Smith CP, Firth D, Bennett S, Howard C, Chisholm P. Ketoacidosis occurring in newly diagnosed and established diabetic children. *Acta Paediatr.* 1998;87(5):537–541.
181. Rewers A, Chase HP, Mackenzie T, et al. Predictors of acute complications in children with type 1 diabetes. *JAMA.* 2002;287(19):2511–2518.

182. Cengiz E, Xing D, Wong JC, et al. Severe hypoglycemia and diabetic ketoacidosis among youth with type 1 diabetes in the T1D Exchange clinic registry. *Pediatr Diabetes*. 2013;14(6):447–454.
183. Maahs DM, Hermann JM, Holman N, et al. Rates of diabetic ketoacidosis: international comparison with 49,859 pediatric patients with type 1 diabetes from England, Wales, the U.S., Austria, and Germany. *Diabetes Care*. 2015;38(10):1876–1882.
184. Morris AD, Boyle DI, McMahon AD, Greene SA, MacDonald TM, Newton RW. Adherence to insulin treatment, glycaemic control, and ketoacidosis in insulin-dependent diabetes mellitus. The DARTS/MEMO Collaboration. Diabetes Audit and Research in Tayside Scotland. Medicines Monitoring Unit. *Lancet*. 1997;350(9090):1505–1510.
185. Hermann JM, Meusers M, Bachran R, et al. Self-reported regular alcohol consumption in adolescents and emerging adults with type 1 diabetes: A neglected risk factor for diabetic ketoacidosis? Multicenter analysis of 29 630 patients from the DPV registry. *Pediatr Diabetes*. 2017;18(8). 917–823.
186. Kitabchi AE, Nyenwe EA. Hyperglycemic crises in diabetes mellitus: diabetic ketoacidosis and hyperglycemic hyperosmolar state. *Endocrinol Metab Clin North Am*. 2006;35(4):725–751.
187. Rosenbloom AL. Hyperglycemic hyperosmolar state: an emerging pediatric problem. *J Pediatr*. 2010;156(2):180–184.
188. Fournier SH, Weinzimer SA, Levitt Katz LE. Hyperglycemic hyperosmolar non-ketotic syndrome in children with type 2 diabetes. *Pediatr Diabetes*. 2005;6(3):129–135.
189. Canarie MF, Bogue CW, Banasiak KJ, Weinzimer SA, Tamborlane WV. Decompensated hyperglycemic hyperosmolarity without significant ketoacidosis in the adolescent and young adult population. *J Pediatr Endocrinol Metab*. 2007;20(10):1115–1124.
190. Klingensmith GJ, Connor CG, Ruedy KJ, et al. Presentation of youth with type 2 diabetes in the Pediatric Diabetes Consortium. *Pediatr Diabetes*. 2016;17(4):266–273.
191. McDonnell CM, Pedreira CC, Vadmalayan B, Cameron FJ, Werther GA. Diabetic ketoacidosis, hyperosmolarity and hypernatremia: are high-carbohydrate drinks worsening initial presentation? *Pediatr Diabetes*. 2005;6(2):90–94.
192. Kleinman ME, Chameides L, Schexnayder SM, et al. Pediatric advanced life support: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Pediatrics*. 2010;126(5). e1361–1399.
193. Kleinman ME, de Caen AR, Chameides L, et al. Pediatric basic and advanced life support: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations. *Pediatrics*. 2010;126(5). e1261–1318.
194. Koves IH, Neutze J, Donath S, et al. The accuracy of clinical assessment of dehydration during diabetic ketoacidosis in childhood. *Diabetes Care*. 2004;27(10):2485–2487.
195. Ugale J, Mata A, Meert KL, Sarnaik AP. Measured degree of dehydration in children and adolescents with type 1 diabetic ketoacidosis. *Pediatr Crit Care Med*. 2012;13(2):e103–e107.
196. Sottosanti M, Morrison GC, Singh RN, et al. Dehydration in children with diabetic ketoacidosis: a prospective study. *Arch Dis Child*. 2012;97(2):96–100.
197. Steiner MJ, DeWalt DA, Byerley JS. Is this child dehydrated? *JAMA*. 2004;291(22):2746–2754.
198. Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet*. 1974;2(7872):81–84.
199. Tasker RC, Lutman D, Peters MJ. Hyperventilation in severe diabetic ketoacidosis. *Pediatr Crit Care Med*. 2005;6(4):405–411.
200. Malone JI, Brodsky SJ. The value of electrocardiogram monitoring in diabetic ketoacidosis. *Diabetes Care*. 1980;3(4):543–547.
201. Soler NG, Bennett MA, Fitzgerald MG, Malins JM. Electrocardiogram as a guide to potassium replacement in diabetic ketoacidosis. *Diabetes*. 1974;23(7):610–615.
202. Flood RG, Chiang VW. Rate and prediction of infection in children with diabetic ketoacidosis. *Am J Emerg Med*. 2001;19(4):270–273.
203. Monroe KW, King W, Atchison JA. Use of PRISM scores in triage of pediatric patients with diabetic ketoacidosis. *Am J Manag Care*. 1997;3(2):253–258.
204. Vanelli M, Chiari G, Capuano C. Cost effectiveness of the direct measurement of 3-beta-hydroxybutyrate in the management of diabetic ketoacidosis in children. *Diabetes Care*. 2003;26(3):959.
205. Ham MR, Okada P, White PC. Bedside ketone determination in diabetic children with hyperglycemia and ketosis in the acute care setting. *Pediatr Diabetes*. 2004;5(1):39–43.
206. Rewers A, McFann K, Chase HP. Bedside monitoring of blood beta-hydroxybutyrate levels in the management of diabetic ketoacidosis in children. *Diabetes Technol Ther*. 2006;8(6):671–676.
207. Prisco F, Picardi A, Iafusco D, et al. Blood ketone bodies in patients with recent-onset type 1 diabetes (a multicenter study). *Pediatr Diabetes*. 2006;7(4):223–228.
208. Noyes KJ, Crofton P, Bath LE, et al. Hydroxybutyrate near-patient testing to evaluate a new end-point for intravenous insulin therapy in the treatment of diabetic ketoacidosis in children. *Pediatr Diabetes*. 2007;8(3):150–156.
209. Yu HY, Agus M, Kellogg MD. Clinical utility of Abbott Precision Xceed Pro(R) ketone meter in diabetic patients. *Pediatr Diabetes*. 2011;12(7):649–655.
210. Halperin ML, Goldstein MB. *Fluid, Electrolyte, and Acid-Base Physiology*. 3rd ed. Philadelphia: Saunders; 1999.
211. Atchley D, Loeb R, Richards Jr D, Benedict E, Driscoll M. On diabetic ketoacidosis: A detailed study of electrolyte balances following the withdrawal and reestablishment of insulin therapy. *J Clin Invest*. 1933;12:297–326.
212. Nabarro J, Spencer A, Stowers J. Metabolic studies in severe diabetic ketosis. *Q J Med*. 1952;82:225–248.
213. Brown TB. Cerebral oedema in childhood diabetic ketoacidosis: is treatment a factor? *Emerg Med J*. 2004;21(2):141–144.
214. Glaser NS, Ghetti S, Casper TC, Dean JM, Kuppermann N. Pediatric diabetic ketoacidosis, fluid therapy, and cerebral injury: the design of a factorial randomized controlled trial. *Pediatr Diabetes*. 2013;14:435–446.
215. Edge JA, Jakes RW, Roy Y, et al. The UK case-control study of cerebral oedema complicating diabetic ketoacidosis in children. *Diabetologia*. 2006;2049(9):2002–2009.
216. Kuppermann N, Ghetti S, Schunk JE, et al. Clinical trial of fluid infusion rates for pediatric diabetic ketoacidosis. *N Engl J Med*. 2018;378(24):2275–2287.
217. Mel JM, Werther GA. Incidence and outcome of diabetic cerebral edema in childhood: are there predictors? *J Paediatr Child Health*. 1995;31(1):17–20.
218. Harris GD, Fiordalisi I, Harris WL, Mosovich LL, Finberg L. Minimizing the risk of brain herniation during treatment of diabetic ketoacidemia: a retrospective and prospective study. *J Pediatr*. 1990;117:22–31.
219. Harris GD, Fiordalisi I. Physiologic management of diabetic ketoacidemia. A 5-year prospective pediatric experience in 231 episodes. *Arch Pediatr Adolesc Med*. 1994;148(10):1046–1052.
220. Toledo JD, Modesto V, Peinador M, et al. Sodium concentration in rehydration fluids for children with ketoacidotic diabetes: effect on serum sodium concentration. *J Pediatr*. 2009;154:895–900.
221. White PC, Dickson BA. Low morbidity and mortality in children with diabetic ketoacidosis treated with isotonic fluids. *J Pediatr*. 2013;163(3):761–766.
222. Yung M, Letton G, Keeley S. Controlled trial of Hartmann's solution versus 0.9% saline for diabetic ketoacidosis. *J Paediatr Child Health*. 2017;53(1):12–17.
223. Fiordalisi I, Novotny WE, Holbert D, Finberg L, Harris GD. An 18-yr prospective study of pediatric diabetic ketoacidosis: an approach to minimizing the risk of brain herniation during treatment. *Pediatr Diabetes*. 2007;8(3):142–149.
224. Halperin ML, Maccari C, Kamel KS, Carlotti AP, Bohn D. Strategies to diminish the danger of cerebral edema in a pediatric patient presenting with diabetic ketoacidosis. *Pediatr Diabetes*. 2006;7(4):191–195.
225. Hoom EJ, Carlotti AP, Costa LA, et al. Preventing a drop in effective plasma osmolality to minimize the likelihood of cerebral edema during treatment of children with diabetic ketoacidosis. *J Pediatr*. 2007;150(5):467–473.
226. Duck SC, Wyatt DT. Factors associated with brain herniation in the treatment of diabetic ketoacidosis. *J Pediatr*. 1988;113:10–14.
227. Adroque HJ, Wilson H, Boyd 3rd AE, Suki WN, Eknayan G. Plasma acid-base patterns in diabetic ketoacidosis. *N Engl J Med*. 1982;307(26):1603–1610.
228. Adroque HJ, Eknayan G, Suki WK. Diabetic ketoacidosis: role of the kidney in the acid-base homeostasis re-evaluated. *Kidney Int*. 1984;25(4):591–598.

229. Taylor D, Durward A, Tibby SM, et al. The influence of hyperchloremia on acid base interpretation in diabetic ketoacidosis. *Intens Care Med.* 2006;32(2):295–301.
230. Durward A, Skellett S, Mayer A, Taylor D, Tibby SM, Murdoch IA. The value of the chloride: sodium ratio in differentiating the aetiology of metabolic acidosis. *Intens Care Med.* 2001;27(5):828–835.
231. Oh MS, Carroll HJ, Goldstein DA, Fein IA. Hyperchloremic acidosis during the recovery phase of diabetic ketosis. *Ann Intern Med.* 1978;89(6):925–927.
232. Oh MS, Banerji MA, Carroll HJ. The mechanism of hyperchloremic acidosis during the recovery phase of diabetic ketoacidosis. *Diabetes.* 1981;30(4):310–313.
233. Oh MS, Carroll HJ, Uribarri J. Mechanism of normochloremic and hyperchloremic acidosis in diabetic ketoacidosis. *Nephron.* 1990;54(1):1–6.
234. Basnet S, Venepalli PK, Andoh J, Verhulst S, Koirala J. Effect of normal saline and half normal saline on serum electrolytes during recovery phase of diabetic ketoacidosis. *J Intens Care Med.* 2014;29(1):38–42.
235. von Oettingen JE, Rhodes ET, Wolfsdorf JI. Resolution of ketoacidosis in children with new onset diabetes: Evaluation of various definitions. *Diabetes Res Clin Pract.* 2017;135:76–84.
236. Chua HR, Venkatesh B, Stachowski E, et al. Plasma-Lyte 148 vs 0.9% saline for fluid resuscitation in diabetic ketoacidosis. *J Crit Care.* 2012;27(2):138–145.
237. Waldhausl W, Kleinberger G, Korn A, Dudczak R, Bratusch-Marrain P, Nowotny P. Severe hyperglycemia: effects of rehydration on endocrine derangements and blood glucose concentration. *Diabetes.* 1979;28(6):577–584.
238. Owen OE, Licht JH, Sapir DG. Renal function and effects of partial rehydration during diabetic ketoacidosis. *Diabetes.* 1981;30(6):510–518.
239. Luzi L, Barrett EJ, Groop LC, Ferrannini E, DeFronzo RA. Metabolic effects of low-dose insulin therapy on glucose metabolism in diabetic ketoacidosis. *Diabetes.* 1988;37(11):1470–1477.
240. Kitabchi AE, Umpierrez GE, Fisher JN, Murphy MB, Stentz FB. Thirty years of personal experience in hyperglycemic crises: diabetic ketoacidosis and hyperglycemic hyperosmolar state. *J Clin Endocrinol Metab.* 2008;93(5):1541–1552.
241. Nallasamy K, Jayashree M, Singhi S, Bansal A. Low-dose vs standard-dose insulin in pediatric diabetic ketoacidosis: a randomized clinical trial. *JAMA Pediatr.* 2014;168(11):999–1005.
242. Burghen GA, Etteldorf JN, Fisher JN, Kitabchi AQ. Comparison of high-dose and low-dose insulin by continuous intravenous infusion in the treatment of diabetic ketoacidosis in children. *Diabetes Care.* 1980;3(1):15–20.
243. Lindsay R, Bolte RG. The use of an insulin bolus in low-dose insulin infusion for pediatric diabetic ketoacidosis. *Pediatr Emerg Care.* 1989;5(2):77–79.
244. Van der Meulen JA, Klip A, Grinstein S. Possible mechanism for cerebral oedema in diabetic ketoacidosis. *Lancet.* 1987;2(8554):306–308.
245. Della Manna T, Steinmetz L, Campos PR, et al. Subcutaneous use of a fast-acting insulin analog: An alternative treatment for pediatric patients with diabetic ketoacidosis. *Diabetes Care.* 2005;28(8):1856–1861.
246. Savoldelli RD, Farhat SC, Manna TD. Alternative management of diabetic ketoacidosis in a Brazilian pediatric emergency department. *Diabetol Metab Syndr.* 2010;2:41.
247. Mul D, Molendijk E. Treatment of mild to moderate ketoacidosis in children and adolescents with subcutaneous insulin. *Arch Dis Child.* 2014;100(1):106–108.
248. Cohen M, Leibovitz N, Shilo S, Zuckerman-Levin N, Shavit I, Shehadeh N. Subcutaneous regular insulin for the treatment of diabetic ketoacidosis in children. *Pediatr Diabetes.* 2017;18(4):290–296.
249. Danowski T, Peters J, Rathbun J, Quashnock J, Greenman L. Studies in diabetic acidosis and coma, with particular emphasis on the retention of administered potassium. *J Clin Invest.* 1949;28:1–9.
250. Butler A, Talbot N, Burnett C, Stanbury J, MacLachlan E. Metabolic studies in diabetic coma. *Trans Assoc Am Physicians.* 1947;60:102–109.
251. Adroge HJ, Lederer ED, Suki WN, Eknayan G. Determinants of plasma potassium levels in diabetic ketoacidosis. *Medicine (Baltimore).* 1986;65(3):163–172.
252. DeFronzo RA, Felig P, Ferrannini E, Wahren J. Effect of graded doses of insulin on splanchnic and peripheral potassium metabolism in man. *Am J Physiol.* 1980;238(5):E421–E427.
253. Tattersall RB. A paper which changed clinical practice (slowly). Jacob Holler on potassium deficiency in diabetic acidosis (1946). *Diabet Med.* 1999;16(12):978–984.
254. Davis SM, Maddux AB, Alonso GT, Okada CR, Mourani PM, Maahs DM. Profound hypokalemia associated with severe diabetic ketoacidosis. *Pediatr Diabetes.* 2016;17:61–65.
255. Riley MS, Schade DS, Eaton RP. Effects of insulin infusion on plasma phosphate in diabetic patients. *Metabolism.* 1979;28(3):191–194.
256. Knochel JP. The pathophysiology and clinical characteristics of severe hypophosphatemia. *Arch Intern Med.* 1977;137(2):203–220.
257. Alberti KG, Emerson PM, Darley JH, Hockaday TD. 2,3-Diphosphoglycerate and tissue oxygenation in uncontrolled diabetes mellitus. *Lancet.* 1972;2(7774):391–395.
258. Fisher JN, Kitabchi AE. A randomized study of phosphate therapy in the treatment of diabetic ketoacidosis. *J Clin Endocrinol Metab.* 1983;57(1):177–180.
259. Singhal PC, Kumar A, Desroches L, Gibbons N, Mattana J. Prevalence and predictors of rhabdomyolysis in patients with hypophosphatemia. *Am J Med.* 1992;92(5):458–464.
260. Zipf WB, Bacon GE, Spencer ML, Kelch RP, Hopwood NJ, Hawker CD. Hypocalcemia, hypomagnesemia, and transient hypoparathyroidism during therapy with potassium phosphate in diabetic ketoacidosis. *Diabetes Care.* 1979;2(3):265–268.
261. Winter RJ, Harris CJ, Phillips LS, Green OC. Diabetic ketoacidosis. Induction of hypocalcemia and hypomagnesemia by phosphate therapy. *Am J Med.* 1979;67(5):897–900.
262. Green SM, Rothrock SG, Ho JD, et al. Failure of adjunctive bicarbonate to improve outcome in severe pediatric diabetic ketoacidosis. *Ann Emerg Med.* 1998;31(1):41–48.
263. Lever E, Jaspan JB. Sodium bicarbonate therapy in severe diabetic ketoacidosis. *Am J Med.* 1983;75(2):263–268.
264. Ohman Jr JL, Marliss EB, Aoki TT, Munichoodappa CS, Khanna VV, Kozak GP. The cerebrospinal fluid in diabetic ketoacidosis. *N Engl J Med.* 1971;284(6):283–290.
265. Narins RG, Cohen JJ. Bicarbonate therapy for organic acidosis: the case for its continued use. *Ann Intern Med.* 1987;106(4):615–618.
266. Shankar V, Haque A, Churchwell KB, Russell W. Insulin glargine supplementation during early management phase of diabetic ketoacidosis in children. *Intens Care Med.* 2007;33(7):1173–1178.
267. Harrison VS, Rustico S, Palladino AA, Ferrara C, Hawkes CP. Glargine co-administration with intravenous insulin in pediatric diabetic ketoacidosis is safe and facilitates transition to a subcutaneous regimen. *Pediatr Diabetes.* 2016;18(8):742–748.
268. Glaser N, Barnett P, McCaslin I, et al. Risk factors for cerebral edema in children with diabetic ketoacidosis. The Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. *N Engl J Med.* 2001;344(4):264–269.
269. Edge JA, Hawkins MM, Winter DL, Dunger DB. The risk and outcome of cerebral oedema developing during diabetic ketoacidosis. *Arch Dis Child.* 2001;85(1):16–22.
270. Lawrence SE, Cummings EA, Gaboury I, Daneman D. Population-based study of incidence and risk factors for cerebral edema in pediatric diabetic ketoacidosis. *J Pediatr.* 2005;146(5):688–692.
271. Ghetti S, Lee JK, Sims CE, Demaster DM, Glaser NS. Diabetic ketoacidosis and memory dysfunction in children with type 1 diabetes. *J Pediatr.* 2010;156(1):109–114.
272. Cameron FJ, Scratch SE, Nadebaum C, et al. Neurological consequences of diabetic ketoacidosis at initial presentation of type 1 diabetes in a prospective cohort study of children. *Diabetes Care.* 2014;37(6):1554–1562.
273. Cato MA, Mauras N, Mazaika P, et al. Longitudinal evaluation of cognitive functioning in young children with type 1 diabetes over 18 months. *J Int Neuropsychol Soc.* 2016;22(3):293–302.
274. Semenkovich K, Bischoff A, Doty T, et al. Clinical presentation and memory function in youth with type 1 diabetes. *Pediatr Diabetes.* 2016;17(7):492–499.
275. Antenor-Dorsey JA, Meyer E, Rutlin J, et al. White matter microstructural integrity in youth with type 1 diabetes. *Diabetes.* 2013;62(2):581–589.

276. Siller AF, Lugar H, Rutlin J, et al. Severity of clinical presentation in youth with type 1 diabetes is associated with differences in brain structure. *Pediatr Diabetes*. 2017;18(8):686–695.
277. Harris GD, Fiordalisi I, Finberg L. Safe management of diabetic ketoacidemia. *J Pediatr*. 1988;113:65–67.
278. Glaser NS, Marcin JP, Wootton-Gorges SL, et al. Correlation of clinical and biochemical findings with diabetic ketoacidosis-related cerebral edema in children using magnetic resonance diffusion-weighted imaging. *J Pediatr*. 2008;153(4):541–546.
279. Glaser NS, Tancredi DJ, Marcin JP, et al. Cerebral hyperemia measured with near infrared spectroscopy during treatment of diabetic ketoacidosis in children. *J Pediatr*. 2013;163(4):1111–1116.
280. Lam TI, Anderson SE, Glaser N, O'Donnell ME. Bumetanide reduces cerebral edema formation in rats with diabetic ketoacidosis. *Diabetes*. 2005;54(2):510–516.
281. Glaser NS, Wootton-Gorges SL, Marcin JP, et al. Mechanism of cerebral edema in children with diabetic ketoacidosis. *J Pediatr*. 2004;145(2):164–171.
282. Couch RM, Acott PD, Wong GW. Early onset fatal cerebral edema in diabetic ketoacidosis. *Diabetes Care*. 1991;14(1):78–79.
283. Close TE, Cepinskas G, Omatsu T, et al. Diabetic ketoacidosis elicits systemic inflammation associated with cerebrovascular endothelial cell dysfunction. *Microcirculation*. 2013;20(6):534–543.
284. Hoffman WH, Passmore GG, Hannon DW, et al. Increased systemic Th17 cytokines are associated with diastolic dysfunction in children and adolescents with diabetic ketoacidosis. *PLoS One*. 2013;8(8). e71905.
285. Omatsu T, Cepinskas G, Clarson C, et al. CXCL1/CXCL8 (GROalpha/IL-8) in human diabetic ketoacidosis plasma facilitates leukocyte recruitment to cerebrovascular endothelium in vitro. *Am J Physiol Endocrinol Metab*. 2014;306(9):E1077–E1084.
286. Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. *Diabetes*. 2004;53(8):2079–2086.
287. Garro A, Chodobski A, Szmydynger-Chodobska J, et al. Circulating matrix metalloproteinases in children with diabetic ketoacidosis. *Pediatr Diabetes*. 2017;18(2):95–102.
288. Yuen N, Anderson SE, Glaser N, Tancredi DJ, O'Donnell ME. Cerebral blood flow and cerebral edema in rats with diabetic ketoacidosis. *Diabetes*. 2008;57(10):2588–2594.
289. Glaser N, Yuen N, Anderson SE, Tancredi DJ, O'Donnell ME. Cerebral metabolic alterations in rats with diabetic ketoacidosis: effects of treatment with insulin and intravenous fluids and effects of bumetanide. *Diabetes*. 2010;59(3):702–709.
290. Glaser NS, Wootton-Gorges SL, Buonocore MH, et al. Frequency of sub-clinical cerebral edema in children with diabetic ketoacidosis. *Pediatr Diabetes*. 2006;7(2):75–80.
291. Sperling MA. Cerebral edema in diabetic ketoacidosis: an underestimated complication? *Pediatr Diabetes*. 2006;7(2):73–74.
292. Rosenbloom AL. Intracerebral crises during treatment of diabetic ketoacidosis. *Diabetes Care*. 1990;13(1):22–33.
293. Edge JA. Cerebral oedema during treatment of diabetic ketoacidosis: are we any nearer finding a cause? *Diabetes Metab Res Rev*. 2000;16(5):316–324.
294. Muir AB, Quisling RG, Yang MC, Rosenbloom AL. Cerebral Edema in Childhood Diabetic Ketoacidosis: Natural history, radiographic findings, and early identification. *Diabetes Care*. 2004;27(7):1541–1546.
295. Decourcey DD, Steil GM, Wypij D, Agus MS. Increasing use of hypertonic saline over mannitol in the treatment of symptomatic cerebral edema in pediatric diabetic ketoacidosis: an 11-year retrospective analysis of mortality*. *Pediatr Crit Care Med*. 2013;14(7):694–700.
296. Kronan K, Normal ME. Renal and electrolyte emergencies. In: Fleisher GR, Ludwig S, eds. *Textbook of Emergency Medicine*. 4th ed. Philadelphia: Lippincott, Williams and Wilkins; 2000.
297. Matz R. Management of the hyperosmolar hyperglycemic syndrome. *Am Fam Physician*. 1999;60(5):1468–1476.
298. Delaney MF, Zisman A, Kettle WM. Diabetic ketoacidosis and hyperglycemic hyperosmolar nonketotic syndrome. *Endocrinol Metab Clin North Am*. 2000;29(4):683–705. V.
299. Gutierrez JA, Bagatell R, Samson MP, Theodorou AA, Berg RA. Femoral central venous catheter-associated deep venous thrombosis in children with diabetic ketoacidosis. *Crit Care Med*. 2003;31(1):80–83.
300. Mannix R, Tan ML, Wright R, Baskin M. Acute pediatric rhabdomyolysis: causes and rates of renal failure. *Pediatrics*. 2006;118(5):2119–2125.
301. Hollander AS, Olney RC, Blackett PR, Marshall BA. Fatal malignant hyperthermia-like syndrome with rhabdomyolysis complicating the presentation of diabetes mellitus in adolescent males. *Pediatrics*. 2003;111(6 Pt 1):1447–1452.
302. Morales AE, Rosenbloom AL. Death caused by hyperglycemic hyperosmolar state at the onset of type 2 diabetes. *J Pediatr*. 2004;144(2):270–273.
303. Kilbane BJ, Mehta S, Backeljauw PF, Shanley TP, Crimmins NA. Approach to management of malignant hyperthermia-like syndrome in pediatric diabetes mellitus. *Pediatr Crit Care Med*. 2006;7(2):169–173.
304. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329(14):977–986.
305. Diabetes Control and Complications Trial Research Group. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. *J Pediatr*. 1994;125(2):177–188.
306. White NH, Cleary PA, Dahms W, Goldstein D, Malone J, Tamborlane WV. Beneficial effects of intensive therapy of diabetes during adolescence: outcomes after the conclusion of the Diabetes Control and Complications Trial (DCCT). *J Pediatr*. 2001;139(6):804–812.
307. DiMeglio LA, Acerini CL, Codner E, Craig ME, Hofer SE, Pillay K, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: Glycemic control targets and glucose monitoring for children, adolescents, and young adults with diabetes. *Pediatr Diabetes*. 2018;19(Suppl 27):105–114.
308. Maura N, Mazaika P, Buckingham B, Weinzimer S, White NH, Tsalikian E, et al. Diabetes Research in Children Network (DirecNet). Longitudinal assessment of neuroanatomical and cognitive differences in young children with type 1 diabetes: association with hyperglycemia. *Diabetes*. 2015;64(5):1770–1779.
309. Maahs DM, Hermann JM, DuBose SN, Miller KM, Heidtmann B, DiMeglio LA, et al. DPV Initiative; T1D Exchange Clinic Network. Contrasting the clinical care and outcomes of 2,622 children with type 1 diabetes less than 6 years of age in the United States T1D Exchange and German/Austrian DPV registries. *Diabetologia*. 2014;57(8):1578–1585.
310. Danne T, Phillip M, Buckingham BA, Jarosz-Chobot P, Saboo B, Urakami T, et al. ISPAD clinical consensus guidelines 2018: Insulin treatment in children and adolescents with diabetes. *Pediatr Diabetes*. 2018;19(suppl 27):115–135.
311. Vajo Z, Fawcett J, Duckworth WC. Recombinant DNA technology in the treatment of diabetes: insulin analogs. *Endocr Rev*. 2001;22(5):706–717.
312. Swan KL, Weinzimer SA, Dziura JD, Steil GM, Voskanyan GR, Steffen AT, et al. Effect of puberty on the pharmacodynamic and pharmacokinetic properties of insulin pump therapy in youth with type 1 diabetes. *Diabetes Care*. 2008;31(1):44–46.
313. Cengiz E, Swan KL, Tamborlane WV, Sherr JL, Martin M, Weinzimer SA. The alteration of aspart insulin pharmacodynamics when mixed with detemir insulin. *Diabetes Care*. 2012;35(4):690–692.
314. Cengiz E, Tamborlane WV, Martin-Fredricksen M, Dziura J, Weinzimer SA. Early pharmacokinetic and pharmacodynamic effects of mixing lispro with glargine insulin: results of glucose clamp studies in youth with type 1 diabetes. *Diabetes Care*. 2010;33(5):1009–1012.
315. Weinzimer SA, Ternand C, Howard C, Chang CT, Becker DJ, Laffel LM. A randomized trial comparing continuous subcutaneous insulin infusion of insulin aspart versus insulin lispro in children and adolescents with type 1 diabetes. *Diabetes Care*. 2008;31(2):210–215.
316. van Bon AC, Bode BW, Sert-Langeron C, DeVries JH, Charpentier G. Insulin glulisine compared to insulin aspart and to insulin lispro administered by continuous subcutaneous insulin infusion in patients with type 1 diabetes: a randomized controlled trial. *Diabetes Technol Ther*. 2011;13(6):607–614.

317. Bangstad, H.J., Danne, T., Deeb, L., Jarosz-Chobot, P., Urakami, T., Hanas, R. (2009). Insulin treatment in children and adolescents with diabetes. *Pediatr Diabetes*, 10(suppl), 82–99.
318. Russell-Jones D, Bode BW, De Block C, et al. Fast-acting insulin aspart improves glycemic control in basal-bolus treatment for type 1 diabetes: results of a 26-week multicenter, active-controlled, treat-to-target, randomized, parallel-group trial (onset 1). *Diabetes Care*. 2017;40(7):943–950.
319. Bode BW, Iotova V, Kovarenko M, et al. Efficacy and safety of fast-acting insulin aspart compared with insulin aspart, both in combination with insulin degludec, in children and adolescents with type 1 diabetes: The Onset 7 Trial. *Diabetes Care*. 2019;42(7):1255–1262.
320. Lepore G, Dodesini AR, Nosari I, Trevisan R. Both continuous subcutaneous insulin infusion and a multiple daily insulin injection regimen with glargine as basal insulin are equally better than traditional multiple daily insulin injection treatment. *Diabetes Care*. 2003;26(4):1321–1322.
321. Danne T, Datz N, Endahl L, Haahr H, Nestoris C, Westergaard L, et al. Insulin detemir is characterized by a more reproducible pharmacokinetic profile than insulin glargine in children and adolescents with type 1 diabetes: results from a randomized, double-blind, controlled trial. *Pediatr Diabetes*. 2008;9(6):554–560.
322. Heise T, Nosek L, Ronn BB, Endahl L, Heinemann L, Kapitza C, et al. Lower within-subject variability of insulin detemir in comparison to NPH insulin and insulin glargine in people with type 1 diabetes. *Diabetes*. 2004;53(6):1614–1620.
323. Battelino T, Bosnyak Z, Danne T, et al. In range: comparison of the second-generation basal insulin analogues glargine 300U/ml and degludec 100U/ml in persons with type 1 diabetes using continuous glucose monitoring-study design. *Diabetes Ther*. 2020;11(4):1017–1027.
324. Codner E, Acerini CL, Craig ME, Hofer SE, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: Limited Care Guidance Appendix. *Pediatr Diabetes*. 2018;19(Suppl 27):328–338.
325. Kordonouri O, Pankowska E, Rami B, Kapellen T, Coutant R, Hartmann R, et al. Sensor-augmented pump therapy from the diagnosis of childhood type 1 diabetes: results of the Paediatric Onset Study (ONSET) after 12 months of treatment. *Diabetologia*. 2010;53(12):2487–2495.
326. Sherr J, Tamborlane WV, Tsalikian E, et al. Hypoglycemia and lower glucose variability. *Diabetes Care*. 2012;35:817–820.
327. Kordonouri O, Hartmann R, Remus K, Blasig S, Sadeghian E, Danne T. Benefit of supplementary fat plus protein counting as compared with conventional carbohydrate counting for insulin bolus calculation in children with pump therapy. *Pediatr Diabetes*. 2012;13(7):540–544.
328. Wood JR, Miller KM, Maahs DM, Beck RW, DiMeglio LA, Libman IM, et al. Most youth with type 1 diabetes in the T1D Exchange Clinic Registry do not meet American Diabetes Association or International Society for Pediatric and Adolescent Diabetes clinical guidelines. *Diabetes Care*. 2013;36(7):2035–2037.
329. Attia N, Jones TW, Holcombe J, Tamborlane WV. Comparison of human regular and lispro insulins after interruption of continuous subcutaneous insulin infusion and in the treatment of acutely decompensated IDDM. *Diabetes Care*. 1998;21(5):817–821.
330. Sherr JL, Tauschmann M, Battelino T, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetes Technologies. *Pediatr Diabetes*. 2018;19(Suppl 27):302–325.
331. Weinzimer SA, Steil GM, Swan KL, Dziura J, Kurtz N, Tamborlane WV. Fully automated closed-loop insulin delivery versus semiautomated hybrid control in pediatric patients with type 1 diabetes using an artificial pancreas. *Diabetes Care*. 2008;31(5):934.
332. Miller KM, Beck RW, Bergenstal RM, Goland RS, Haller MJ, McGill JB, et al. Evidence of a strong association between frequency of self-monitoring of blood glucose and hemoglobin A1c levels in T1D exchange clinic registry participants. *Diabetes Care*. 2013;36(7):2009–2014.
333. Szymowska A, Schwandt A, Svensson J, et al. Insulin pump therapy in children with type 1 diabetes: analysis of data from the SWEET registry. *Pediatr Diabetes*. 2016;17 Suppl 23:38–45. <https://doi.org/10.1111/pedi.12416>.
334. Tamborlane WV, Sikes KA. Insulin therapy in children and adolescents. *Endocrinol Metab Clin North Am*. 2012;41(1):145–160.
335. Misso ML, Egberts KJ, Page M, O'Connor D, Shaw J. Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. *Cochrane Database Syst Rev*. 2010;2010(1). Cd005103.
336. Pankowska E, Blazik M, Dziechciarz P, Szymowska A, Szajewska H. Continuous subcutaneous insulin infusion vs. multiple daily injections in children with type 1 diabetes: a systematic review and meta-analysis of randomized control trials. *Pediatr Diabetes*. 2009;10(1):52–58.
337. Fox LA, Buckloh LM, Smith SD, Wysocki T, Mauras N. A randomized controlled trial of insulin pump therapy in young children with type 1 diabetes. *Diabetes Care*. 2005;28(6):1277–1281.
338. Weintrob N, Benzaquen H, Galatzer A, Shalitin S, Lazar L, Fayman G, et al. Comparison of continuous subcutaneous insulin infusion and multiple daily injection regimens in children with type 1 diabetes: a randomized open crossover trial. *Pediatrics*. 2003;112(3 Pt 1):559–564.
339. Lawrence JM, Yi-Frazier JP, Black MH, Anderson A, Hood K, Imperatore G, et al. Demographic and clinical correlates of diabetes-related quality of life among youth with type 1 diabetes. *J Pediatr*. 2012;161(2). 201–207.e2.
340. Knight S, Northam E, Donath S, Gardner A, Harkin N, Taplin C, et al. Improvements in cognition, mood and behaviour following commencement of continuous subcutaneous insulin infusion therapy in children with type 1 diabetes mellitus: a pilot study. *Diabetologia*. 2009;52(2):193–198.
341. Knight SJ, Northam EA, Cameron FJ, Ambler GR. Behaviour and metabolic control in children with Type 1 diabetes mellitus on insulin pump therapy: 2-year follow-up. *Diabetic Med*. 2011;28(9):1109–1112.
342. Pickup JC. Insulin-pump therapy for type 1 diabetes mellitus. *N Engl J Med*. 2012;366(17):1616–1624.
343. Shalitin S, Gil M, Nimri R, de Vries L, Gavan MY, Phillip M. Predictors of glycaemic control in patients with Type 1 diabetes commencing continuous subcutaneous insulin infusion therapy. *Diabetic Med*. 2010;27(3):339–347.
344. de Vries L, Grushka Y, Lebenthal Y, Shalitin S, Phillip M. Factors associated with increased risk of insulin pump discontinuation in pediatric patients with type 1 diabetes. *Pediatr Diabetes*. 2011;12(5):506–512.
345. Nimri R, Weintrob N, Benzaquen H, Ofan R, Fayman G, Phillip M. Insulin pump therapy in youth with type 1 diabetes: a retrospective paired study. *Pediatrics*. 2006;117(6):2126–2131.
346. Sulmont V, Souchon PF, Gouillard-Darnaud C, Fartura A, Salmon-Musial AS, Lambrecht E, et al. Metabolic control in children with diabetes mellitus who are younger than 6 years at diagnosis: continuous subcutaneous insulin infusion as a first line treatment? *J Pediatr*. 2010;157(1):103–107.
347. Bratina N, Battelino T. Insulin pumps and continuous glucose monitoring (CGM) in preschool and school-age children: how schools can integrate technology. *Pediatr Endocrinol Rev*. 2010;7(Suppl 3):417–421.
348. Bachran R, Beyer P, Klinkert C, Heidtmann B, Rosenbauer J, Holl RW. Basal rates and circadian profiles in continuous subcutaneous insulin infusion (CSII) differ for preschool children, prepubertal children, adolescents and young adults. *Pediatr Diabetes*. 2012;13(1):1–5.
349. Holterhus PM, Bokelmann J, Riepe F, Heidtmann B, Wagner V, Rami-Merhar B, et al. Predicting the optimal basal insulin infusion pattern in children and adolescents on insulin pumps. *Diabetes Care*. 2013;36(6):1507–1511.
350. Nabhan ZM, Rardin L, Meier J, Eugster EA, Dimeglio LA. Predictors of glycemic control on insulin pump therapy in children and adolescents with type 1 diabetes. *Diabetes Res Clin Pract*. 2006;74(3):217–221.
351. Danne T, von Schutz W, Lange K, Nestoris C, Datz N, Kordonouri O. Current practice of insulin pump therapy in children and adolescents – the Hannover recipe. *Pediatr Diabetes*. 2006;7(Suppl 4):25–31.
352. Battelino T. Risk and benefits of continuous subcutaneous insulin infusion (CSII) treatment in school children and adolescents. *Pediatr Diabetes*. 2006;7(Suppl 4):20–24.
353. Battelino T. CSII and continuous glucose monitoring in children and adolescents. In: Pickup J, ed. *ODL Insulin Pump Therapy and*

- Continuous Glucose Monitoring*. Oxford: Oxford University Press; 2009:53–68.
354. Shalitin S, Phillip M. Hypoglycemia in type 1 diabetes: a still unresolved problem in the era of insulin analogs and pump therapy. *Diabetes Care*. 2008;31(Suppl 2):S121–124.
 355. Danne T, Battelino T, Jarosz-Chobot P, Kordonouri O, Pankowska E, Ludvigsson J, et al. Establishing glycaemic control with continuous subcutaneous insulin infusion in children and adolescents with type 1 diabetes: experience of the PedPump Study in 17 countries. *Diabetologia*. 2008;51(9):1594–1601.
 356. Enander R, Gundeval C, Stromgren A, Chaplin J, Hanas R. Carbohydrate counting with a bolus calculator improves postprandial blood glucose levels in children and adolescents with type 1 diabetes using insulin pumps. *Pediatr Diabetes*. 2012;13(7):545–551.
 357. O'Connell MA, Gilbertson HR, Donath SM, Cameron FJ. Optimizing postprandial glycemia in pediatric patients with type 1 diabetes using insulin pump therapy: impact of glycemic index and prandial bolus type. *Diabetes Care*. 2008;31(8):1491–1495.
 358. Scaramuzza AE, Iafusco D, Santoro L, Bosetti A, De Palma A, Spiri D, et al. Timing of bolus in children with type 1 diabetes using continuous subcutaneous insulin infusion (TiBoDi Study). *Diabetes Technol Ther*. 2010;12(2):149–152.
 359. Cobry E, McFann K, Messer L, Gage V, VanderWel B, Horton L, et al. Timing of meal insulin boluses to achieve optimal postprandial glycemic control in patients with type 1 diabetes. *Diabetes Technol Ther*. 2010;12(3):173–177.
 360. Vanderwel BW, Messer LH, Horton LA, McNair B, Cobry EC, McFann KK, et al. Missed insulin boluses for snacks in youth with type 1 diabetes. *Diabetes Care*. 2010;33(3):507–508.
 361. Wilkinson J, McFann K, Chase HP. Factors affecting improved glycaemic control in youth using insulin pumps. *Diabetic Med*. 2010;27(10):1174–1177.
 362. Aman J, Skinner TC, de Beaufort CE, Swift PG, Aanstoot HJ, Cameron F. Associations between physical activity, sedentary behavior, and glycemic control in a large cohort of adolescents with type 1 diabetes: the Hvidoere Study Group on Childhood Diabetes. *Pediatr Diabetes*. 2009;10(4):234–239.
 363. Weinzimer SA, Beck RW, Chase HP, et al. Accuracy of newer-generation home blood glucose meters in a Diabetes Research in Children Network (DirecNet) in patient exercise study. *Diabetes Technol Ther*. 2005;7:675–680.
 364. Tsalikian E, Kollman C, Tamborlane WB, Beck RW, Fiallo-Scharer R, Fox L, et al. Prevention of hypoglycemia during exercise in children with type 1 diabetes by suspending basal insulin. *Diabetes Care*. 2006;29(10):2200–2204.
 365. Admon G, Weinstein Y, Falk B, Weintrob N, Benzaquen H, Ofan R, et al. Exercise with and without an insulin pump among children and adolescents with type 1 diabetes mellitus. *Pediatrics*. 2005;116(3):e348–355.
 366. Scrimgeour L, Cobry E, McFann K, Burdick P, Weimer C, Slover R, et al. Improved glycemic control after long-term insulin pump use in pediatric patients with type 1 diabetes. *Diabetes Technol Ther*. 2007;9(5):421.
 367. Jakisch BI, Wagner VM, Heidtmann B, Lepler R, Holterhus PM, Kapellen TM, et al. Comparison of continuous subcutaneous insulin infusion (CSII) and multiple daily injections (MDI) in paediatric Type 1 diabetes: a multicentre matched-pair cohort analysis over 3 years. *Diabetic Med*. 2008;25(1):80–85.
 368. Guerci B, Meyer L, Salle A, Charrie A, Dousset B, Ziegler O, et al. Comparison of metabolic deterioration between insulin analog and regular insulin after a 5-hour interruption of a continuous subcutaneous insulin infusion in type 1 diabetic patients. *J Clin Endocrinol Metab*. 1999;84(8):2673–2678.
 369. Finfer S, Chittock DR, Su SY, Blair D, Foster D, Dhingra V, et al. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med*. 2009;360(13):1283–1297.
 370. Van Herpe T, Vanhosebrouck K, Mesotten D, De Moor B, Van den Bergh G. Glycemic control in the pediatric intensive care unit of Leuven: two years of experience. *J Diabetes Sci Technol*. 2012;6(1):15–21.
 371. Maahs DM, Horton LA, Chase HP. The use of insulin pumps in youth with type 1 diabetes. *Diabetes Technol Ther*. 2010;12(Suppl 1):S59–65.
 372. Bratina N. Establishing a good pump clinic: successes and challenges. *Diabetes Manag*. 2013;3(2):131–143.
 373. Cengiz E, Xing D, Wong JC, Wolfsdorf JL, Haymond MW, Rewers A, et al. Severe hypoglycemia and diabetic ketoacidosis among youth with type 1 diabetes in the T1D Exchange clinic registry. *Pediatr Diabetes*. 2013;14(6):447–454.
 374. Shorer M, David R, Schoenberg-Taz M, Levavi-Lavi I, Phillip M, Meyerovitch J. Role of parenting style in achieving metabolic control in adolescents with type 1 diabetes. *Diabetes Care*. 2011;34(8):1735–1737.
 375. Ziegler R, Heidtmann B, Hilgard D, et al. Frequency of SMBG correlates with HbA1c and acute complications in children and adolescents with type 1 diabetes. *Pediatr Diabetes*. 2011;12(1):11–17.
 376. Dovc K, Battelino T. Evolution of diabetes technology. *Endocrinol Metab Clin*. 2020;9(1):1–16.
 377. Tauschmann M, Hovorka R. Technology in the management of type 1 diabetes mellitus - current status and future prospects. *Nat Rev Endocrinol*. 2018;14(8):464–475.
 378. Sundberg F, Forsander G. Continuous glucose monitoring in healthy children aged 2-8 years. *Diabetes Technol Ther*. 2018;20(2):113–116.
 379. Cengiz E. Analysis of a remote system to closely monitor glycemia and insulin pump delivery—is this the beginning of a wireless transformation in diabetes management? *J Diabetes Sci Technol*. 2013;7(2):362–364.
 380. Piona C, Dovc K, Mutlu GY, et al. Non-adjunctive flash glucose monitoring system use during summer-camp in children with type 1 diabetes: the free-summer study. *Pediatr Diabetes*. 2018;19(7):1285–1293.
 381. Barnard KD, Kropff J, Choudhary P, et al. Acceptability of Implantable Continuous Glucose Monitoring Sensor. *J Diabetes Sci Technol*. 2018;12(3):634–638.
 382. Ludwig-Seibold CU, Holder M, Rami B, et al. Continuous glucose monitoring in children, adolescents, and adults with type 1 diabetes mellitus: analysis from the prospective DPV diabetes documentation and quality management system from Germany and Austria. *Pediatr Diabetes*. 2012;13(1):12–14.
 383. Wong JC, Foster NC, Maahs DM, et al. Real-time continuous glucose monitoring among participants in the T1D Exchange clinic registry. *Diabetes Care*. 2014;37(10):2702–2709.
 384. DeSalvo DJ, Miller KM, Hermann JM, Maahs DM, Hofer SE, Clements MA, et al. T1D Exchange and DPV Registries. Continuous glucose monitoring and glycemic control among youth with type 1 diabetes: International comparison from the T1D Exchange and DPV Initiative. *Pediatr Diabetes*. 2018;19(7):1271–1275.
 385. Szypowska A, Ramotowska A, Dzygalo K, et al. Beneficial effect of real-time continuous glucose monitoring system on glycemic control in type 1 diabetic patients: systematic review and meta-analysis of randomized trials. *Eur J Endocrinol*. 2012;166(4):567–574.
 386. Battelino T, Phillip M. Real-time continuous glucose monitoring in randomized control trials. *Pediatr Endocrinol Rev*. 2010;7(Suppl 3):401–404.
 387. Phillip M, Danne T, Shalitin S, et al. Consensus Forum Participants. Use of continuous glucose monitoring in children and adolescents. *Pediatr Diabetes*. 2012;13(3):215–228.
 388. Tamborlane WV, Beck RW, Bode BW, et al. Continuous glucose monitoring and intensive treatment of type 1 diabetes. *N Engl J Med*. 2008;359(14):1464–1476.
 389. Beck RW, Buckingham B, Miller K, et al. Factors predictive of use and of benefit from continuous glucose monitoring in type 1 diabetes. *Diabetes Care*. 2009;32(11):1947–1953.
 390. Wong JC, Foster NC, Maahs DM, et al. Real-time continuous glucose monitoring among participants in the T1D Exchange clinic registry. *Diabetes Care*. 2014;37(10):2702–2709.
 391. Bergenstal RM, Tamborlane WV, Ahmann A, et al. Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes. *N Engl J Med*. 2010;363(4):311–320.
 392. Bergenstal RM, Tamborlane WV, Ahmann A, et al. Sensor-augmented pump therapy for A1C reduction (STAR 3) study: results from the 6-month continuation phase. *Diabetes Care*. 2011;34(11):2403–2405.
 393. Chase HP, Beck RW, Xing D, et al. Continuous glucose monitoring in youth with type 1 diabetes: 12-month follow-up of the

- Juvenile Diabetes Research Foundation continuous glucose monitoring randomized trial. *Diabetes Technol Ther.* 2010;12(7):507–515.
394. Battelino T, Phillip M, Bratina N, et al. Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes. *Diabetes Care.* 2011;34(4):795–800.
 395. Battelino T, Conget I, Olsen B, et al. The use and efficacy of continuous glucose monitoring in type 1 diabetes treated with insulin pump therapy: a randomised controlled trial. *Diabetologia.* 2012;55(12):3155–3162.
 396. Jeha GS, Karaviti LP, Anderson B, et al. Continuous glucose monitoring and the reality of metabolic control in preschool children with type 1 diabetes. *Diabetes Care.* 2004;27(12):2881–2886.
 397. Gandrud LM, Xing D, Kollman C, et al. The Medtronic Minimed Gold continuous glucose monitoring system: an effective means to discover hypo- and hyperglycemia in children under 7 years of age. *Diabetes Technol Ther.* 2007;9(4):307–316.
 398. Mauras N, Beck R, Xing D, et al. A randomized clinical trial to assess the efficacy and safety of real-time continuous glucose monitoring in the management of type 1 diabetes in young children aged 4 to <10 years. *Diabetes Care.* 2012;35(2):204–210.
 399. Tsalikian E, Fox L, Weinzimer S, et al. Feasibility of prolonged continuous glucose monitoring in toddlers with type 1 diabetes. *Pediatr Diabetes.* 2012;13(4):301–307.
 400. Dovč K, Bratina N, Battelino T. A new horizon for glucose monitoring. *Horm Res Paediatr.* 2005;83(3):149–156.
 401. Kordonouri O, Vazeou A, Scharf M, et al. SWEET Group. Striving for control: lessons learned from a successful international Type 1 Diabetes Youth Challenge. *Acta Diabetol.* 2017;54(4):403–409.
 402. Dovc K, Cargnelutti K, Sturm A, et al. Continuous glucose monitoring use and glucose variability in pre-school children with type 1 diabetes. *Diabetes Res Clin Pract.* 2018;147:76–80.
 403. Bratina N, Battelino T. Insulin pumps and continuous glucose monitoring (CGM) in preschool and school-age children: how schools can integrate technology. *Pediatr Endocrinol Rev.* 2010;7(Suppl 3):417–421.
 404. Bratina N, Forsander G, Annan F, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Management and support of children and adolescents with type 1 diabetes in school. *Pediatr Diabetes.* 2018;19(Suppl 27):287–301.
 405. Goss PW, Middlehurst A, Acerini CL, et al. ISPAD Position Statement on type 1 diabetes in schools. *Pediatr Diabetes.* 2018;19(7):1338–1341.
 406. Choudhary P, Ramasamy S, Green L, et al. Real-time continuous glucose monitoring significantly reduces severe hypoglycemia in hypoglycemia-unaware patients with type 1 diabetes. *Diabetes Care.* 2013;36(12):4160–4162.
 407. van Beers CA, DeVries JH, Kleijer SJ, et al. Continuous glucose monitoring for patients with type 1 diabetes and impaired awareness of hypoglycaemia (IN CONTROL): a randomised, open-label, crossover trial. *Lancet Diabetes Endocrinol.* 2016;4(11):893–902.
 408. Heinemann L, Freckmann G, Ehrmann D, et al. Real-time continuous glucose monitoring in adults with type 1 diabetes and impaired hypoglycaemia awareness or severe hypoglycaemia treated with multiple daily insulin injections (HypoDE): a multicentre, randomised controlled trial. *Lancet.* 2018;391(10128):1367–1377.
 409. Beck RW, Hirsch IB, Laffel L, et al. The effect of continuous glucose monitoring in well-controlled type 1 diabetes. *Diabetes Care.* 2009;32(8):1378–1383.
 410. Buse JB, Kudva YC, Battelino T, et al. Effects of sensor-augmented pump therapy on glycemic variability in well-controlled type 1 diabetes in the STAR 3 study. *Diabetes Technol Ther.* 2012;14(7):644–647.
 411. El-Laboudi AH, Godsland IF, Johnston DG, et al. Measures of glycemic variability in type 1 diabetes and the effect of real-time continuous glucose monitoring. *Diabetes Technol Ther.* 2016;18(12):806–812.
 412. Kordonouri O, Pankowska E, Rami B, et al. Sensor-augmented pump therapy from the diagnosis of childhood type 1 diabetes: results of the Paediatric Onset Study (ONSET) after 12 months of treatment. *Diabetologia.* 2010;53(12):2487–2495.
 413. Langendam M, Luijck YM, Hooft L, et al. Continuous glucose monitoring systems for type 1 diabetes mellitus. *Cochrane Database Syst Rev.* 2012;1. CD008101.
 414. Ludvigsson J, Hanas R. Continuous subcutaneous glucose monitoring improved metabolic control in pediatric patients with type 1 diabetes: a controlled crossover study. *Pediatrics.* 2003;111(5):933–938.
 415. Deiss D, Bolinder J, Riveline JP, et al. Improved glycemic control in poorly controlled patients with type 1 diabetes using real-time continuous glucose monitoring. *Diabetes Care.* 2006;29(12):2730–2732.
 416. Golicki DT, Golicka D, Groele L, et al. Continuous glucose monitoring system in children with type 1 diabetes mellitus: a systematic review and meta-analysis. *Diabetologia.* 2008;51(2):233–240.
 417. Foster NC, Miller KM, Tamborlane WV, et al. Network TDEC. Continuous glucose monitoring in patients with type 1 diabetes using insulin injections. *Diabetes Care.* 2016;39(6):e81–e2.
 418. Lind M, Polonsky W, Hirsch IB, et al. Continuous glucose monitoring vs conventional therapy for glycemic control in adults with type 1 diabetes treated with multiple daily insulin injections: The GOLD randomized clinical trial. *JAMA.* 2017;317(4):379–387.
 419. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, et al. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. *Lancet.* 2016;388(10057):2254–2263.
 420. Aleppo G, Ruedy KJ, Riddlesworth TD, et al. REPLACE-BG: a randomized trial comparing continuous glucose monitoring with and without routine blood glucose monitoring in adults with well-controlled type 1 diabetes. *Diabetes Care.* 2017;40(4):538–545.
 421. Campbell FM, Murphy NP, Stewart C, et al. Outcomes of using flash glucose monitoring technology by children and young people with type 1 diabetes in a single arm study. *Pediatr Diabetes.* 2018;19(7):1294–1301.
 422. Hansen EA, Klee P, Dirlwanger M, et al. Accuracy, satisfaction and usability of a flash glucose monitoring system among children and adolescents with type 1 diabetes attending a summer camp. *Pediatr Diabetes.* 2018;19(7):1276–1284.
 423. Giani E, Macedoni M, Barilli A, et al. Performance of the flash glucose monitoring system during exercise in youth with type 1 diabetes. *Diabetes Res Clin Pract.* 2018;146:321–329.
 424. Kovatchev BP, Patek SD, Ortiz EA, et al. Assessing sensor accuracy for non-adjunct use of continuous glucose monitoring. *Diabetes Technol Ther.* 2015;17(3):177–186.
 425. Edelman SV. Regulation catches up to reality. *J Diabetes Sci Technol.* 2017;11(1):160–164.
 426. Shapiro AR. FDA approval of nonadjunctive use of continuous glucose monitors for insulin dosing: a potentially risky decision. *JAMA.* 2017;318(16):1541–1542.
 427. Beck RW. Continuous glucose monitors for insulin dosing. *JAMA.* 2018;319(13):1383.
 428. Pettus J, Edelman SV. Recommendations for using real-time continuous glucose monitoring (rtCGM) data for insulin adjustments in type 1 diabetes. *J Diabetes Sci Technol.* 2017;11(1):138–1347.
 429. Forlenza GP, Argento NB, Laffel LM. Practical considerations on the use of continuous glucose monitoring in pediatrics and older adults and nonadjunctive use. *Diabetes Technol Ther.* 2017;19(S3):S13–S20.
 430. Kropff J, Choudhary P, Neupane S, et al. Accuracy and longevity of an implantable continuous glucose sensor in the PRECISE Study: a 180-day, prospective, multicenter, Pivotal Trial. *Diabetes Care.* 2017;40(1):63–68.
 431. Christiansen MP, Klaff LJ, Brazg R, et al. A prospective multicenter evaluation of the accuracy of a novel implanted continuous glucose sensor: PRECISE II. *Diabetes Technol Ther.* 2018;20(3):197–206.
 432. Heinemann L, Franc S, Phillip M, et al. Reimbursement for continuous glucose monitoring: a European view. *J Diabetes Sci Technol.* 2012;6(6):1498–1502.
 433. Battelino T, Danne T, Bergenstal RM, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the International Consensus on Time in Range. *Diabetes Care.* 2019;42(8):1593–1604.
 434. Scheiner G. *Practical CGM: A Guide to Improving Outcomes Through Continuous Glucose Monitoring.* Arlington, VA: American Diabetes Association, Inc.; 2015.
 435. Laffel LM, Aleppo G, Buckingham BA, et al. A practical approach to using trend arrows on the Dexcom G5 CGM system to manage

- children and adolescents with diabetes. *J Endocr Soc.* 2017;1(12):1461–1476.
436. Edge J, Acerini C, Campbell F, et al. An alternative sensor-based method for glucose monitoring in children and young people with diabetes. *Arch Dis Child.* 2017;102(6):543–549.
 437. Benassi K, Drobny J, Aye T. Real-time continuous glucose monitoring systems in the classroom/school environment. *Diabetes Technol Ther.* 2013;15(5):409–412.
 438. Erie C, Van Name MA, Weyman K, et al. Schooling diabetes: use of continuous glucose monitoring and remote monitors in the home and school settings. *Pediatr Diabetes.* 2018;19(1):92–97.
 439. Markowitz JT, Pratt K, Aggarwal J, et al. Psychosocial correlates of continuous glucose monitoring use in youth and adults with type 1 diabetes and parents of youth. *Diabetes Technol Ther.* 2012;14(6):523–526.
 440. Hirsch IB, Battelino T, Peters AL, et al. *Role of Continuous Glucose Monitoring in Diabetes Treatment.* Arlington, Va.: American Diabetes Association; 2018.
 441. Heinemann L, Kamann S. Adhesives used for diabetes medical devices: a neglected risk with serious consequences? *J Diabetes Sci Technol.* 2016;10(6):1211–1215.
 442. Englert K, Ruedy K, Coffey J, et al. Skin and adhesive issues with continuous glucose monitors: a sticky situation. *J Diabetes Sci Technol.* 2014;8(4):745–751.
 443. Hirsch IB, Ableseth J, Bode BW, et al. Sensor-augmented insulin pump therapy: results of the first randomized treat-to-target study. *Diabetes Technol Ther.* 2008;10(5):377–383.
 444. Slover RH, Welsh JB, Criego A, et al. Effectiveness of sensor-augmented pump therapy in children and adolescents with type 1 diabetes in the STAR 3 study. *Pediatr Diabetes.* 2012;13(1):6–11.
 445. Cengiz E, Sherr JL, Weinzimer SA, et al. Clinical equipoise: an argument for expedited approval of the first small step toward an autonomous artificial pancreas. *Expert Rev Med Devices.* 2012;9(4):315–317.
 446. Garg S, Brazg RL, Bailey TS, et al. Reduction in duration of hypoglycemia by automatic suspension of insulin delivery: the in-clinic ASPIRE study. *Diabetes Technol Ther.* 2012;14(3):205–209.
 447. Bergenstal RM, Klonoff DC, Garg SK, et al. Threshold-based insulin-pump interruption for reduction of hypoglycemia. *N Engl J Med.* 2013;369(3):224–232.
 448. Ly TT, Nicholas JA, Retterath A, et al. Effect of sensor-augmented insulin pump therapy and automated insulin suspension vs standard insulin pump therapy on hypoglycemia in patients with type 1 diabetes: a randomized clinical trial. *JAMA.* 2013;310(12):1240–1247.
 449. Danne T, Kordonouri O, Holder M, et al. Prevention of hypoglycemia by using low glucose suspend function in sensor-augmented pump therapy. *Diabetes Technol Ther.* 2011;13(11):1129–1134.
 450. Agrawal P, Welsh JB, Kannard B, et al. Usage and effectiveness of the low glucose suspend feature of the Medtronic Paradigm Veo insulin pump. *J Diabetes Sci Technol.* 2011;5(5):1137–1141.
 451. Sherr JL, Collazo MP, Cengiz E, et al. Safety of nighttime 2-hour suspension of basal insulin in pump-treated type 1 diabetes even in the absence of low glucose. *Diabetes Care.* 2014;37(3):773–779.
 452. Maahs DM, Calhoun P, Buckingham BA, et al. A randomized trial of a home system to reduce nocturnal hypoglycemia in type 1 diabetes. *Diabetes Care.* 2014;37(7):1885–1891.
 453. Buckingham BA, Raghinaru D, Cameron F, et al. Predictive low-glucose insulin suspension reduces duration of nocturnal hypoglycemia in children without increasing ketosis. *Diabetes Care.* 2015;38(7):1197–1204.
 454. Calhoun PM, Buckingham BA, Maahs DM, et al. Efficacy of an overnight predictive low-glucose suspend system in relation to hypoglycemia risk factors in youth and adults with type 1 diabetes. *J Diabetes Sci Technol.* 2016;10(6):1216–1221.
 455. Buckingham BA, Bailey TS, Christiansen M, et al. Evaluation of a predictive low-glucose management system in-clinic. *Diabetes Technol Ther.* 2017;19(5):288–292.
 456. Battelino T, Nimri R, Dovc K, et al. Prevention of hypoglycemia with predictive low glucose insulin suspension in children with type 1 diabetes: a randomized controlled trial. *Diabetes Care.* 2017;40(6):764–770.
 457. Abraham MB, Nicholas JA, Smith GJ, et al. Reduction in hypoglycemia with the predictive low-glucose management system: a long-term randomized controlled trial in adolescents with type 1 diabetes. *Diabetes Care.* 2018;41(2):303–310.
 458. Scaramuzza AE, Arnaldi C, Cherubini V, et al. Use of the predictive low glucose management (PLGM) algorithm in Italian adolescents with type 1 diabetes: CareLink data download in a real-world setting. *Acta Diabetol.* 2017;54(3):317–319.
 459. Forlenza GP, Li Z, Buckingham BA, et al. Predictive low-glucose suspend reduces hypoglycemia in adults, adolescents, and children with type 1 diabetes in an at-home randomized crossover study: results of the PROLOG Trial. *Diabetes Care.* 2018;41(10):2155–2161.
 460. Danne T, Phillip M, Buckingham BA, et al. ISPAD Clinical Practice Consensus Guidelines 2018: insulin treatment in children and adolescents with diabetes. *Pediatr Diabetes.* 2018;19(Suppl 27):115–135.
 461. Beck RW, Raghinaru D, Wadwa RP, et al. Frequency of morning ketosis after overnight insulin suspension using an automated nocturnal predictive low glucose suspend system. *Diabetes Care.* 2014;37(5):1224–1229.
 462. Scaramuzza AE, Arnaldi C, Cherubini V, et al. Recommendations for the use of sensor-augmented pumps with predictive low-glucose suspend features in children: the importance of education. *Pediatr Diabetes.* 2017;18(8):883–889.
 463. Steil G, Rebrin K, Mastrototaro JJ. Metabolic modelling and the closed-loop insulin delivery problem. *Diabetes Res Clin Pract.* 2006;74(Suppl 2):S183–S186.
 464. Hovorka R, Canonico V, Chassin LJ, et al. Nonlinear model predictive control of glucose concentration in subjects with type 1 diabetes. *Physiol Meas.* 2004;25(4):905–920.
 465. Mauseth R, Wang Y, Dassau E, et al. Proposed clinical application for tuning fuzzy logic controller of artificial pancreas utilizing a personalization factor. *J Diabetes Sci Technol.* 2010;4(4):913–922.
 466. Atlas E, Nimri R, Miller S, et al. MD-Logic artificial pancreas system: a pilot study in adults with type 1 diabetes mellitus. *Diabetes Care.* 2010;33(5):1072–1076.
 467. Steil GM, Rebrin K, Darwin C, et al. Feasibility of automating insulin delivery for the treatment of type 1 diabetes. *Diabetes.* 2006;55(12):3344–3350.
 468. Hovorka R, Allen JM, Elleri D, et al. Manual closed-loop insulin delivery in children and adolescents with type 1 diabetes: a phase 2 randomized crossover trial. *Lancet.* 2010;375(9716):743–751.
 469. Phillip M, Battelino T, Atlas E, et al. Nocturnal glucose control with an artificial pancreas at a diabetes camp. *N Engl J Med.* 2013;368(9):824–833.
 470. Ly TT, Breton MD, Keith-Hynes P, et al. Overnight glucose control with an automated, unified safety system in children and adolescents with type 1 diabetes at diabetes camp. *Diabetes Care.* 2014;37(8):2310–2316.
 471. Ly TT, Buckingham BA, DeSalvo DJ, et al. Day-and-night closed-loop control using the unified safety system in adolescents with type 1 diabetes at camp. *Diabetes Care.* 2016;39(8):e106–e107.
 472. Ly TT, Keenan DB, Roy A, et al. Automated overnight closed-loop control using a proportional-integral-derivative algorithm with insulin feedback in children and adolescents with type 1 diabetes at diabetes camp. *Diabetes Technol Ther.* 2016;18(6):377–384.
 473. Ly TT, Roy A, Grosman B, et al. Day and night closed-loop control using the integrated Medtronic hybrid closed-loop system in type 1 diabetes at diabetes camp. *Diabetes Care.* 2015;38(7):1205–1211.
 474. Breton MD, Chernavsky DR, Forlenza GP, et al. Closed-loop control during intense prolonged outdoor exercise in adolescents with type 1 diabetes: the Artificial Pancreas Ski Study. *Diabetes Care.* 2017;40(12):1644–1650.
 475. Russell SJ, El-Khatib FH, Sinha M, et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N Engl J Med.* 2014;371(4):313–325.
 476. Kovatchev BP, Renard E, Cobelli C, et al. Feasibility of outpatient fully integrated closed-loop control first studies of wearable artificial pancreas. *Diabetes Care.* 2013;36(7):1851–1858.
 477. Sharifi A, De Bock MI, Jayawardene D, et al. Glycemia, treatment satisfaction, cognition, and sleep quality in adults and adolescents with type 1 diabetes when using a closed-loop system overnight versus sensor-augmented pump with low-glucose suspend function: a randomized crossover study. *Diabetes Technol Ther.* 2016;18(12):772–783.

478. Weinzimer SA, Steil GM, Swan KL, et al. Fully automated closed-loop insulin delivery versus semiautomated hybrid control in pediatric patients with type 1 diabetes using an artificial pancreas. *Diabetes Care*. 2008;31(5):934–939.
479. Castle JR, Engle JM, El Youssef J, et al. Novel use of glucagon in a closed-loop system for prevention of hypoglycemia in type 1 diabetes. *Diabetes Care*. 2010;33(6):1282–1287.
480. El-Khatib FH, Russell SJ, Magyar KL, et al. Autonomous and continuous adaptation of a bi-hormonal bionic pancreas in adults and adolescents with type 1 diabetes. *J Clin Endocrinol Metab*. 2014;99(5):1701–1711.
481. Haidar A, Legault L, Dallaire M, et al. Glucose-responsive insulin and glucagon delivery (dual-hormone artificial pancreas) in adults with type 1 diabetes: a randomized crossover controlled trial. *Can Med Assoc J*. 2013;185(4):297–305.
482. Russell SJ, El-Khatib FH, Nathan DM, et al. Blood glucose control in type 1 diabetes with a bi-hormonal bionic endocrine pancreas. *Diabetes Care*. 2012;35(11):2148–2155.
483. Van Bon AC, Jonker LD, Koebrugge R, et al. Feasibility of a bi-hormonal closed-loop system to control postexercise and postprandial glucose excursions. *J Diabetes Sci Technol*. 2012;6(5):1114–1122.
484. DeBoer MD, Breton MD, Wakeman C, et al. Performance of an artificial pancreas system for young children with type 1 diabetes. *Diabetes Technol Ther*. 2017;19(5):293–298.
485. Sharifi A, De Bock MI, Jayawardene D, et al. Glycemia, treatment satisfaction, cognition, and sleep quality in adults and adolescents with type 1 diabetes when using a closed-loop system overnight versus sensor-augmented pump with low-glucose suspend function: a randomized crossover study. *Diabetes Technol Ther*. 2016;18(12):772–783.
486. de Bock MI, Roy A, Cooper MN, et al. Feasibility of outpatient 24-hour closed-loop insulin delivery. *Diabetes Care*. 2015;38(11):e186–187.
487. Haidar A, Rabasa-Lhoret R, Legault L, et al. Single-and dual-hormone artificial pancreas for overnight glucose control in type 1 diabetes. *J Clin Endocrinol*. 2016;101(1):214–223.
488. Hovorka R, Elleri D, Thabit H, et al. Overnight closed-loop insulin delivery in young people with type 1 diabetes: a free-living, randomized clinical trial. *Diabetes Care*. 2014;37(5):1204–1211.
489. Nimri R, Muller I, Atlas E, et al. Night glucose control with MD-Logic artificial pancreas in home setting: a single blind, randomized crossover trial -interim analysis. *Pediatr Diabetes*. 2014;15(2):91–99.
490. Nimri R, Muller I, Atlas E, et al. MD-Logic overnight control for 6 weeks of home use in patients with type 1 diabetes: randomized crossover trial. *Diabetes Care*. 2014;37(11):3025–3032.
491. Spaic T, Driscoll M, Raghinaru D, et al. Predictive hyperglycemia and hypoglycemia minimization: in-home evaluation of safety, feasibility, and efficacy in overnight glucose control in type 1 diabetes. *Diabetes Care*. 2017;40(3):359–366.
492. Tauschmann M, Allen JM, Wilinska ME, et al. Day-and-night hybrid closed-loop insulin delivery in adolescents with type 1 diabetes: a free-living, randomized clinical trial. *Diabetes Care*. 2016;39(7):1168–1174.
493. Thabit H, Elleri D, Leelarathna L, et al. Unsupervised home use of an overnight closed-loop system over 3–4 weeks: a pooled analysis of randomized controlled studies in adults and adolescents with type 1 diabetes. *Diabetes Obes Metab*. 2015;17(5):452–458.
494. Thabit H, Tauschmann M, Allen JM, et al. Home use of an artificial beta cell in type 1 diabetes. *N Engl J Med*. 2015;373(22):2129–2140.
495. Tauschmann M, Allen JM, Wilinska ME, et al. Home use of day-and-night hybrid closed-loop insulin delivery in suboptimally controlled adolescents with type 1 diabetes: a 3-week, free-living, randomized crossover trial. *Diabetes Care*. 2016;39(11):2019–2025.
496. Anderson SM, Raghinaru D, Pinsky JE, et al. Multinational home use of closed-loop control is safe and effective. *Diabetes Care*. 2016;39(7):1143–1150.
497. Blauw H, van Bon A, Koops R, et al. Performance and safety of an integrated bi-hormonal artificial pancreas for fully automated glucose control at home. *Diabetes Obes Metab*. 2016;18(7):671–677.
498. Del Favero S, Bruttomesso D, Di Palma F, et al. First use of model predictive control in outpatient wearable artificial pancreas. *Diabetes Care*. 2014;37(5):1212–1215.
499. El-Khatib FH, Balliro C, Hillard MA, et al. Home use of a bi-hormonal bionic pancreas versus insulin pump therapy in adults with type 1 diabetes: a multicentre randomised crossover trial. *Lancet*. 2017;389(10067):369–380.
500. Kropff J, Del Favero S, Place J, et al. 2-month evening and night closed-loop glucose control in patients with type 1 diabetes under free-living conditions: a randomised crossover trial. *Lancet Diabetes Endocrinol*. 2015;3(12):939–947.
501. Leelarathna L, Dellweg S, Mader JK, et al. Day and night home closed-loop insulin delivery in adults with type 1 diabetes: three-center randomized crossover study. *Diabetes Care*. 2014;37(7):1931–1937.
502. Thabit H, Lubina-Solomon A, Stadler M, et al. Home use of closed-loop insulin delivery for overnight glucose control in adults with type 1 diabetes: a 4-week, multicentre, randomised crossover study. *Lancet Diabetes Endocrinol*. 2014;2(9):701–709.
503. Sherr, J.L., Cengiz, E., Palerm, C.C., et al. Reduced hypoglycemia and increased time in target using closed-loop insulin delivery during nights with or without antecedent afternoon exercise in type 1 diabetes. *Diabetes Care*, 36(10), 2909–2914.
504. Bruttomesso D. Toward automated insulin delivery. *N Engl J Med*. 2019;38(18):1774–1775.
505. Troncone A, Bonfanti R, Iafusco D, et al. Evaluating the experience of children with type 1 diabetes and their parents taking part in an artificial pancreas clinical trial over multiple days in a diabetes camp setting. *Diabetes Care*. 2016;39(12):2158–2164.
506. Barnard KD, Wysocki T, Allen JM, et al. Closing the loop overnight at home setting: psychosocial impact for adolescents with type 1 diabetes and their parents. *BMJ Open Diabetes Res Care*. 2014;2(1). e000025.
507. Barnard KD, Wysocki T, Thabit H, et al. Psychosocial aspects of closed-and open-loop insulin delivery: closing the loop in adults with type 1 diabetes in the home setting. *Diabetes Med*. 2015;32(5):601–608.
508. Weissberg-Benchell J, Hessler D, Polonsky WH, et al. Psychosocial impact of the bionic pancreas during summer camp. *J Diabetes Sci Technol*. 2016;10(4):840–844.
509. Schatz D. 2016 Presidential address: diabetes at 212—confronting the invisible disease. *Diabetes Care*. 2016;39(10):1657–1663.
510. Diabetesatlas.org. Brussels, Belgium: International Diabetes Federation IDF Diabetes Atlas – 9th edition; 2017. Available at www.diabetesatlas.org. Last accessed 22 November 2018.
511. Beck RW, Tamborlane WV, Bergenstal RM, et al. For the T1D exchange clinic network. The T1D exchange clinic registry. *J Clin Endocrinol Metab*. 2012;97(12):4383–4389.
512. Vigersky RA, Fish L, Hogan P, et al. The Clinical Endocrinology Workforce: current status and future projections of supply and demand. *J Clin Endocrinol Metab*. 2014;99(9):3112–3121.
513. Comellas MJ, Albiñana E, Artes M, et al. Evaluation of a new digital automated glycemic pattern detection tool. *Diabetes Technol Ther*. 2017;19:633–640.
514. Mobilemarketingmagazine.com. 2.4bn smartphone users in 2017 says marketer. Available at <https://mobilemarketingmagazine.com/24bn-smartphone-users-in-2017-says-emarketer>. Last accessed 22 November 2018.
515. Graetz, I., Gordon, N., Fung, V., et al. The digital divide and patient portals: internet access explained differences in patient portal use for secure messaging by age, race, and income. *Med Care*, 54(8), 772–779.
516. Diabetetechnology.org. Diabetes Technology Society: Standard for Wireless Diabetes Device Security (DTSec); 2016. Available at <https://www.diabetestechnology.org/dtsec-standard-final.pdf>. Last accessed 22 November 2018.
517. Nimri R, Dassau E, Segall T, et al. Adjusting insulin doses in patients with type 1 diabetes that use insulin pump and continuous glucose monitoring - Variations among countries and physicians. *Diabetes Obes Metab*. 2018;20(10):2458–2466.
518. Device classification under section 513(f)(2)(de novo) <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?id=den170043>.

519. Rigla M, García-Sáez G, Pons B, et al. Artificial intelligence methodologies and their application to diabetes. *J Diabetes Sci Technol*. 2018;12(2):303–310.
520. Zimmermann E, Lenart A, Da Rocha Fernandes JD, et al. Use of big data algorithms to characterize patients with T2D on basal insulin (BI) who add a glucagon-like peptide-1 receptor agonist (GLP-1 ra) and predict their a1c response. *Diabetes*. 2018;67(supplement 1):100.
521. Mei J, Zhao S, Jin F, et al. Deep diabetologist: learning to prescribe hypoglycemic medications with recurrent neural networks. *Stud Health Technol Inform*. 2017;245:1277.
522. Elliot M, Blackburn MC, Heard KM, et al. Sustainability of a real-time informatics alert to prevent inpatient severe hypoglycemia. *Diabetes*. 2018;67(supplement 1):397.
523. Mcconnell MV, Shcherbina A, Pavlovic A, et al. Feasibility of obtaining measures of lifestyle from a smartphone app: the myheart counts cardiovascular health study. *JAMA Cardiol*. 2017;2(1):67–76.
524. Openaps. The open artificial pancreas system project n.d. <https://openaps.org/>.
525. Tidepool N.D. <https://tidepool.org>.
526. <http://www.diabetesforecast.org/2017-mar-apr/diabetes-applications.html>. Last accessed 13 December 2018.
527. <https://www.healthline.com/health/diabetes/top-iphone-android-apps>. Last accessed 13 December 2018.
528. [Diabetestechology.org](http://www.diabetestechology.org). Diabetes Technology Society: Standard for Wireless Diabetes Device Security (DTSec); 2016. Available at <https://www.diabetestechology.org/dtsec-standard-final.pdf>. Last accessed 22 November 2018.
529. Danne T, Nimri R, Battelino T, et al. International Consensus on Use of Continuous Glucose Monitoring. *Diabetes Care*. 2017;40(12):1631–1640.
530. Petrie JR, Peters AL, Bergenstal RM, et al. Improving the clinical value and utility of CGM systems: issues and recommendations - a joint statement of the European association for the study of diabetes and the American Diabetes Association diabetes technology working group. *Diabetes Care*. 2017;40(12):1614–1621.
531. Gregory RP, Davis DL. Use of carbohydrate counting for meal planning in type I diabetes. *Diabetes Educator*. 1994;20(5):406–409.
532. Aslander-van Vliet E, Smart C, Waldron S. Nutrition management of childhood and adolescent diabetes. *Pediatr Diabetes*. 2007;8:323–339.
533. Ahern JA, Gatcomb PM, Held NA, Petit Jr WA, Tamborlane WV. Exaggerated hyperglycemia after a pizza meal in well-controlled diabetes. *Diabetes Care*. 1993;16(4):578–580.
534. Pankowska E, Blazik M. Bolus calculator with nutrition database software, a new concept of prandial insulin programming for pump users. *J Diabetes Sci Technol*. 2010;4(3):571–576.
535. American Diabetes Association Task Force for Writing Nutrition Principles and Recommendations for the Management of Diabetes and Related Complications. American Diabetes Association position statement: evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *J Am Diet Assoc*. 2002;102(1):109–118.
536. Beck RW, Tamborlane WV, Bergenstal RM, Miller KM, DuBose SN, Hall CA. The T1D Exchange clinic registry. *J Clin Endocrinol Metab*. 2012;97(12):4383–4389.
537. Corbin KD, Driscoll KA, Pratley RE, Smith SR, Maahs DM, Mayer-Davis EJ, Advancing Care for Type 1 Diabetes and Obesity Network (ACTION). Obesity in type 1 diabetes: pathophysiology, clinical impact, and mechanisms. *Endocr Rev*. 2018;39(5):629–663.
538. Riddell MC, Gallen IW, Smart CE, Taplin CE, Adolfsson P, Lumb AN, et al. Exercise management in type 1 diabetes: a consensus statement. *Lancet Diabetes Endocrinol*. 2017;5(5):377–390. Review. Erratum in: *Lancet Diabetes Endocrinol*. 2017, 5(5), e3.
539. Adolfsson P, Riddell MC, Taplin CE, Davis EA, Fournier PA, Annan F, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Exercise in children and adolescents with diabetes. *Pediatr Diabetes*. 2018;19(Suppl 27):205–226.
540. Tansey MJ, Tsalikian E, Beck RW, Mauras N, Buckingham BA, Weinzimer SA, et al. The effects of aerobic exercise on glucose and counterregulatory hormone concentrations in children with type 1 diabetes. *Diabetes Care*. 2006;29(1):20–25.
541. Tsalikian E, Mauras N, Beck RW, Tamborlane WV, Janz KF, Chase HP, et al. Impact of exercise on overnight glycemic control in children with type 1 diabetes mellitus. *J Pediatr*. 2005;147(4):528–534.
542. McMahon SK, Ferreira LD, Ratnam N, Davey RJ, Youngs LM, Davis EA, et al. Glucose requirements to maintain euglycemia after moderate-intensity afternoon exercise in adolescents with type 1 diabetes are increased in a biphasic manner. *J Clin Endocrinol Metab*. 2007;92(3):963–968.
543. Wood MA, Shulman DI, Forlenza GP, Bode BW, Pinhas-Hamiel O, Buckingham BA, et al. In-clinic evaluation of the Mini-Med 670G system "suspend before low" feature in children with type 1 diabetes. *Diabetes Technol Ther*. 2018;20(11):731–737.
544. Tamborlane WV. Triple jeopardy: nocturnal hypoglycemia after exercise in the young with diabetes. *J Clin Endocrinol Metab*. 2007;92(3):815–816.
545. Sherr J, Xing D, Ruedy KJ, Beck RW, Kollman C, Buckingham B, et al. Lack of association between residual insulin production and glucagon response to hypoglycemia in youth with short duration of type 1 diabetes. *Diabetes Care*. 2013;36(6):1470–1476.
546. Cengiz E, Connor CG, Ruedy KJ, Beck RW, Kollman C, Klingensmith GJ, et al. Pediatric diabetes consortium T1D New Onset (NeOn) study: clinical outcomes during the first year following diagnosis. *Pediatr Diabetes*. 2014;15(4):287–293.
547. The DCCT Research Group. Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual beta-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT). *J Clin Endocrinol Metab*. 1987;65(1):30–36.
548. The Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual beta-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial. *Ann Intern Med*. 1998;128(7):517–523.
549. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*. 2002;346(22):1692–1698.
550. Haynes A, Hermann JM, Miller KM, Hofer SE, Jones TW, Beck RW, et al. T1D Exchange, WACDD and DPV registries. Severe hypoglycemia rates are not associated with HbA1c: a cross-sectional analysis of 3 contemporary pediatric diabetes registry databases. *Pediatr Diabetes*. 2016;18(7):643–650.
551. Karges B, Schwandt A, Heidtmann B, Kordonouri O, Binder E, Schierloh U, et al. Association of insulin pump therapy vs insulin injection therapy with severe hypoglycemia, ketoacidosis, and glycemic control among children, adolescents, and young adults with type 1 diabetes. *JAMA*. 2017;318(14):1358–1366.
552. American Diabetes Association. Minimizing hypoglycemia in diabetes. *Diabetes Care*. 2015;38:1583–1591.
553. Seaquist ER, Anderson J, Childs B, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care*. 2013;36:1384–1395.
554. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. *Pediatr Diabetes*. 2018;19(Suppl 27):178–192.
555. Jones TW, Boulware SD, Kraemer DT, Caprio S, Sherwin RS, Tamborlane WV. Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. *Diabetes*. 1991;40(3):358–363.
556. Ryan C, Gurtunca N, Becker D. Hypoglycemia: a complication of diabetes therapy in children. *Pediatr Clin North Am*. 2005;52(6):1705–1733.
557. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes*. 2005;54(12):3592–3601.
558. Cryer PE. Diverse causes of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*. 2004;350(22):2272–2279.
559. Tsalikian E, Tamborlane W, Xing D, Becker DM, Mauras N, Fiallo-Scharer R, et al. Blunted counterregulatory hormone responses to hypoglycemia in young children and adolescents with well-controlled type 1 diabetes. *Diabetes Care*. 2009;32(11):1954.

560. Jones TW, Porter P, Sherwin RS, Davis EA, O'Leary P, Frazer F, et al. Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med*. 1998;338(23):1657–1662.
561. Craig ME, Prinz N, Boyle CT, Campbell FM, Jones TW, Hofer SE, et al. Australasian Diabetes Data Network (ADDN); T1D Exchange Clinic Network (T1DX); National Paediatric Diabetes Audit (NPDA) and the Royal College of Paediatrics and Child Health; Prospective Diabetes Follow-up Registry (DPV) initiative. Prevalence of celiac disease in 52,721 youth with type 1 diabetes: international comparison across three continents. *Diabetes Care*. 2017;40(8):1034–1040.
562. Freemark M, Levitsky LL. Screening for celiac disease in children with type 1 diabetes: two views of the controversy. *Diabetes Care*. 2003;26(6):1932–1939.
563. Springer D, Dziura J, Tamborlane WV, Steffen AT, Ahern JH, Vincent M, et al. Optimal control of type 1 diabetes mellitus in youth receiving intensive treatment. *J Pediatr*. 2006;149(2):227–232.
564. Palau-Collazo MM, Rose P, Sikes K, Kim G, Benavides V, Urban A, et al. Effectiveness of a Spanish language clinic for Hispanic youth with type 1 diabetes. *Endocr Pract*. 2013;19(5):800–804.
565. Doyle EA, Weinzimer SA, Steffen AT, Ahern JA, Vincent M, Tamborlane WV. A randomized, prospective trial comparing the efficacy of continuous subcutaneous insulin infusion with multiple daily injections using insulin glargine. *Diabetes Care*. 2004;27(7):1554–1558.
566. Grey MJ, Genel M, Tamborlane WV. Psychosocial adjustment of latency-aged diabetics: determinants and relationship to control. *Pediatrics*. 1980;65(1):69–73.
567. Silverstein J, Klingensmith G, Copeland K, Plotnick L, Kaufman F, Laffel L, et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. *Diabetes Care*. 2005;28(1):186.
568. Codner E, Acerini CL, Craig ME, Hofer SE, Maahs DM. ISPAD Clinical Practice Consensus Guidelines 2018: What is new in diabetes care? *Pediatr Diabetes*. 2018;19(Suppl 27):5–6.
569. Griffin ME, Feder A, Tamborlane WV. Lipoatrophy associated with lispro insulin in insulin pump therapy: an old complication, a new cause? *Diabetes Care*. 2001;24(1):174.
570. Wood JR, Kaminski BM, Kollman C, Beck RW, Hall CA, Yun JP, et al. Accuracy and precision of the Axis-Shield Afinion hemoglobin A1c measurement device. *J Diabetes Sci Technol*. 2012;6(2):380–386.
571. Donaghue, K.C., Marcovecchio, M.L., Wadwa, R.P., Chew, E.Y., Wong, T.Y., Calliari, L.E., et al. (2018). ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes*, 19 Suppl 27, 262–274.
572. Huo B, Steffen AT, Swan K, Sikes K, Weinzimer SA, Tamborlane WV. Clinical outcomes and cost-effectiveness of retinopathy screening in youth with type 1 diabetes. *Diabetes Care*. 2007;30(2):362–363.
573. Jefferies C, Rhodes E, Rachmiel M, Chizo AJ, Kapellen T, Abdulla MA, Hofer SE. SPAD Clinical Practice Consensus Guidelines 2018: Management of children and adolescents with diabetes requiring surgery. *Pediatr Diabetes*. 2018;19(Suppl 27):227–236.
574. Rhodes ET, Ferrari LR, Wolfsdorf JI. Perioperative management of pediatric surgical patients with diabetes mellitus. *Anesth Analg*. 2005;101(4):986–999. table of contents.
575. Betts P, Brink SJ, Swift PG, Silink M, Wolfsdorf J, Hanas R. Management of children with diabetes requiring surgery. *Pediatr Diabetes*. 2007;8(4):242–247.
576. Isermann B, Ritzel R, Zorn M, Schilling T, Nawroth PP. Autoantibodies in diabetes mellitus: current utility and perspectives. *Exp Clin Endocrinol Diabetes*. 2007;115(8):483–490.
577. Kim MS, Quintos JB. Mauriac syndrome: growth failure and type 1 diabetes mellitus. *Pediatr Endocrinol Rev*. 2008;5(Suppl 4):989–993.
578. Mauras N, Merimee T, Rogol AD. Function of the growth hormone-insulin-like growth factor I axis in the profoundly growth-retarded diabetic child: Evidence for defective target organ responsiveness in the Mauriac syndrome. *Metabolism*. 1991;40:1106–1111.
579. MacDonald MJ, et al. Discovery of a genetic metabolic cause for Mauriac syndrome in type 1 diabetes. *Diabetes*. 2016;65:2051–2059.
580. Mitchell DM. Growth in patients with type 1 diabetes. *Curr Opin Endocrinol Diabetes Obes*. 2017;24(1):67–72.
581. Rosenbloom AL. Limited joint mobility in childhood diabetes: discovery, description, and decline. *J Clin Endocrinol Metab*. 2013;98:466–473.
582. Hanna W, Friesen D, Bombardier C, Gladman D, Hanna A. Pathologic features of diabetic thick skin. *J Am Acad Dermatol*. 1987;16:546–553.
583. Diabetes Control and Complications Trial Research Group, Nathan, D.M., et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;329:977–986.
584. Genuth S. Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. *Endocr Pract*. 2006;12(Suppl 1):34–41.
585. Benitez-Aguirre P, et al. Sex differences in retinal microvasculature through puberty in type 1 diabetes: are girls at greater risk of diabetic microvascular complications? *Invest Ophthalmol Vis Sci*. 2015;56:571–577.
586. Amin R, et al. Risk of microalbuminuria and progression to macroalbuminuria in a cohort with childhood onset type 1 diabetes: prospective observational study. *BMJ*. 2008;336:697–701.
587. Donaghue KC, et al. Do all prepubertal years of diabetes duration contribute equally to diabetes complications? *Diabetes Care*. 2003;26:1224–1229.
588. Sawicki PT, et al. Smoking is associated with progression of diabetic nephropathy. *Diabetes Care*. 1994;17:126–131.
589. Gallego PH, Craig ME, Hing S, Donaghue KC. Role of blood pressure in development of early retinopathy in adolescents with type 1 diabetes: prospective cohort study. *BMJ*. 2008;337:a918.
590. Marcovecchio ML, et al. Prevalence of abnormal lipid profiles and the relationship with the development of microalbuminuria in adolescents with type 1 diabetes. *Diabetes Care*. 2009;32:658–663.
591. Donaghue KC, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. *Pediatr Diabetes*. 2018;19(Suppl 2):262–274.
592. Sauder KA, et al. Co-occurrence of early diabetes-related complications in adolescents and young adults with type 1 diabetes: an observational cohort study. *Lancet Child Adolesc Heal*. 2019;3(1):35–43.
593. Afkarian M. Diabetic kidney disease in children and adolescents. *Pediatr Nephrol*. 2015;30:65–74. quiz 70–1.
594. Tervaert TWC, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol*. 2010;21:556–563.
595. Drummond K, Mauer M, International Diabetic Nephropathy Study Group. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. *Diabetes*. 2002;51:1580–1587.
596. Pavenstädt H, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev*. 2003;83:253–307.
597. Torbjörnsdotter TB, Staffansdotter Sohlman Perrin NE, Jaremko GA, Berg UB. Widening of foot processes in normoalbuminuric adolescents with type 1 diabetes. *Pediatr Nephrol*. 2005;20:750–758.
598. Thomas MC, Burns WC, Cooper ME. Tubular changes in early diabetic nephropathy. *Adv Chronic Kidney Dis*. 2005;12:177–186.
599. Al-Kafaji G, Golbahar J. High glucose-induced oxidative stress increases the copy number of mitochondrial DNA in human mesangial cells. *Biomed Res Int*. 2013;7:54946.
600. Thallas-Bonke V, Cooper ME. Tandem inhibition of PKC in Diabetic nephropathy: it takes two to tango? *Diabetes*. 2013;62:1010–1011.
601. Mukhi D, Nishad R, Menon RK, Pasupulati AK. Novel actions of growth hormone in podocytes: implications for diabetic nephropathy. *Front Med*. 2017;4:102.
602. Ichihara A, et al. Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol*. 2006;17:1950–1961.
603. Saraheimo M, Teppo A-M, Forsblom C, Fagerudd J, Groop P-H. Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. *Diabetologia*. 2003;46:1402–1407.

604. Thomson SC, Vallon V, Blantz RC. Kidney function in early diabetes: the tubular hypothesis of glomerular filtration. *Am J Physiol Renal Physiol*. 2004;286:F8–15.
605. Hansen KW, et al. Normoalbuminuria ensures no reduction of renal function in type 1 (insulin-dependent) diabetic patients. *J Intern Med*. 1992;232:161–167.
606. Ellis EN, et al. Renal structural-functional relationships in early diabetes mellitus. *Pediatr Nephrol*. 1997;11:584–591.
607. Marcovecchio ML, et al. Renal and cardiovascular risk according to tertiles of urinary albumin-to-creatinine ratio: The Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AddIT). *Diabetes Care*. 2018;41:1963–1969.
608. Perkins BA, et al. Regression of microalbuminuria in type 1 diabetes. *N Engl J Med*. 2003;348:2285–2293.
609. Lambers Heerspink HJ, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. *J Am Soc Nephrol*. 2010;21:1355–1360.
610. American Diabetes Association. Microvascular complications and foot care: standards of medical care in diabetes-2018. *Diabetes Care*. 2018;41:S105–S118.
611. Flynn JT, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. 2017;140.
612. ACE Inhibitors in Diabetic Nephropathy Trialist Group. Should all patients with type 1 diabetes mellitus and microalbuminuria receive angiotensin-converting enzyme inhibitors? *A meta-analysis of individual patient data* *Ann Intern Med*. 2001;134:370–379.
613. Flaxman SR, et al. Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. *Lancet Glob Health*. 2017;5:e1221–e1234.
614. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. IX. Four-year incidence and progression of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol (Chicago, Ill 1960)*. 1989;107:237–243.
615. Downie E, et al. Continued reduction in the prevalence of retinopathy in adolescents with type 1 diabetes: role of insulin therapy and glycemic control. *Diabetes Care*. 2011;34:2368–2373.
616. Cunha-Vaz J, Bernardes R, Lobo C. Blood-retinal barrier. *Eur J Ophthalmol*. 2011;21(Suppl 6). S3–9.
617. Santiago AR, Boia R, Aires ID, Ambrósio AF, Fernandes R. Sweet stress: coping with vascular dysfunction in diabetic retinopathy. *Front Physiol*. 2018;9:820.
618. Ting DSW, et al. Development and validation of a deep learning system for diabetic retinopathy and related eye diseases using retinal images from multiethnic populations with diabetes. *JAMA*. 2017;318:2211–2223.
619. Feldman-Billard S, Llarger É, Massin P, Standards for screening and surveillance of ocular complications in people with diabetes SFD study group. Early worsening of diabetic retinopathy after rapid improvement of blood glucose control in patients with diabetes. *Diabetes Metab*. 2018;44:4–14.
620. Daneman D, et al. Progressive retinopathy with improved control in diabetic dwarfism (Mauriac's syndrome). *Diabetes Care*. 1981;4(3):360–365.
621. Best RM, Chakravarthy U. Diabetic retinopathy in pregnancy. *Br J Ophthalmol*. 1997;81:249–251.
622. Evans JR, Michelessi M, Virgili G. Laser photocoagulation for proliferative diabetic retinopathy. *Cochrane database Syst Rev*. 2014;. CD011234.
623. Sivaprasad S, et al. Clinical efficacy of intravitreal aflibercept versus panretinal photocoagulation for best corrected visual acuity in patients with proliferative diabetic retinopathy at 52 weeks (CLARITY): a multicentre, single-blinded, randomised, controlled, phase 2b, n. *Lancet (London, England)*. 2017;389:2193–2203.
624. Tan GS, Cheung N, Simó R, Cheung GCM, Wong TY. Diabetic macular oedema. *Lancet Diabetes Endocrinol*. 2017;5:143–155.
625. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. *JAMA*. 2007;298:902–916.
626. Mah JK, Pacaud D. Diabetic neuropathy in children. *Handb Clin Neurol*. 2014;126:123–143.
627. Pop-Busui R, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care*. 2017;40:136–154.
628. Nelson D, et al. Comparison of conventional and non-invasive techniques for the early identification of diabetic neuropathy in children and adolescents with type 1 diabetes. *Pediatr Diabetes*. 2006;7:305–310.
629. Jaiswal M, et al. Peripheral neuropathy in adolescents and young adults with type 1 and type 2 diabetes from the SEARCH for Diabetes in Youth follow-up cohort: a pilot study. *Diabetes Care*. 2013;36:3903–3908.
630. Solders G, et al. Nerve conduction and autonomic nerve function in diabetic children. A 10-year follow-up study. *Acta Paediatr*. 1997;86:361–366.
631. Verrotti A, Chiarelli F, Blasetti A, Morgese G. Autonomic neuropathy in diabetic children. *J Paediatr Child Health*. 1995;31:545–548.
632. Boulton AJM, Kempner P, Ametov A, Ziegler D. Whither pathogenetic treatments for diabetic polyneuropathy? *Diabetes Metab Res Rev*. 2013;29:327–333.
633. Omladic JS, Ozimic AS, Vovk A, et al. Acute hyperglycemia and spatial working memory in adolescents with type 1 diabetes. *Diabetes Care*. 2020;43:1–4.
634. Fox LA, Hershey T, Mauras N, et al. Persistence of abnormalities in white matter in children with type 1 diabetes. *Diabetologia*. 2018;61(7):1538–1547.
635. Bjornstad P, Donaghue KC, Maahs DM. Macrovascular disease and risk factors in youth with type 1 diabetes: time to be more attentive to treatment? *Lancet Diabetes Endocrinol*. 2018;6:809–820.
636. Chan CL, et al. The role of glycemia in insulin resistance in youth with type 1 and type 2 diabetes. *Pediatr Diabetes*. 2017;18:470–477.
637. Libman IM, Arslanian SA. Prevention and treatment of type 2 diabetes in youth. *Horm Res*. 2007;67(1):22–34.
638. Gungor N, Hannon T, Libman I, Bacha F, Arslanian S. Type 2 diabetes mellitus in youth: the complete picture to date. *Pediatr Clin North Am*. 2005;52(6):1579–1609.
639. Weiss R, Caprio S. Altered glucose metabolism in obese youth. *Pediatr Endocrinol Rev*. 2006;3(3):233–238.
640. Savoye M, Shaw M, Dziura J, Tamborlane WV, Rose P, Guandalini C, et al. Effects of a weight management program on body composition and metabolic parameters in overweight children: a randomized controlled trial. *JAMA*. 2007;297(24):2697–2704.
641. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*. 2004;350(23):2362–2374.
642. Weiss R, Caprio S, Trombetta M, Taksali SE, Tamborlane WV, Bonadonna R. Beta-cell function across the spectrum of glucose tolerance in obese youth. *Diabetes*. 2005;54(6):1735–1743.
643. Kralisch S, Sommer G, Deckert CM, Linke A, Bluher M, Stumvoll M, et al. Adipokines in diabetes and cardiovascular diseases. *Minerva Endocrinol*. 2007;32(3):161–171.
644. Yi P, Park JS, Melton DA. Betatrophin: a hormone that controls pancreatic beta cell proliferation. *Cell*. 2013;153(4):747–758.
645. Spiegelman BM. Banting Lecture 2012: Regulation of adipogenesis: toward new therapeutics for metabolic disease. *Diabetes*. 2013;62(6):1774–1782.
646. Imamura M, Maeda S. Genetics of type 2 diabetes: the GWAS era and future perspectives [Review]. *Endocr J*. 2011;58(9):723–739.
647. Xu M, Bi Y, Cui B, Hong J, Wang W, Ning G. The new perspectives on genetic studies of type 2 diabetes and thyroid diseases. *Curr Genomics*. 2013;14(1):33–48.
648. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science (New York, NY)*. 2007;316(5829):1336–1341.
649. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science (New York, NY)*. 2007;316(5829):1341–1345.
650. Pinhas-Hamiel O, Standiford D, Hamiel D, Dolan LM, Cohen R, Zeitler PS. The type 2 family: a setting for development and treatment of adolescent type 2 diabetes mellitus. *Arch Pediatr Adolesc Med*. 1999;153(10):1063–1067.
651. Rosenbloom AL. Distinguishing type 1 and type 2 diabetes at diagnosis. What is the problem? *Pediatr Diabetes*. 2007;8(2):51–52.

652. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet.* 2007;8(9):657–662.
653. Hattersley AT. Prime suspect: the TCF7L2 gene and type 2 diabetes risk. *J Clin Invest.* 2007;117(8):2077–2079.
654. Salonen JT, Uimari P, Aalto JM, Pirskanen M, Kaikkonen J, Todorova B, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am J Hum Genet.* 2007;81(2):338–345.
655. Geijselaers SLC, Sep SJS, Stehouwer CDA, et al. Glucose regulation, cognition, and brain MRI in type 2 diabetes: a systematic review. *Lancet Diabetes Endocrinol.* 2015;3(1):75–89.
656. Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, et al. Prevalence of diabetes in U.S. youth in 2009: the SEARCH for diabetes in youth study. *Diabetes Care.* 2014;37(2):402–408.
657. Zeitler P, Hirst K, Pyle L, Linder B, Copeland K, Arslanian S, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. *N Engl J Med.* 2012;366(24):2247–2256.
658. Bjerregaard LG, Jensen BW, Angquist L, Osler M, Sorensen TIA, Baker JL. Change in overweight from childhood to early adulthood and risk of type 2 diabetes. *N Engl J Med.* 2018;378(14):1302–1312.
659. Khattab A, Sperling MA. Obesity in adolescents and youth: the case for and against bariatric surgery. *J Pediatr.* 2019;207:18–22.
660. Solomon DH, Winkelmayer WC. Cardiovascular risk and the thiazolidinediones: déjà vu all over again? *JAMA.* 2007;298(10):1216–1218.
661. Rich S. Still a geneticists nightmare. *Nature.* 2016;536:37.
662. Jyoti R, et al. A gene atlas of type 2 diabetes mellitus associated complex disorders. *Sci Rep.* 2017;7:6892.
663. Tamborlane WV, Barrieno-Pere M, Fainberg V, et al. Liraglutide in children and adolescents with type 2 diabetes. *New Engl J Med.* 2019;381:637–646.
664. Hattersley AH, Patel KA. Precision Medicine: Learning from monogenic diabetes. *Diabetologia.* 2017;60:769–777.
665. Bonnefond A, Froguel P. Rare and common genetic events in Type 2 diabetes: What should biologists know. *Cell Metab.* 2015;21:357–368.
666. Karaa A, Goldstein A. The spectrum of clinical presentation, diagnosis, and management of mitochondrial forms of diabetes. *Pediatr Diabetes.* 2015;16(1):1–9.
667. Mancuso M, Filosto M, Choub A, Tentorio M, Broglio L, Padovani A, et al. Mitochondrial DNA-related disorders. *Biosci Rep.* 2007;27(1–3):31–37.
668. Urano F. Wolfram Syndrome: diagnosis, management and treatment. *Curr Diabetes Rep.* 2016;16:6.
669. Barrett TG, Scott-Brown M, Seller A, Bednarz A, Poulton K, Poulton J. The mitochondrial genome in Wolfram syndrome. *J Med Genet.* 2000;37(6):463–466.
670. Astuti D, Sabir A, Fulton P, et al. Monogenic diabetes syndromes: Locus-specific database for Alstrom, Wolfram and Thiamine-responsive megaloblastic anemia. *Hum Mutat.* 2017;38(7):764–777.
671. Shaw-Smith C, Flanagan SE, Patch AM, et al. Recessive SLC19A2 mutations are a cause of neonatal diabetes mellitus in thiamine-responsive megaloblastic anemia. *Pediatr Diabetes.* 2012;13(4):314–321.
672. Liu MZ, He HY, Luo JQ, et al. Drug-induced hyperglycemia and diabetes: pharmacogenomics perspectives. *Arch Pharm Res.* 2018;41(7):725–736.
673. Sharif A, Cohnsey S. Post-transplantation diabetes; State of the Art. *Lancet Diabetes Endocrinol.* 2016;4(4):337–349.
674. Chen J, Huang XF, Shao, et al. Molecular mechanism of antipsychotic drug-induced diabetes. *Front Neurosci.* 2017;11:643.
675. Moheet A, Moran A. CF-related diabetes: Containing the metabolic miscreant of cystic fibrosis. *Pediatr Pulmonol.* 2017;52(S48):S37–S43.
676. Teinturier C, Toumade MF, Caillat-Zucman S, Boitard C, Amoura Z, Bougneres PF, et al. Diabetes mellitus after abdominal radiation therapy. *Lancet (London, England).* 1995;346(8975):633–634.
677. Jun HS, Yoon JW. A new look at viruses in type 1 diabetes. *Diabetes Metabol Res Rev.* 2003;19(1):8–31.
678. Andreoli S, Bergstein J. Exocrine and endocrine pancreatic insufficiency and calcinosis after hemolytic uremic syndrome. *J Pediatr.* 1987;110(5):816–817.
679. Krogvold L, Edwin B, Buanes T, et al. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes.* 2015;64(5):1682–1687.
680. Altindis E, Cai W, Sagaguchi M, et al. Viral insulin-like peptides activate human insulin and IGF-1 receptor signaling: A paradigm shift for host-microbe interactions. *Proc Natl Acad Sci U S A.* 2018;115:2461–2466.
681. Low L, Chernauek SD, Sperling MA. Acromegaloid patients with type A insulin resistance: parallel defects in insulin and insulin-like growth factor-I receptors and biological responses in cultured fibroblasts. *J Clin Endocrinol Metab.* 1989;69(2):329–337.
682. Flier JS, Kahn CR, Roth J. Receptors, antireceptor antibodies and mechanisms of insulin resistance. *N Engl J Med.* 1979;300(8):413–419.
683. Chernauek SD. Mendelian genetic causes of the short child born small for gestational age. *J Endocrinol Invest.* 2006;29(1 Suppl):16–20.
684. Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S, et al. Clinical course of genetic diseases of the insulin receptor (type A and Rabson-Mendenhall syndromes): a 30-year prospective. *Medicine.* 2004;83(4):209–222.
685. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol.* 2014;6. a009191.
686. Hegele RA, Joy TR, Al-Attar SA, Rutt BK. Thematic review series: Adipocyte Biology. Lipodystrophies: windows on adipose biology and metabolism. *J Lipid Res.* 2007;48(7):1433–1444.
687. Brown RJ, Araujo-Vilar D, Cheung PT, et al. The diagnosis and management of lipodystrophy syndromes: a Multi-Society Practice guideline. *J Clin Endocrinol Metab.* 2016;101(12):4500–4511.
688. Crino A, Di Giorgio G, Manco M, Grugni G, Maggioni A. Effects of growth hormone therapy on glucose metabolism and insulin sensitivity indices in prepubertal children with Prader-Willi syndrome. *Horm Res.* 2007;68(2):83–90.
689. Giménez-Barcons M, Casteràs A, Armengol Mdel P, et al. Autoimmune predisposition in Down syndrome may result from a partial central tolerance failure due to insufficient intrathymic expression of AIRE and peripheral antigens. *J Immunol.* 2014;193(8):3872–3879.
690. Bakalov VK, Cooley MM, Quon MJ, Luo ML, Yanovski JA, Nelson LM, et al. Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab.* 2004;89(7):3516–3520.
691. Schmidt F, Kapellen TM, Wiegand S, et al. Diabetes mellitus in children and adolescents with genetic syndromes. *Exp Clin Endocrinol Diabetes.* 2012;120(10):579–585.
692. Aoki N. Klinefelter's syndrome, autoimmunity, and associated endocrinopathies. *Intern Med. (Tokyo, Japan).* 1999;38(11):838–839.
693. Minton JA, Owen KR, Ricketts CJ, Crabtree N, Shaikh G, Ehtisham S, et al. Syndromic obesity and diabetes: changes in body composition with age and mutation analysis of ALMS1 in 12 United Kingdom kindreds with Alstrom syndrome. *J Clin Endocrinol Metab.* 2006;91(8):3110–3116.
694. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *N Engl J Med.* 2011;364(16):1533–1543.
695. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nat Rev Endocrinol.* 2012;8(11):639–649.
696. Sperling MA. ATP-sensitive potassium channels—neonatal diabetes mellitus and beyond. *N Engl J Med.* 2006;355(5):507–510.
697. De Franco E, Flanagan SE, Houghton JA, et al. The effect of early, comprehensive genomic testing on clinical care in neonatal diabetes: an international cohort study. *Lancet.* 2015;386:957–963.
698. Gloyd AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS, et al. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med.* 2004;350(18):1838–1849.
699. Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, et al. Switching from insulin to oral sulfonylureas in

- patients with diabetes due to Kir6.2 mutations. *N Engl J Med*. 2006;355(5):467–477.
700. Babenko AP, Polak M, Cave H, Busiah K, Czernichow P, Scharfmann R, et al. Activating mutations in the ABCC8 gene in neonatal diabetes mellitus. *N Engl J Med*. 2006;355(5):456–466.
701. Hattersley AT, Ashcroft FM. Activating mutations in Kir6.2 and neonatal diabetes: new clinical syndromes, new scientific insights, and new therapy. *Diabetes*. 2005;54(9):2503–2513.
702. Ellard S, Flanagan SE, Girard CA, Patch AM, Harries LW, Parrish A, et al. Permanent neonatal diabetes caused by dominant, recessive, or compound heterozygous SUR1 mutations with opposite functional effects. *Am J Hum Genet*. 2007;81(2):375–382.
703. Ashcroft FM, Rorsman P. Diabetes mellitus and the β cell: the last ten years. *Cell*. 2012;148(6):1160–1171.
704. Bansal V, Gassenhuber J, Phillips T, et al. Spectrum of mutations in monogenic diabetes genes identified from high-throughput DNA sequencing of 6888 individuals. *BMC Med*. 2017;15(1):213.
705. Yang Y, Chan L. Monogenic diabetes: what it teaches us on the common forms of type 1 and type 2. *Diabetes Endocr Rev*. 2016;37(3):190–222.
706. Mackay, D.J.G., Temple, I.K. Human imprinting disorders: Principles, practice, problems and progress. *Eur J Med Genet*, 60(11), 618–626.
707. Yeung RO, Hannah-Shmouni F, Niederhoffer K, Walker MA. Not quite type 1 or type 2, what now? Review of monogenic, mitochondrial, and syndromic diabetes. *Rev Endocr Metab Disord*. 2018;19(1):35–52.
708. Vaxillaire M, Froguel P. Monogenic diabetes: Implementation of translational genomic research towards precision medicine. *J Diabetes*. 2016;8(6):782–795.
709. Silver K, Shetty A. IPF-1 gene variation and the development of type 2 diabetes. *Mol Genet Metab*. 2002;75(3):287–289.
710. Osbak KK, Colclough K, Saint-Martin C. Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. *Hum Mutat*. 2009;30(11):1512–1526.
711. Julier C, Nicolino M. Wolcott-Rallison syndrome. *Orphanet J Rare Dis*. 2010;5:29.
712. Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR. PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab*. 2006;4(6):491–497.
713. Zimmet P, Alberti KG, Kaufman F, Tajima N, Silink M, Arslanian S, et al. The metabolic syndrome in children and adolescents - an IDF consensus report. *Pediatr Diabetes*. 2007;8(5):299–306.
714. Zeitler P, Arslanian S, Fu J, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Type 2 diabetes mellitus in youth. *Pediatr Diabetes*. 2018;19(Suppl 27):28–46.
715. Robertson RP, Davis C, Larsen J, Stratta R, Sutherland DER. Pancreas and islet transplantation in type 1 diabetes. (POSITION STATEMENT: Reviews/Commentaries/ADA Statements). *Diabetes Care*. 2006;29(4):935.
716. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. 2006;355(13):1318–1330.
717. McCall M, Shapiro AM. Islet cell transplantation. *Semin Pediatr Surg*. 2014;23(2):83–90.
718. Desai T, Shea LD. Advances in islet encapsulation technologies. *Nat Rev Drug Discov*. 2017;16(5):338–350.
719. Bottino R, Trucco M. Clinical implementation of islet transplantation: a current assessment. *Pediatr Diabetes*. 2015;16(6):393–401.
720. Malmegrim KC, de Azevedo JT, Arruda LC, et al. Immunological balance is associated with clinical outcome after autologous hematopoietic stem cell transplantation in type 1 diabetes. *Front Immunol*. 2017;22(8):167.

CHAPTER OUTLINE

INTRODUCTION	884
MECHANISMS UNDERLYING TOLERANCE	884
Introduction	884
Central T-Cell Tolerance and AIRE	885
Peripheral T-Cell Tolerance	886
B-Cell Tolerance	887
Autoimmune Diseases	887
Defects in Tolerance That Cause Autoimmune Disease	888
Checkpoint Inhibitors and Autoimmunity	889
CLASSIFICATION OF THE AUTOIMMUNE POLYGLANDULAR SYNDROMES	889
GENETICS OF APS I AND APS II	890
CLINICAL ASPECTS	891
APS I	891
APS II	893
IPEX Syndrome	893
DIAGNOSTIC APPROACH AND FOLLOW-UP	894
TREATMENT	896
AUTOANTIBODIES IN AUTOIMMUNE POLYGLANDULAR SYNDROMES	896
Nonorgan-Specific Autoantibodies	896
Organ-Specific Autoantibodies	896
SUMMARY	898
References	898

INTRODUCTION

The autoimmune polyglandular syndromes (APS) are uncommon constellations of organ-specific autoimmune diseases characterized by the occurrence of more than one autoimmune disease in an affected individual (Table 22.1). Although autoimmune endocrine disorders commonly affect single organs, multiorgan autoimmune involvement of both endocrine and nonendocrine organs and tissues, secondary to loss of self-tolerance, is a characteristic feature of APS.

Tolerance is a physiological state in which one's immune system recognizes self-antigens and does not mount an immune response to self-antigens.¹⁻⁴ If tolerance is not established or is lost, autoimmunity and subsequent disease may result. Although breakdown in self-tolerance remains mostly unexplained, improved understanding of the complex interplay between genetics and the environment, and the resultant aberrant immunological processes has identified a number of possible mechanisms. To comprehend these mechanisms, a brief overview of how self-tolerance is established and maintained is essential.

MECHANISMS UNDERLYING TOLERANCE

Introduction

The body's first line of immune defense, after physiological barriers against invading foreign pathogens, is the innate arm of the immune system.⁵ Innate immunity is a nonspecific response mediated by expression of germ line genes, which does not require prior exposure to an antigen and provides immediate defense.⁵ However, the innate immune system does not exhibit immunologic memory and does not provide long-lasting protection. The next line of defense against foreign antigens is the adaptive immune system.⁶ Although the adaptive immune system assumes all exogenous antigens are potentially harmful, it produces antigen-specific responses. In a normal adaptive immune response; the host organism must differentiate self- from nonself-antigens; mount an immune response; eliminate or remove the inciting antigen; and protect the host from injury, organ dysfunction, and even death.¹⁻⁴ Discrimination of self/nonself is carried out by the adaptive (specific) immune system by a mechanism that uses specific T- and B-cell surface receptors.⁷⁻⁹ T-cell and B-cell receptors recognize distinctive antigen peptides and epitopes, respectively, and are the keys to the specificity of the adaptive immune response. Whereas B cells and their receptors recognize soluble antigens on cell surfaces of pathogens, T cells and their receptors (TCRs) perceive short polypeptides only when presented by antigen presenting cells (APCs), using specialized cell-surface molecules encoded by the major histocompatibility complex (MHC).¹⁰⁻¹³ The human MHC is termed the *human leukocyte antigen* (HLA) complex. Class I MHC (e.g., HLA-A, HLA-B, and HLA-C molecules) is found on all nucleated cells, and presents endogenous peptides derived from the cytoplasm (such as from a pathogen infected cell) to CD8 T cells. Class II MHC (e.g., HLA-DP, HLA-DQ, and HLA-DR) molecules, on the other hand, present foreign antigens (peptides) that have been endocytosed and processed by APCs (such as B cells, dendritic cells, and macrophages) to CD4 T cells.

T cells are initially classified based on their cluster of differentiation (CD) surface proteins that bind differentially to antigens presented on class I (binds to CD8) and class II (binds to CD4) MHC. For example, CD8 T cells, also called *cytotoxic* T cells when activated, typically recognize antigens presented via class I MHC, and mediate an immune response specific to that antigen. Conversely, CD4 T cells, serving as helper or regulatory T cells, are activated when presented with peptides via class II MHC. Regulation of T-cell self-tolerance occurs at two distinct but interdependent levels: central tolerance and peripheral tolerance (described later) (Fig. 22.1). Central tolerance occurs in the thymus, whereas peripheral tolerance occurs in both lymphoid and nonlymphoid tissues. Although many of the mechanisms involved in self-tolerance remain poorly understood, almost 2 decades of characterizing the autoimmune regulatory gene (AIRE) has improved our understanding of the pathways in the establishment and maintenance of self-tolerance.¹⁴ The AIRE gene encodes a transcription factor in the thymic medulla, which plays a critical role in establishing

TABLE 22.1 The Autoimmune Polyglandular Syndromes I and II

	APS I	APS II
Comparative frequency	Less common	More common
Onset	Infancy/early childhood	Late childhood, adulthood
Heredity	Autosomal recessive	Polygenic
Gender	Males = females	Female predominance
Genetics	<i>AIRE</i> gene; no HLA association	HLA associated; DR/DQ
Hypoparathyroidism	77%–89%	<5%
Mucocutaneous candidiasis	73%–100%	None
Ectodermal dysplasia	77%	None
Addison disease	60%–86%	70%–100%
Type 1 diabetes	4%–18%	41%–52%
Autoimmune thyroid disease	8%–40%	70%
Pernicious anemia	12%–15%	2%–25%
Gonadal failure		
Females	30%–60%	3.5%–10%
Males	7%–17%	5%
Vitiligo	4%–13%	4%–5%
Alopecia	27%	2%
Autoimmune hepatitis	10%–15%	Rare
Malabsorption	10%–18%	Rare

HLA, Human leukocyte antigen.

central tolerance (described in detail in the later part of this chapter).^{15,16} Deletions in the mouse *AIRE* homologue result in multiorgan autoimmunity, whereas mutations in the human *AIRE* gene result in APS I.^{17,18}

Tolerance is initially developed in utero with T and B cells playing important roles. T and B cells are produced

continuously throughout life by hematopoietic stem cells of the bone marrow, with the T-cell precursors migrating to the thymus for further maturation. Although the thymus atrophies after puberty,¹⁹ residual thymic tissue may provide for T cell development throughout life.

Although T cells require exposure to small doses of antigen to achieve tolerance during their thymic development, larger doses of antigen are required to induce B-cell tolerance, and B-cell tolerance is often short lived. Tolerance is immunologically specific and induced in developing lymphocytes early in life; however, it can also be induced in mature lymphocytes when costimulatory signals are absent at the time of peptide interaction with TCR.

Central T-Cell Tolerance and AIRE

T cells are primarily educated to distinguish self and nonself when they develop in the thymus.^{20–23} In the thymic cortex, CD4+ CD8+ (double-positive) T cells bearing α/β TCRs that are able to bind to self-peptide/MHC complexes are selected to survive, whereas T cells whose TCRs fail to bind undergo apoptosis. As many as 99% of developing thymocytes undergo apoptosis and never reach the periphery (see Fig. 22.1). This is referred to as *positive selection* and is carried out by antigen-presenting cortical thymic nurse epithelial cells (cTECs), bearing MHC I and MHC II. T cells that bind to MHC I commit the developing T cell toward a CD8 T-cell pathway, whereas those that bind to MHC II develop into CD4 T cells. Positively selected cells migrate through the corticomedullary junction into the medulla where a secondary checkpoint occurs. Although T cells must be able to bind to MHC/self-peptide to establish tolerance, cells that bind too tightly to self-antigens are capable of inducing autoreactivity and therefore undergo negative selection and apoptosis.²⁴ This process of positive selection of T cells for positive selection of MHC binding T cells

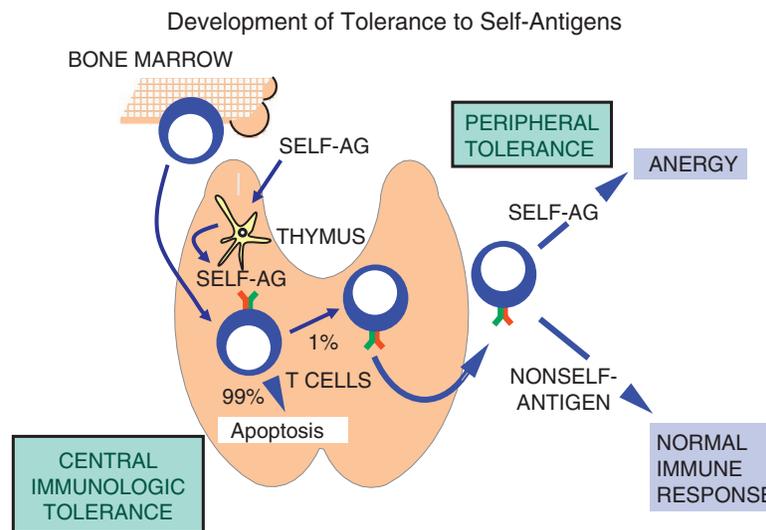


Fig. 22.1 Normal tolerance pathways. T-cell precursors initially arise in the bone marrow. These progenitors enter the thymus where developing T cells encounter self-antigens (*SELF-AG*) derived from circulating proteins or generated within the thymus (e.g., via the action of medullary thymic epithelial cells [mTECs]; drawn as a yellow-colored dendritic cell). Ectopic expression of various proteins in the thymus, including hormones such as insulin, is facilitated by the action of the autoimmune regulator gene (*AIRE*). Strong self-antigen stimulation of receptors on developing T cells induces apoptosis, with ultimately approximately 99% of all developing T cells dying in this manner. This is “central immunologic tolerance” (also known as “thymic” tolerance; *bottom left*) where strongly anti-self-reactive T cells are eliminated. One percent of T cells will progress to become either CD4 T cells or CD8 T cells. These naïve T cells that leave the thymus can be subsequently tolerized to self-antigens if they encounter self-antigens without the normal costimulatory signals (B7.1/B7.2-CD28; see Fig. 22.2). Induction of tolerance outside the thymus is termed “peripheral tolerance” (*top right*), and is a complementary mechanism to central tolerance. Peripheral tolerance is functionally expressed as anergy: autoreactive cells are present but are inactive (*top right*). However, if a nonself-antigen is encountered, a normal immune response ensues (*bottom right*).

(in the thymic cortex) and negative selection of T cells tightly bound to self-antigens (in the medulla), accounts for central (thymic) immunological tolerance.^{3,16,25} The single positive T cells expressing either CD4 or CD8 then migrate to the periphery and secondary lymphoid organs.

Medullary thymic epithelial cells (mTECs) are specifically involved in the process of negative selection of T cells and express an enormously diverse range of peripheral tissue-specific antigens (TSAs) for presentation to the developing T cells. This has been referred to as *promiscuous gene expression* (PGE) and is mediated by AIRE, a transcriptional regulator. Notably, AIRE is expressed in a small population of mature mTECs, with high levels of CD80 and MHC II. Unlike traditional transcriptional regulators, it does not bind to deoxyribonucleic acid (DNA) segments but activates ribonucleic acid (RNA) polymerases, and elongates TSA RNA transcripts. This process is also mediated by interaction of AIRE with other transcriptional regulators.²⁶ Another transcriptional regulator that is involved in negative T-cell selection in the thymus is the FEZ family zinc finger protein 2 (FEZF2), which is involved in AIRE-independent TSA expression in mTECs.²⁶ Studies have shown that AIRE and FEZF2 share complementary and parallel function in establishing and maintaining central tolerance. In addition to regulation of TSA expression in mTECs, AIRE is involved in thymic selection and differentiation of autoreactive CD4 T cells into regulatory T cells (Tregs),²⁷ and upregulation of chemokines that aid in thymocyte migration.¹⁴ Extrathymic expression of AIRE in tissues, such as the bone marrow, has also been shown to aid in peripheral tolerance by inducing energy of CD4 and CD8 T cells.²⁸

Peripheral T-Cell Tolerance

Once naïve T cells enter the circulation or secondary lymphoid organs (e.g., lymph nodes and spleen) and recognize specific antigens, they require additional cosignals to become activated.⁷ The first signal involves the interaction of antigen-peptides bound to the MHC molecules on the surface of APCs with TCRs on the surface of CD4 and CD8 T-cells. CD4 and CD8 molecules on these T-cell subsets serve as antigen-nonspecific coreceptors binding to nonpolymorphic portions of the class II and class I MHC molecules, respectively. The second signal is antigen non-specific and is provided by the B7.1 (CD80) and B7.2 (CD86) molecules of the APC interacting with the CD28 molecule on the T-cell surface (Fig. 22.3). In addition to CD80/CD86/CD28 interactions, there are other costimulatory signals that have important roles in T-cell development.²⁹

When T cells perceive both signals (MHC-antigen with TCR and B7-CD28), a cascade of intracellular signaling events occur, leading to T-cell activation. Activated cytotoxic CD8 T cells mediate direct lysis of target cells, whereas activated CD4 T cells lead to expression of numerous cytokines, cytokine receptors, and cytotoxic T-lymphocyte antigen 4 (CTLA-4). CTLA-4 is homologous to CD28 and competes with CD28 for binding to B7.1/B7.2. CTLA-4 expression by the activated T cell and its interaction with B7.1/B7.2 provide an immunosuppressive/immunoregulatory signal to the T-cell, thereby downregulating the T-cell responses. Thus CTLA-4 and CD28 though homologous, act antithetically: B7.1/B7.2-CD28 turns on T cells, whereas B7.1/B7.2-CTLA-4 downregulates the T cell (see Fig. 22.2).

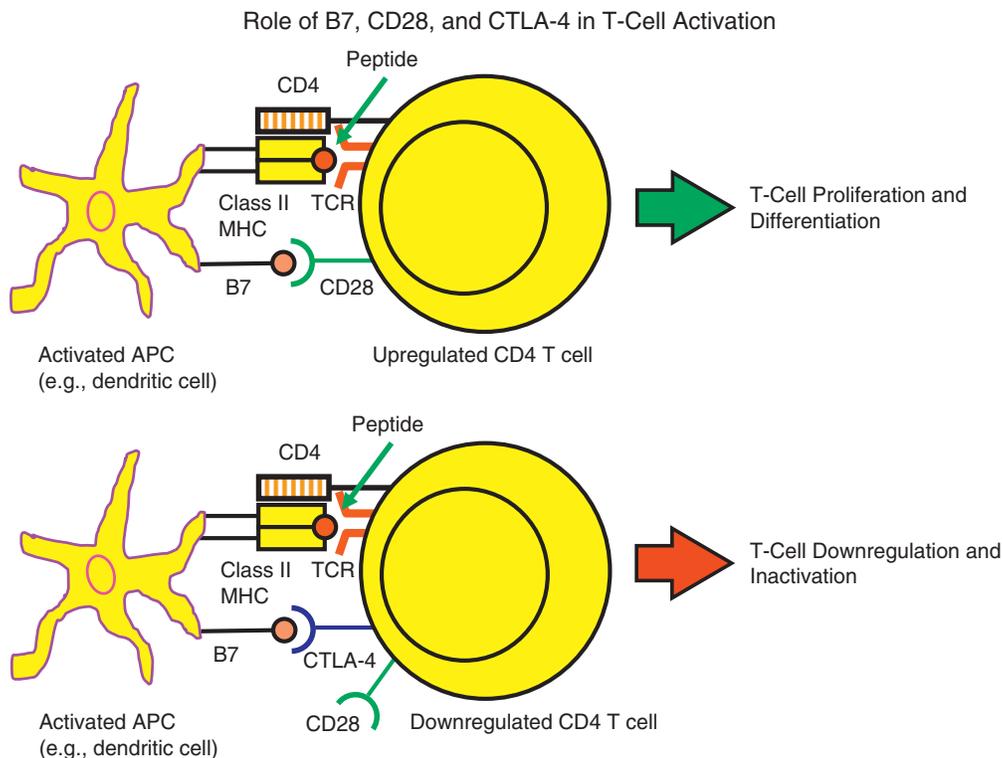


Fig. 22.2 Role of B7, CD28, and CTLA-4 in T-Cell Activation. Activated antigen presenting cells (APCs) present antigen peptides via major histocompatibility complex (MHC) molecules (in this drawing, class II MHC) and express B7 [including B7.1 (CD80) and B7.2 (CD86)] co-stimulators. When B7.1 or B7.2 is bound by CD28, and MHC plus peptide is bound by the T cell receptor, T cell proliferation and differentiation of naïve T cells ensues (top image). Conversely, activated T cells express CTLA-4 and bind to B7 (either B7.1 or B7.2) inducing down regulation and inactivation of T-cells (bottom image).

The requirement for two signals to activate naïve T cells mainly accounts for peripheral T-cell tolerance. When the naïve T cell perceives antigen peptide presented by MHC molecules without the necessary costimulatory signal (e.g., B7.1/B7.2-CD28), the T cell becomes unresponsive. This state of unresponsiveness is termed *anergy*; anergic T cells are generally not restimulated with antigen peptide displayed by the APCs. T cells may also undergo apoptosis (programmed cell death) to be removed completely from the T-cell repertoire. Tolerance may also exist because the TCR does not encounter the relevant peptide, and this has been termed *T-cell ignorance*.

Another mechanism whereby T-cell tolerance is mediated is the interaction of programmed death 1 (PD-1) receptor on T cells and its ligands PD-L1 and PD-L2.³⁰ These interactions bring about inhibition of T-cell effector functions in an antigen-specific manner. PD-1 signaling can also mediate the conversion of naïve T cells to Tregs.³¹

Helper CD4 T cells are classically divided into two distinct lineages: (1) Th1 cells, which activate cell-mediated and some antibody responses, and (2) Th2 cells, which predominantly activate antibody-mediated responses.³² However, additional T-cell lineages exist (i.e., Th17 cells, T-follicular helper cells, and regulatory T cells) and recent data have described remarkable plasticity in their cytokine expression, suggesting shifting T-cell functionalities, depending on environmental cues.³³ Although overtly simplistic, Th1 subsets secrete predominantly proinflammatory cytokines, such as interleukin (IL)-2, interferon-gamma (IFN- γ), tumor necrosis factor-beta (TNF- β), induce IL-12 secretion from dendritic cells,³⁴ and activate macrophages and CD8 T cells to eliminate intracellular pathogens. Upon activation, CD8 T cells, often with the help of Th1 cells supplying IFN- γ to upregulate B7 expression on APCs, become functional cytotoxic T killer cells.³⁵ Conversely, Th2 cells elaborate IL-4, IL-5, IL-6, IL-10, and IL-13, which aids antibody and eosinophil production.³⁵ There is also crosstalk between Th1 and Th2 cells; for example, IFN- γ from Th1 cells suppresses Th2 cells, and IL-10 from Th2 cells inhibits Th1 cells.

Tregs are a subset of T cells, which play a critical role in suppressing the activity of effector T cells that escape negative selection to self-antigens in the thymus.³⁶ Functional Tregs are able to anergize previously self-reactive T-effector cells, resulting in improved tolerance to self. Tregs originating from the thymus are termed as *central* or *natural Tregs* and carry surface CD4⁺CD25⁺ and intracellular forkhead transcription factor (FOXP3⁺) markers, which are specific to CD4⁺CD25⁺ Treg cell population. FOXP3 is a transcription factor that is required for developing α/β TCR-positive T cells to differentiate into Tregs in the thymus. First identified in the *Scurfy* mouse, a mouse model of immune dysfunction and polyendocrinopathy, abnormal FOXP3 expression is now known to be responsible for failure in immune tolerance in humans affected with a similar polyendocrinopathy, as further discussed later.^{37–39} Abnormal FOXP3 expression in humans leads to an extremely rare, X-linked inherited, and typically fatal autoimmune lymphoproliferative disease known as IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, and X-linked inheritance).^{40,41} Defects in FOXP3 responsible for IPEX map to Xp11.23-Xq13.3.

B-Cell Tolerance

Naïve B cells, during the early stage of development in the bone marrow, express surface immunoglobulin (Ig)M, which serves as B-cell receptors (BCRs). Upon interacting with self-antigens, naïve immature B cells undergo negative selection, either through clonal deletion or anergy, whereby B cells enter a state of unresponsiveness and have a reduced life span.⁴² Another

process mediating B-cell central tolerance is receptor editing, whereby genetic rearrangement of the Ig chain leads to generation of BCRs with new antigen specificities. B cells with non-autoreactive BCRs are positively selected and continue to the periphery.⁴³ If self-reactive B cells escape into the periphery, they undergo anergy. Anergized B cells do not die immediately but have a shorter half-life. Naïve mature B cells require T-cell help for realization of their full potential through affinity maturation and class switching. The absence of T-cell help also leads to B-cell tolerance.

Autoimmune Diseases

The organ-specific nature of many autoimmune diseases results from abnormal immune system recognition of tissue-specific self-antigens. In many autoimmune endocrinopathies, the target molecule is either a tissue-specific or tissue-limited (i.e., the protein is not unique to one tissue but is clearly restricted in its distribution) enzyme or cell-surface receptor.^{44,45} (Table 22.2).

The criteria for classification of a disease as autoimmune are not universally agreed upon.⁴⁶ However, major criteria that are generally accepted as strong evidence of autoimmune disease include: (1) detection of autoantibodies or autoreactive T cells, including lymphocytic infiltration of the targeted tissue or organ;

TABLE 22.2 Autoantigens in Autoimmune Endocrine and Associated Diseases

Disease	Autoantigens	Putative Autoantigens
Mucocutaneous Candidiasis		IL-17A IL-17F IL-22
Hypoparathyroidism		NALP5 CASR
Addison disease	P450c21	P450c17 P450scc
Hashimoto thyroiditis	Thyroid peroxidase Thyroglobulin	
Graves disease	Thyrotropin receptor	
Diabetes	Insulin Glutamic acid Decarboxylase 65 IA-2 (ICA 512) IA-2 β ZnT8	Proinsulin Carboxypeptidase H ICA69 Glima 38
Premature gonadal failure	P450scc	P450c17 3 β hydroxysteroid dehydrogenase
Pernicious anemia	H ⁺ /K ⁺ ATPase pump Intrinsic factor	
Myasthenia gravis	Acetylcholine receptor α chain	
Vitiligo		Tyrosinase Tyrosinase-related protein 2 L-amino acid decarboxylase
Celiac disease	Endomysium transglutaminase	Reticulin Deamidate gliadin
Autoimmune hepatitis	Liver kidney microsome 1	L-amino acid decarboxylase Tryptophan hydroxylase

CaSR, Calcium sensing receptor; IA-2, protein tyrosine phosphatase-like protein; ICA, islet cell autoantigen; IL, interleukin; NALP5, NACHT leucine rich repeat protein 5; ZnT8, zinc transporter 8.

(2) disease transfer with antibodies or T cells; (3) disease recurrence in transplanted tissue; and (4) ability to abrogate the disease process with immunosuppression or immunomodulation. Few, if any, human autoimmune diseases meet all these criteria. Further information that is supportive of, but not diagnostic for an autoimmune disease, include: (1) increased disease frequency in women compared with men, (2) the presence of other organ-specific autoimmune diseases in affected individuals, and (3) increased frequencies of particular HLA alleles in affected individuals.

Defects in Tolerance That Cause Autoimmune Disease

Several different hypotheses explaining defects in tolerance have been proposed.⁴⁷ Autoimmunity may develop because (1) tolerance never developed to specific self-antigens or (2) established tolerance was lost (Fig. 22.3). If self-antigen is not efficiently presented in the thymus, tolerance may not be established during T-cell education within the thymic cortex.⁴⁸ For example, *AIRE* mutations lead to lack of expression of ectopic self-antigens by mTECs and their presentation to developing T cells. This leads to escape of autoreactive T cells into the periphery and ultimately multiorgan autoimmunity. Another example is the insulin gene (*INS*) VNTR (variable number of tandem repeats), which lies around 500 basepairs upstream of the *INS* gene promoter. The *INS* VNTR influences thymic insulin T-cell expression and education based on its length. Specifically, longer VNTRs are associated with increased thymic expression of insulin, and thus a decreased risk of developing type 1 diabetes, whereas shorter VNTR are associated with decreased thymic expression of insulin, failure to delete specific autoreactive T-cell clones, and an increased risk of developing diabetes.⁴⁹

If tolerance has not developed because of intracellular sequestration of an antigen, and thus not expressed in the thymus during T-cell ontogeny, T-cell reactivity in the periphery will not be abolished. However, several antigens initially thought to be sequestered intracellularly have now been shown to circulate in low concentrations in normal individuals. Thyroglobulin, a self-antigen in autoimmune thyroid disease, is known to circulate in low but appreciable quantities in individuals with no serologic evidence of thyroid autoimmunity.

Thyroid follicular cell destruction in Hashimoto thyroiditis is mostly cell mediated and not mediated by humoral factors.

If sequestered antigens do play a role in autoimmune disease, viral infections, trauma, ischemia, or irradiation are all possible mechanisms that could disturb cellular integrity and lead to release of intracellular antigens.⁵⁰ Some sequestered self-antigens may never encounter the immune system, unless there is a breakdown of anatomic barriers within the body. An example is the occurrence of autoimmunity to intraocular proteins, following orbital trauma. Although a rare consequence of orbital damage, initiation of an autoimmune response to released sequestered intraorbital proteins in adjacent lymph nodes can generate autoreactive T cells that can invade and damage the contralateral eye (sympathetic ophthalmia).^{51,52} Removal of the inciting damaged tissues and immunosuppression may be required to sustain vision in the undamaged eye. Similarly, transient autoantibody reactivity to cardiac myosin following myocardial infarction has been described albeit with no pathological consequences.⁵³

Alteration of self-antigens because of infection or neoplasia is another possible theory explaining some types of autoimmunity. As environmental triggers, viral infections could lead to modification of self-proteins and neoantigen expression. Alternatively, a self-antigen may be partially degraded, leading to a “new” antigenic target for the adaptive immune system. This new antigen is recognized as foreign by the immune system, and the immune response to these new antigens results in autoimmunity. Some cells/tissues may suffer unintended autoimmune damage when substances bind to the cells and elicit an initial immune response. For example, certain drugs bind to red blood cells and result in an immune hemolytic anemia. If an antibody response to the red-cell-bound drug is elicited, the antigen-antibody complex present on the red blood cell can lead to red blood cell destruction. This can occur either through red blood cell phagocytosis by the monocyte-macrophage system or via complement-mediated lysis of the red blood cell. Thus the red blood cell becomes an innocent bystander to the antidrug humoral immune response. Theoretically, this could also occur with viruses that serendipitously attach to tissues.

Molecular mimicry is another mechanism to explain development of autoimmunity.^{50,54} Following exposure to a dietary, viral, or bacterial antigen (e.g., infection) and similarity (molecular mimicry) between the self-antigen and the foreign

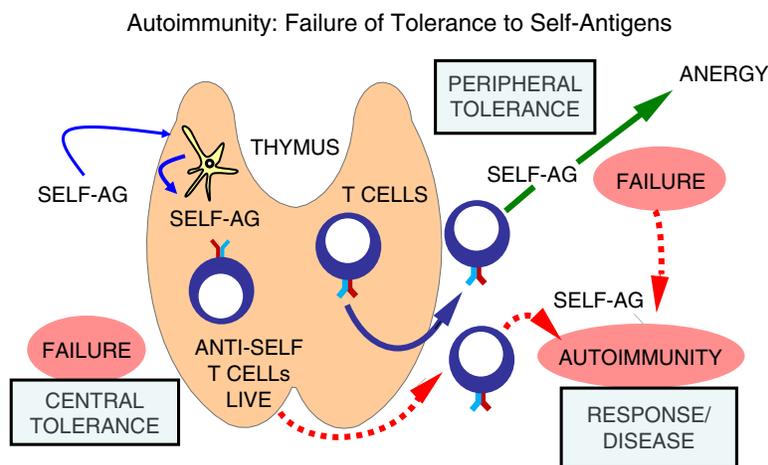


Fig. 22.3 Autoimmunity: Failure of Tolerance to Self-Antigens. With the failure of central tolerance (bottom left), anti-self T cells survive that should not normally survive. When such anti-self T cells leave the thymus, they are able to produce autoimmunity (red arrows). Alternatively, with a failure of peripheral tolerance (top right), if anergy (green arrow) does not occur after contact with self-antigen, an autoimmune response or disease can occur (red arrow).

antigen, the immune response to the foreign antigen leads to cross-reactivity with self-antigen, autoimmunity, and disease.^{54–57} For this theory to work, tolerance must not previously exist to the self-antigen. This might be true if the self-antigen is truly sequestered and the immune system has never developed tolerance to the self-antigen. Alternatively, the self-antigen peptides may be present in very low concentration to elicit an immune response and initial tolerogenicity has not occurred. Only after infection or novel dietary exposure would there be a sufficient degree of immunization to the exogenous antigen (which is similar to a self-antigen) and subsequent immune autoreactivity. If the self-antigen is a cell-surface antigen, the “pathogen-induced” autoantibodies could fix to self and produce disease via complement fixation, or the antibodies could act as opsonins for fixed or circulating phagocytes (antibody-dependent cell cytotoxicity). In rheumatic fever, associated comorbidities such as carditis and Sydenham chorea are thought to be autoimmune manifestations secondary to similarities in the structural components of group A *Streptococcus* with collagen (I and IV) and fibronectin in human cardiac connective tissues⁵⁸ and tubulin in human brain cells,⁵⁹ respectively.

Some cases of autoimmunity may result from superantigens, which can be secreted by certain pathogenic bacteria and viruses. Superantigens are polyclonal T-cell stimulators that can cross-link TCR β chains and MHC molecules, and activate as many as a third of T cells in the body. This can initiate a nonspecific T-cell immune response, including against self-antigens. In such cases, systemic disease can develop from massive cytokine release (e.g., systemic inflammatory response syndrome [SIRS]). This is the case in toxic shock syndrome, wherein a staphylococcal exotoxin acts as a superantigen. Mycobacterial antigens have also been proposed as possible superantigens in Crohn disease.⁶⁰ This theory requires that T cells bearing antiself TCRs have not been deleted or become permanently anergic. These T cells may be stimulated and proliferate to develop an autoimmune response if they encounter the specific self-antigen.

Similar to polyclonal T-cell activation, polyclonal B-cell stimulation has also been implicated in humoral autoimmunity. Autoreactive B cells arise routinely as part of the naïve B-cell repertoire, and can be found in healthy individuals. If an autoreactive clone of B cells encounters a self-antigen and a costimulator (which might be nonspecific, e.g., a virus, such as Epstein-Barr virus, or a bacterial product, such as lipopolysaccharide), autoantibodies could be produced, bypassing the need for T-cell help.

Autoimmune human disease likely results from an interaction of environmental and genetic factors.^{61–63} Environmental factors implicated include: wheat gliadin ingestion and celiac disease, penicillamine exposure and myasthenia gravis, methimazole and autoimmune hypoglycemia from insulin autoantibodies (reported primarily in Japanese patients), and amiodarone and thyroiditis. Cancer has also been associated with the development of autoimmunity: thymoma and myasthenia gravis,⁶⁴ ovarian teratoma and *N*-methyl-D-aspartate receptor-mediated encephalitis,⁶⁵ and breast cancer and stiff-person syndrome.⁶⁶ Despite remarkable improvements in our understanding of immunology, the mechanisms whereby the complex interaction of genes, environment, and immune system lead to autoimmunity remain to be fully elucidated.

Checkpoint Inhibitors and Autoimmunity

Tumor cells can manipulate the inherent immune tolerance mechanism to disrupt antitumor immunity. An efficient way of escaping antitumor activity is by increasing checkpoint pathways, which suppress T-cell responses. An example is the

interaction of CTLA-4 (present on activated T-cells) with B7.1/B7.2 of APCs that downregulates T cell response. CTLA-4 can also remove B7 molecules from APCs, through a process called *transendocytosis* and prevent binding of CD28 costimulatory molecules, and thus bring about T-cell anergy. Another example is the interaction of PD-1 on T cells with its ligands PD-L1 and PD-L2.^{67–71} PD-1/PD-L1/PD-L2 interactions inhibit T-cell proliferation and production of proinflammatory cytokines (TNF- α , IFN- γ , and IL-2), allowing immune checkpoint pathways to promote a tolerogenic environment.

Checkpoint inhibitors, now increasingly used in anticancer therapy, include monoclonal antibodies targeting the CTLA-4 and PD-1 pathway (both the PD-1 receptor and PD-L1 ligand) and thus removing the restraint on antitumor activity (Fig. 22.4). Blockade of CTLA-4 enhances costimulatory signals and leads to naïve T cells having increased effector T-cell responses to tumor cells, whereas PD-1 pathway blockade leads to increased T-cell proliferation and a proinflammatory milieu that aids in antitumor activity. In addition, there are other immune pathways impacted by blockade of these pathways. The discovery of immune checkpoint inhibitors was a breakthrough in anticancer therapy, resulting in the 2018 Nobel Prize for Medicine awarded to Drs. James Allison and Tasuku Honzo, two pioneers in this field.

Given that checkpoint inhibitors have the ability to eliminate a tolerogenic environment, it is not surprising that these immunomodulatory agents can bring about immune-related adverse effects (irAEs), and such irAEs have even been referred to as the *Achilles heel of cancer immunotherapy*.^{67,71,72} Hypophysitis, hepatitis, dermatitis, colitis, type 1 diabetes, thyroiditis, adrenalitis, and myocarditis have been reported following treatment with ipilimumab (CTLA-4 antibody) and/or PD-1 antibody (nivolumab) therapy. Other potential mechanisms leading to irAEs after use of checkpoint inhibitors include: (1) cross-presentation, where tumor antigens released after antitumor activity are picked up by APCs, initiating secondary immune responses, and (2) epitope spreading (after release of tumor antigens), whereby there is continuous acquisition of neo antigens and recruitment of untargeted T cells. Despite the success of checkpoint inhibitors as anticancer drugs, these irAEs remain a concern. There are few studies in cancer patients with and without preexisting autoimmune conditions, and the long-term effects of checkpoint inhibitor use remain unknown. Newer modes of anticancer immunotherapy that have been investigated to lessen irAEs include increasing the efficacy and the use of vaccines against tumor neoantigens.^{73,74}

Having provided a review of basic immunological concepts related to central and peripheral tolerance, we will shift our focus for the remainder of the chapter toward the clinical and pathological aspects of APS.

CLASSIFICATION OF THE AUTOIMMUNE POLYGLANDULAR SYNDROMES

APS I, also known as *autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy* (APECED) is an autosomal recessive disorder characterized by several autoimmune disorders with significant heterogeneity in its presentation. The syndrome is caused by a mutation in the *AIRE* gene on chromosome 21q22.3.^{17,18}

The presence of two of the following three conditions are prerequisites for the diagnosis of APS I: (1) adrenocortical failure (Addison disease) or serologic evidence of adrenalitis (adrenal autoantibodies), (2) hypoparathyroidism, and (3) chronic mucocutaneous candidiasis.^{61,62,75–78} APS II is defined by the coexistence of at least two of these three endocrinopathies: (1) autoimmune adrenocortical insufficiency or serologic evidence of adrenalitis, (2) autoimmune thyroiditis, and/or

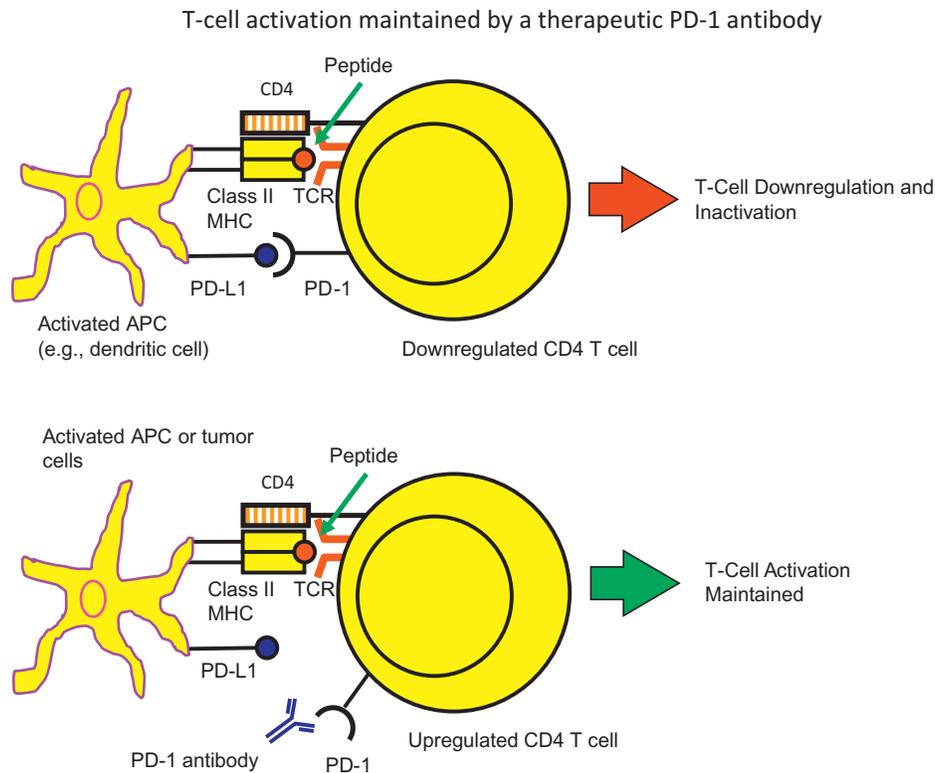


Fig. 22.4 T cell activation maintained by a therapeutic PD-1 antibody. Whereas the binding of PD-L1 on an APC to PD-1 (pictured) down-regulates activated T cells (CD4 T cells in this drawing; top half of the figure), interference in PD-1:PD-L1 signaling via a therapeutic monoclonal antibody to PD-1 (as pictured) or via a therapeutic monoclonal antibody to PD-L1 will maintain T cell activation (bottom half of the figure). Indeed therapeutic anti-PD-1 and anti-PD-L1 monoclonal antibodies have produced remarkable beneficial results in the treatment of some cancers.

(3) type 1 diabetes mellitus or serological evidence of islet autoimmunity.^{61,79–82} Traditionally, the presentation of autoimmune thyroid and adrenal disease has been termed *Schmidt syndrome*, whereas a complete triad of adrenal, thyroid, and islet autoimmunity (or type 1 diabetes mellitus) has been termed *Carpenter syndrome* (Fig. 22.5). The presence of thyroiditis, without adrenal disease, but associated with type 1 diabetes,⁸³ pernicious anemia, vitiligo, or alopecia has been referred to by some authors as *APS III*, whereas additional combinations of autoimmune disease have been referred to as *APS IV* (i.e., vitiligo plus alopecia, type 1 diabetes plus celiac disease, or type 1 diabetes and vitiligo).⁸⁴ Given that they share similar susceptibility genes and immunological features, these subtypes are often considered as extensions of APS II and not necessarily as separate entities.

GENETICS OF APS I AND APS II

APS I is caused by mutations in the *AIRE* gene, and is inherited in an autosomal recessive fashion.⁸⁵ The *AIRE* protein is expressed in the thymus, lymph nodes, and fetal liver, as well as in pancreas, adrenal cortex, and testes. The gene spans 11.9 kb, contains 14 exons, and encodes a 545 amino acid protein.¹⁸ The protein *AIRE* has four major domains—CARD (caspase recruitment domain), SAND (SP100, *AIRE1*, NucP41/P75 and DEAF1), PHD1 (plant homeodomain), and PHD2. The CARD domain is involved in multimerization of the *AIRE* protein into an active state, and its attachment to a target chromatin.⁸⁶ The SAND domain is required for *AIRE* to interact with a transcriptional repressive complex (ATF7ip), which in

turn is critical for *AIRE*-dependent gene expression.⁸⁷ The PHD domains function as sites for multiple binding events and aids *AIRE* transcriptional activity.⁸⁸

Over 98% of patients with APS I have mutations in the *AIRE* gene.^{77,89–91} So far, more than 100 mutations in the *AIRE* gene have been reported, and recessive mutations causing disease are distributed throughout the *AIRE* gene. Although the inheritance is traditionally accepted as autosomal recessive, there are some patients reported with heterozygous dominant mutations (in the gene encoding the SAND domain) and atypical or nonclassical manifestations of APS I.^{92,93} Because *AIRE* is active in multimeric forms, minor changes in amino acid sequences in critical domains of the *AIRE* secondary to heterozygous mutations can lead to defective multimers, and thus a negative dominant effect, whereby the altered gene product inhibits the functioning of the wild-type protein. Such an effect has also been reported for monoallelic mutations in the *AIRE* gene encoding the PHD1 domain.⁹⁴

Unlike APS I, APS II is not inherited as a single gene mutation but displays polygenic inheritance. APS II is much more typical of other autoimmune endocrinopathies, where cases can occur either sporadically, or within families with APS II. Often, different organ-specific manifestations share common genetic associations, and most of these genes code for key regulatory elements in immune system pathways. The *MHC* (*HLA* in humans) class II complex involved in antigen presentation is the most important gene associated with APS II.^{95,96}

A study in 1986 reported an association of *HLA-DR3* and/or *DR4* haplotypes⁹⁵ with Addison disease and type 1 diabetes. Subsequent studies have shown that variants in *DR3-DQ2*

Diagnostic relationships and common associations in the autoimmune polyglandular syndromes

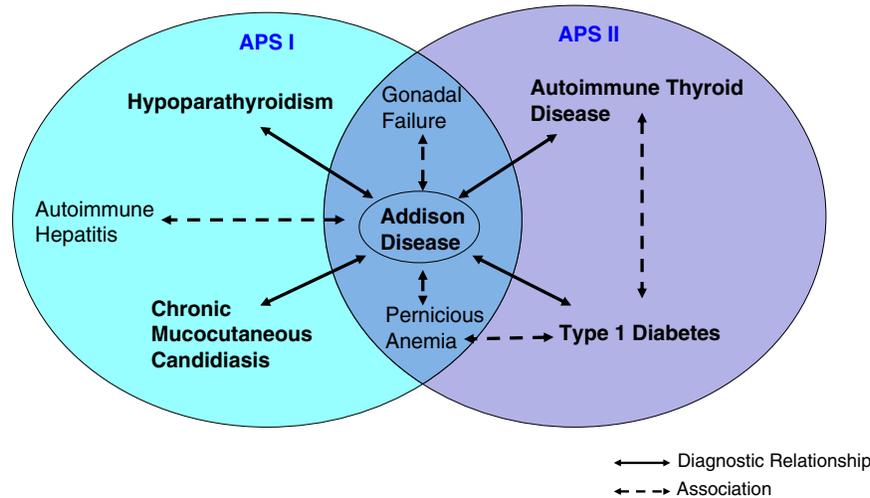


Fig. 22.5 Diagnostic relationships and common associations in the autoimmune polyglandular syndromes. The *solid lines* indicate diagnostic relationships. The *dashed lines* indicate common associations. The diagnosis of APS I depends on the coexistence of Addison disease (or adrenal autoantibodies) plus either hypoparathyroidism or chronic mucocutaneous candidiasis or both. The diagnosis of APS II depends on the coexistence of Addison disease (or adrenal autoantibodies) plus either autoimmune thyroid disease or type 1 diabetes or both (or their associated autoantibodies). Autoimmune hepatitis can occur in people affected with APS I. Gonadal failure or pernicious anemia can be seen in persons affected with either APS I or APS II. Pernicious anemia is also strongly associated with autoimmune thyroid disease (independent of APS II and Addison disease; note: for graphic simplicity, no line is drawn in the figure to indicate such “thyrogastric” autoimmunity).

and *DR4-DQ8* haplotypes confer a risk for type 1 diabetes and Addison disease, as well as autoimmune thyroid disease and celiac disease. This may indicate a common immunogenetic etiopathogenesis and highlights why multiple autoimmune disorders can develop in the same person.⁹⁷ Other components of APS II, such as Graves disease, have shown association with *HLA-DR3* as opposed to Hashimoto thyroiditis, which is associated with *HLA-DR4* or *DR5*.^{98,99} In addition to class II molecules, class I molecules (*HLA-A* and *B*) have also been implicated in the risk for type 1 diabetes.

Because the association of APS II with specific *HLA* alleles is only modest, the role of other susceptibility genes has been studied. Other non-*HLA* genes conferring APS II risk include genes that encode *CTLA-4*,¹⁰⁰ the transcriptional regulator protein *BACH2*,^{101–103} protein tyrosine phosphatase nonreceptor type 22 (*PTPN22*),¹⁰² and *CD25* (the high-affinity *IL-2* receptor).¹⁰⁴

CLINICAL ASPECTS

The major disease components, frequencies, and differences between APS I and II are shown in [Table 22.1](#). The overlap in some disease components between APS I and II are also highlighted in [Fig. 22.5](#).

APS I

Although the disease is not common, with an overall prevalence of less than 1:100,000, it is more prevalent in Finland (1:25,000), Sardinia (1:14,000), and among Iranian Jews in Israel (1:9000).^{75,76,78,105,106} The Finnish cohort of 91 subjects is the largest and most well-characterized APS I group of patients worldwide.⁷⁶

Persistent mucocutaneous candidiasis is usually the first sign (60% of all APS I patients) and appears during the first year or two of life. In the Finnish cohort, 50% developed candidiasis by age 5 years, 94% by age 20 years, and 100% by

40 years of age.⁷⁶ Candidal diaper rash is observed early in life, with vulvovaginal candidiasis often developing at puberty in females. Colonization of the gut by *candida* can lead to intermittent abdominal pain and diarrhea. Retrosternal pain in patients with oral candidiasis may suggest esophageal candidiasis and should be confirmed by esophagoscopy. Infection of the nails with chronic candidiasis may lead to a darkened discoloration, thickening, or erosion. Studies have shown that T cells from *AIRE*-deficient individuals still have a competent proliferative response against *C. albicans*. However, there is defective receptor-mediated internalization of yeast cell wall derivatives by monocytes in *AIRE*-deficient antigen presentation, and this leads to ineffective clearing of mucocutaneous candidiasis, and thus the chronicity. Thus, all patients with refractory mucocutaneous candidiasis should be thoroughly investigated not only for a T-lymphocyte abnormality (absolute lymphocyte count, enumeration of T-cell subpopulations, assessment of T-cell function where possible) but also for the presence of polyendocrinopathies. Chronic oral mucosal candidiasis must be treated aggressively, as there is increased risk of candidiasis associated squamous cell carcinoma (SCC) of the oral mucosa or esophagus; SCC was reported in seven of the 55 Finnish APS I patients over 25 years of age, albeit five of the seven were also smokers.^{75,107}

Oral mucous membranes must be protected from exposures that can increase susceptibility to *candida* infections. Specifically, patients should be advised to avoid hard, sharp, or spicy foods, as well as whitening toothpastes or abrasives. Dentures or orthodontics can provide additional surfaces for *candida* growth.⁷⁵ Fluconazole has good activity against *candida* and is the preferred treatment. Other azoles that have been used include ketoconazole, and miconazole. Data suggest that prolonged use can lead to resistance.^{108,109} With drug resistance, newer azoles, such as itraconazole, voriconazole, or posaconazole can be used. Patients should follow up closely with an infectious disease specialist. Physicians must be cognizant of the possibility of precipitating adrenal insufficiency

or worsening already present adrenal deficiency when using ketoconazole; ketoconazole can inhibit cortisol synthesis.^{110,111} In addition, there have been reports of adrenal insufficiency following use of other azoles (fluconazole, posaconazole) in sick patients.^{112,113}

Hypoparathyroidism is typically the first endocrinopathy to develop in APS I and eventually occurs in more than 85% of patients. Hypoparathyroidism usually presents after the onset of mucocutaneous candidiasis but before puberty, with 33% of APS I patients diagnosed by age 5 years, 66% by age 10 years, and nearly 85% by age 30 years.⁷⁶ Severe hypocalcemia, as evidenced by seizures, carpopedal spasms, muscle twitching, and laryngospasm may be presenting features of APS I, although these symptoms may be masked by the relative hypercalcemia associated with the cooccurrence of adrenal insufficiency. Some of the theories to explain the development of hypercalcemia in adrenal insufficiency include: (1) hypovolemia-induced reduction in glomerular filtration, and hence the amount of calcium filtered through the glomerulus, and (2) increased activity of 1- α hydroxylase, which is normally inhibited by glucocorticoids. Low or inappropriately normal intact parathyroid hormone (PTH) with concurrent hypocalcemia and hyperphosphatemia are diagnostic of hypoparathyroidism. Standard therapy consists of oral calcium salts and calcitriol administration (see Chapter 20 for management). Whereas recent data suggest that once or twice daily administration of subcutaneous recombinant parathyroid hormone (rPTH 1-34 and rPTH 1-84) may provide optimal therapy,^{114–116} especially in those who are poorly controlled on standard therapy, this approach is not yet approved by the US Food and Drug Administration for children in the United States.

Autoimmune adrenocortical insufficiency (Addison disease) is the third major component of APS I and typically occurs after mucocutaneous candidiasis and hypoparathyroidism have been diagnosed. Over 85% of APS I patients eventually develop adrenal insufficiency. Unfortunately, the clinical diagnosis of adrenal insufficiency is often missed initially with the diagnosis commonly made late, or at the time of a life-threatening adrenal crisis. In the Finnish cohort of APS I patients, 40% had Addison disease by 10 years of age and nearly 80% by 30 years of age.⁷⁶ Deficiencies of cortisol, aldosterone, and adrenal androgens may present simultaneously or may evolve over months to years. In addition, the initial symptoms of adrenal insufficiency are often nonspecific, mimicking psychiatric or gastrointestinal disease. These include fatigue, weight loss, myalgias, arthralgias, behavioral changes, nausea and vomiting, abdominal pain, and diarrhea. Cortisol deficiency leads to increased pituitary production of proopiomelanocortin or POMC, a precursor molecule that is cleaved into products including adrenocorticotrophic hormone (ACTH) and melanocyte-stimulating hormone (MSH). Over time, hyperpigmentation (because of elevated MSH) in non-sun-exposed areas, along with postural hypotension, can usually be found on careful examination. Unexplained hypotonic dehydration, with concomitant presence of the mentioned features, should raise the suspicion of Addison disease. Adrenal crisis with hyponatremia, hyperkalemia, acidosis, hypotension, and hypoglycemia may be fatal, unless recognized and treated appropriately and promptly with intravenous glucocorticoids and isotonic fluids (please see specific chapters for more details). As discussed in detail further in the chapter, adrenal autoantibodies are used to predict adrenal cortical failure. If antibody testing is positive, morning cortisol and renin measurements and/or an ACTH stimulation testing may be used diagnostically or monitoring asymptomatic patients.

Autoimmune gonadal failure occurs in over 50% to 60% of women with APS I by age 20 years, whereas less than 25% of males develop testicular insufficiency.^{76,97} Gonadal failure

often presents with primary amenorrhea in young women though menstrual irregularities, polycystic ovaries, or infertility may be presenting features.^{76,117} As with autoimmune adrenalitis, gonadal failure can be predicted by the presence of steroid cell autoantibodies.¹¹⁸

Ectodermal dystrophy, unrelated to hypoparathyroidism or mucocutaneous candidiasis, has been extensively documented in the Finnish cohort. Dental enamel hypoplasia of permanent (but not deciduous) teeth, as well as nail dystrophy (nail pits of 0.5–1 mm), are commonly found. There may be complete absence of the enamel or transverse hypoplastic bands alternating with zones of well-formed enamel. Nearly one-third of the Finnish patients also had calcification of the tympanic membranes¹¹⁹ and 20% to 25% developed keratitis.⁷⁶

As shown in Table 22.1, type 1 diabetes and thyrogastric autoimmunity (a descriptive term for the combination of autoimmune thyroid disease and atrophic gastritis) are associated with APS I, but occur far less frequently than in APS II. When present, thyroiditis is typically atrophic rather than goitrous. Gastric-parietal cell autoimmunity, which leads to atrophic gastritis, with resultant achlorhydria and intrinsic factor deficiency, typically presents as iron-deficiency or vitamin B₁₂-deficient pernicious anemia. Atrophic gastritis occurs in 15% to 30% of APS I cases with a mean age of onset of 16 years.^{76,119} Whereas iron-deficiency anemia is microcytic and vitamin B₁₂-deficient anemia is macrocytic, combined iron and vitamin B₁₂ deficiency can be normocytic. It is also important to recognize that the spinal cord comorbidities (subacute combined degeneration of the spinal cord) of vitamin B₁₂ deficiency can occur in the absence of anemia.

Nonendocrine organ-specific manifestations are less common (~5%–20%) and include alopecia, vitiligo, autoimmune hepatitis, and malabsorption among others. Progression to alopecia totalis (total loss of scalp hair) or universalis (total loss of all body hair, including eyelashes, eyebrows, and scalp hair) usually occurs before puberty. Vitiligo presents initially as small, pale pigment-lacking skin patches. These may be missed, unless specifically sought and ultraviolet light examination of the skin may be necessary. The appearance of clay-colored stools, dark urine, and jaundice suggests chronic active autoimmune hepatitis. Hepatitis occurs in 10% to 15% of APS I patients and is the leading cause of death. Consequently, all patients suspected of having APS I should have their liver function regularly monitored. Autoimmune hepatitis is typically treated with glucocorticoids initially, and later with azathioprine, once there is partial disease control. Malabsorption, which may occur intermittently (and typically of fat), has been linked to hypoparathyroidism (insufficient duodenal mucosal hormone and pancreatic enzymes because of hypocalcemia), bacterial and fungal overgrowth, gluten sensitivity (celiac disease), and IgA deficiency. There have also been rare reports of APS I with hypophysitis (diabetes insipidus, growth hormone deficiency, ACTH deficiency), and nonendocrine manifestations, such as rheumatoid arthritis, Sjögren disease, tubulointerstitial nephritis, autoimmune bronchiolitis, myopathy, and asplenia.^{75,120,121} Recent studies in a European cohort of 112 APS I patients have also uncovered a wide spectrum of phenotypic manifestations and genotypic changes in AIRE.¹²²

A study in a cohort of 35 North and South American subjects revealed that nonendocrine manifestations occur much earlier and more frequently when compared with European cohorts. These nonendocrine manifestations included urticarial eruptions (66%), hepatitis (43%), gastritis (48%), intestinal dysfunction (80%), Sjögren-like syndrome (43%), and pneumonitis (40%), and were present in 80% before the diagnostic dyad criteria of APS were met.¹²³ It is unclear if there are genotype-phenotype correlations that can explain the higher frequency of nonendocrine manifestations in the American cohort.

APS II

APS II is the most common of the autoimmune polyendocrinopathies (excluding the coincidences of type 1 diabetes and autoimmune thyroid disease), with a prevalence of 1 to 4:100,000 and with a polygenic inheritance. Unlike APS I, APS II usually has its onset in adulthood, particularly during the third or fourth decade and is at least 3 times more common in females than males, whereas APS I is equally common in males and females. This female bias in APS II is primarily explained by the coexistence of autoimmune thyroid disease (AITD), which is more common in women. In 1926 Schmidt first described the association of adrenocortical and thyroid gland failure and Carpenter extended this in 1964 to include type 1 diabetes mellitus.^{79,80} In 1957 the autoimmune nature of these diseases was suggested by Doniach and Roitt's discovery of thyroglobulin autoantibodies in patients with Hashimoto thyroiditis.¹²⁴

APS II presents with adrenocortical failure in approximately 50% of cases, though multiple disease components may be present at diagnosis. Although the disease usually has its onset between ages 20 and 50 years, it is not unusual to find cases before or after these ages.^{82,125,126} Type 1 diabetes coexists in nearly 50% of patients with Addison disease, whereas AITD coexists in about two-thirds of patients with Addison disease. Therefore type 1 diabetes and AITD must be pursued vigorously in any patient presenting with Addison disease.

The most common component of APS II is AITD and is seen in almost 70% to 75% of patients.¹²⁷ AITD affects nearly 4.5% of the US population,¹²⁸ with 80% to 90% of all cases occurring in females. AITD increases in incidence during the teen years, peaking in the fifth and sixth decades. Chronic lymphocytic thyroiditis (Hashimoto disease) is by far the most common form of AITD, although Graves disease may also occur. Postpartum thyroiditis has also been considered a transient manifestation of autoimmune thyroiditis following childbirth and can manifest as hypo- or hyperthyroidism. Several studies have reported on the coexistence of islet autoimmunity (3%–8%) or even overt type 1 diabetes and AITD,^{129,130} whereas less than 1% of patients, with otherwise isolated thyroiditis, have serological evidence of adrenal autoimmunity.

Although "polyglandular syndrome" involvement in patients with autoimmune thyroid disease is infrequent, thyroid autoimmunity or a family history of thyroiditis is common in patients with pernicious anemia, vitiligo, alopecia, myasthenia gravis, and Sjögren syndrome.^{131–133} Almost 20% to 40% of vitiligo patients have another component of APS II, with thyrogastic autoimmunity being the most common.^{134,135} Most patients with vitiligo are asymptomatic and evidence of concurrent autoimmunity can be ascertained only by autoantibody screening. Segmental vitiligo with involvement of dermatomal regions is not associated with autoimmunity.¹³⁶ Up to 15% of patients with alopecia (areata, totalis, universalis) and 5% of their first-degree relatives have thyroid disease. More patients with APS I than APS II have cutaneous manifestations, such as vitiligo or alopecia, but because APS II is far more common, most patients with either of the manifestations and another autoimmune disease are categorized as incomplete APS II.

Nearly 30% of patients with myasthenia gravis, an autoimmune disease characterized by presence of anti-acetylcholine receptor autoantibodies and muscle weakness worsening during muscular contraction, have AITD. Both Hashimoto thyroiditis and Graves disease may occur in patients with myasthenia gravis.^{137,138} Interestingly, patients with myasthenia gravis and concomitant AITD tend to have milder expression of their myasthenia, and a lower incidence of thymic disease and acetylcholine receptor α -chain autoantibodies. The incidence of ocular myasthenia is higher in patients with Graves disease.

Type 1 diabetes mellitus, a diagnostic component of APS II, has a peak incidence during the teen years with a smaller but increasing incidence occurring in the preschool years.¹³⁹ Nevertheless, the disease may have its onset at any age. Approximately 10% to 15% of APS patients, incorrectly labeled as type 2 diabetes because of diabetes onset after 40 years of age, actually have slowly progressive autoimmune diabetes (also referred to as *latent autoimmune diabetes of adults* or LADA).¹⁴⁰ Unlike APS II, in which there is a female gender bias despite the occurrence of type 1 diabetes, no gender bias is present in patients with isolated type 1 diabetes. AITD (denoted by the presence of thyroperoxidase and/or thyroglobulin autoantibodies) occurs in 20% to 25% of patients with type 1 diabetes with women representing nearly two-thirds of the autoantibody-positive patients.¹²⁵ Despite the high prevalence of thyroid autoantibodies, less than 20% of patients with thyroid autoantibodies have evidence of thyroid dysfunction defined as an elevated thyroid-stimulating hormone (TSH) concentration. Adrenocortical autoimmunity is much less frequent among patients with type 1 diabetes, with serological evidence reported in 1.5% of cases.^{141,142} Tissue transglutaminase autoantibodies suggestive of celiac disease are present in 3% to 7% patients with type 1 diabetes.¹⁴³ Celiac disease should be suspected in type 1 diabetes patients with unexplained diarrhea, weight loss, failure to gain weight, or failure to thrive, unexplained hypoglycemia should be confirmed by intestinal biopsy.^{144,145}

Gastric parietal cell autoantibodies (PCAs) are present in approximately 10% of females and 5% of males with type 1 diabetes.¹⁴⁶ Although pernicious anemia typically affects women after the fifth decade, children with PCA should be monitored closely for the development of pernicious anemia. Atrophic gastritis may lead to the development of megaloblastic anemia because of lack of intrinsic factor required for absorption of dietary vitamin B₁₂ from the gut. Iron-deficiency anemia may also occur in both adolescents and adults because of impaired ability to absorb iron consequent to decreased acid production (achlorhydria).¹⁴⁷

Approximately 10% of women younger than 40 years of age with APS II develop ovarian failure. Ovarian failure may present as either primary or secondary amenorrhea. In females with biopsy-proven lymphocytic oophoritis, adrenocortical failure or subclinical adrenal autoimmunity is often present.¹⁴⁸ In contrast, progression to gonadal failure is very rare among males with Addison disease.

Pituitary involvement is occasionally seen in APS II.^{149,150} Hypophysitis and empty sella syndrome have been described, leading to isolated failure of secretion of growth hormone, ACTH, TSH, follicle-stimulating hormone (FSH), or luteinizing hormone (LH).

Several nonendocrinological conditions have also been reported in association with APS II. These include ulcerative colitis,¹⁵¹ primary biliary cirrhosis,¹⁵² sarcoidosis,^{153,154} achalasia,¹⁵⁵ myositis,¹⁵⁶ and neuropathy.¹⁵⁷

IPEX Syndrome

IPEX is a syndrome caused by defective Tregs secondary to mutations in the *FOXP3* gene.¹⁵⁸ The *FOXP3* gene encodes a transcriptional factor of the same name. More than 70 *FOXP3* mutations associated with IPEX have been described to date. Because *FOXP3* is located in the X chromosome, the inheritance is X-linked recessive with males being affected, whereas females are carriers.

Patients who have *FOXP3* inactivating mutations resulting in *FOXP3* deficiency develop IPEX, which is characterized by multiorgan autoimmunity that begins very early in life and typically includes the triad of neonatal-onset type 1 diabetes, eczematous dermatitis, and enteropathy (watery diarrhea).

Other autoimmune disorders include thyroiditis, hemolytic anemia, thrombocytopenia, hepatitis, nephropathy, arthritis, and lung disease.^{159–162} Patients with IPEX syndrome often manifest autoantibodies early in life; those with type 1 diabetes often have glutamic acid decarboxylase autoantibodies (GADA), and other islet cell autoantibodies (ICAs) in the neonatal period. Other autoantibodies detected early in life include those against harmonin and villin, proteins found in the microvilli of the intestinal brush border and the renal proximal tubule, and may explain the enteropathy and nephritis found in these patients.

IPEX is often fatal in the first few years of life. To date, only long-term immunosuppression or bone marrow transplantation, with the goal of enhancing regulatory T-cell function, have been effective therapies for IPEX. Sustained expression of *FOXP3* may reprogram effector T cells to act as regulatory T cells.^{163,164} Novel approaches, including treatment with engineered Tregs^{165,166} or targeted gene editing of *FOXP3*,¹⁶⁷ are being investigated as treatment options for IPEX syndrome.

DIAGNOSTIC APPROACH AND FOLLOW-UP

The approach to diagnosing polyglandular syndromes is three-fold: (1) autoantibody screening to (i) verify the autoimmune

nature of the suspected endocrinopathy, and (ii) test for the involvement of other organs and tissues; (2) full assessment of endocrine function in patients with confirmed autoantibodies and autoantibody-negative subjects in whom disease is suspected clinically; and (3) mutation analysis to confirm the diagnosis, and screen siblings and other relatives for their potential carrier status.

Recognition of multiorgan autoimmune diseases before their symptomatic phases is vital to minimize associated morbidity and mortality. A thorough history and physical examination should always be performed, and a high index of suspicion should be maintained. In addition, family history of multiorgan autoimmune disease should increase the suspicion for potential APS.

As discussed later, perhaps the greatest single achievement in the last decade was the discovery of the association of autoantibodies to type 1 interferons and APS I, enabling early diagnosis of APS I patients.¹⁶⁸ Although not indicative of tissue-specific immune attack, autoantibodies to interferon (α and ω) provide a nearly 95% to 100% sensitive and specific screening test for APS I.^{169–171} The presence of autoantibodies to interferons should be followed by confirmatory testing for *AIRE* mutations, as well as testing for other tissue specific autoantibodies (Fig. 22.6). These include 21-hydroxylase or adrenal cortex

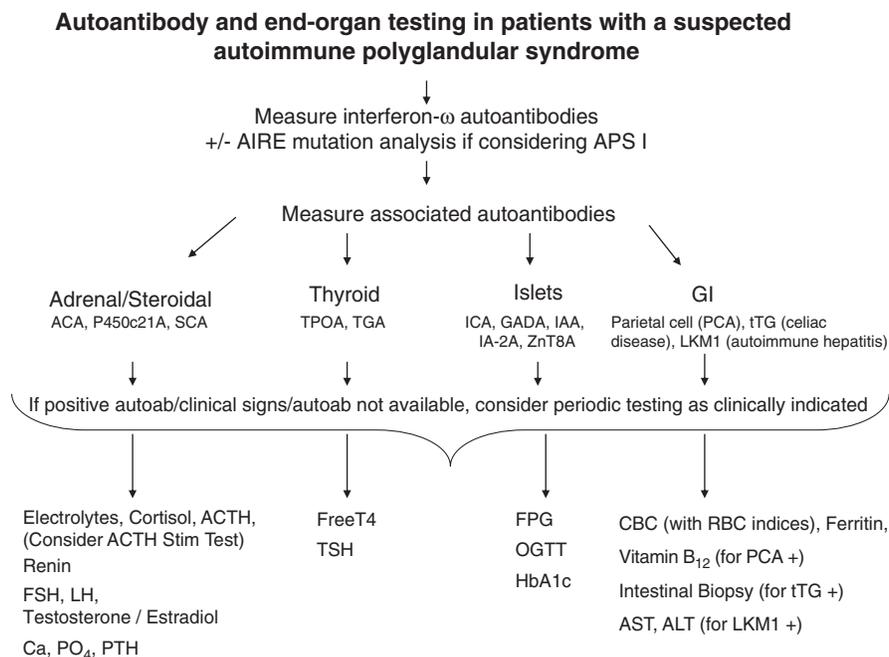


Fig. 22.6 Autoantibody and end-organ testing in patients with a suspected autoimmune polyglandular syndrome. This flow diagram indicates which autoantibodies should be obtained when APS is suspected. Given its sensitivity and specificity, primary screening with interferon- ω autoantibodies (if available) is likely adequate if APS I is suspected. That said, we recommend organ specific autoantibody testing in subjects with a suspected APS related disease (adrenitis, chronic mucocutaneous candidiasis, or autoimmune hypoparathyroidism) at the time of initial diagnosis. We also recommend autoantibody testing in subjects with 2 or more associated autoimmune diseases (i.e. T1D and autoimmune thyroiditis). Mutations in the *AIRE* gene can also be sought. Unfortunately, data are lacking to provide firm recommendations regarding the need for repeat autoantibody testing in patients who are initially autoantibody negative. However, if antibodies are negative, we recommend repeat autoantibody testing in early puberty if clinical signs or symptoms of APS persist. For those who are autoantibody positive, annual testing of end-organ failure should be obtained as shown. When autoantibody assays are unavailable, clinicians must use frequent testing of disease-specific endpoints to identify associated comorbidities. ACA = adrenal cytoplasmic autoantibodies detected by indirect immunofluorescence; P450c21A = Autoantibodies against the adrenal enzyme 21-hydroxylase; SCA = steroidal cell autoantibodies; TPOA = thyroperoxidase autoantibodies; TGA = thyroglobulin autoantibodies; ICA = islet cell cytoplasmic autoantibodies; GADA = glutamic acid decarboxylase autoantibodies; IAA = insulin autoantibodies; IA-2A = insulinoma associated-2 autoantibodies; ZnT8A = zinc transporter-8 autoantibodies; tTG = IgA transglutaminase autoantibodies; LKM1 = liver kidney microsomal type 1 autoantibodies; ACTH = adrenocorticotrophic hormone; FSH = follicle-stimulating hormone; LH = luteinizing hormone; Ca = calcium; PO₄ = phosphate; PTH = parathyroid hormone; T4 = thyroxine; TSH = thyroid stimulating hormone; FPG = fasting plasma glucose, OGTT = oral glucose tolerance test; HbA1c = hemoglobin A1c; CBC = complete blood count.

cytoplasmic autoantibodies (for autoimmune Addison disease), GADA, insulinoma associated autoantibodies (IA-2A), insulin autoantibodies (IAA), zinc transporter 8 (ZnT8) autoantibodies (for type 1 diabetes), thyroperoxidase and thyroglobulin autoantibodies (for AITD), steroidal cell autoantibodies (for ovarian failure), and transglutaminase autoantibodies (for celiac disease). In addition, although not readily available, autoantibodies to 17-hydroxylase, side-chain cleavage enzyme, and 3-hydroxysteroid dehydrogenase can be used to detect gonadal and adrenal autoimmunity. Autoantibodies to NACHT leucine-rich repeat protein 5 (NALP5) and calcium sensing receptor indicate underlying autoimmune hypoparathyroidism (CaSR).^{172,173} However, the specificity of CaSR and NALP5 for diagnosing autoimmune hypoparathyroidism is around 83% and 50%, respectively, whereas sensitivity has been reported to be 39% and 26%, respectively.¹⁷⁴

Other autoantibodies include antibodies to human pituitary enolase, and suggest autoimmune hypophysitis.¹⁷⁵

It should be noted that measurement of some of these autoantibodies might not be available to all clinicians, and require individualized approaches to screening and follow-up. In addition, data suggest a number of autoantibodies to cytokines (IL-17A, IL-17F, and IL-22) may be associated with mucocutaneous candidiasis.¹⁷⁶ Although there is a clear link between the presence of organ-specific autoantibodies and either the presence of preexisting disease, or subsequent progression to disease, the number of associated disorders that may develop and their age of appearance are unpredictable. Consequently, long-term follow-up is necessary in both autoantibody-positive and -negative subjects.

All patients with a single autoimmune disease must be considered at risk for other autoimmune diseases. Whether and when to screen for other autoantibodies is based on the likelihood of finding another autoimmune disease, cost-effectiveness, and the likelihood that screening will prevent morbidity and mortality from other future potential disorders, such as diabetic ketoacidosis, Addisonian crisis, or hypocalcemia with seizures, and so on.

Because of the high incidence of AITD in patients with type 1 diabetes, it is recommended that type 1 diabetes patients have thyroperoxidase and thyroglobulin autoantibodies measured shortly after diagnosis and thyrotropin (TSH) measured biannually.¹⁷⁷ Measuring both thyroid autoantibodies has close to 90% sensitivity for the detection of AITD. The frequency with which thyroid autoantibodies should be repeated remains somewhat controversial. In fact, some clinicians prefer to follow at-risk patients by means of TSH only, given that treatment is typically not initiated until the TSH becomes elevated.

Screening for all components of APS is not recommended in patients with isolated autoimmune thyroid disease. However, several reports have demonstrated an increased incidence of PCA in younger patients with autoimmune thyroiditis.^{147,178} Thus screening for PCA in children with AITD may be considered. In hypothyroid patients with confirmed APS, evidence for adrenal autoimmunity must be sought before starting thyroid hormone replacement therapy, because thyroid hormone

replacement can potentially precipitate an adrenal crisis in patients with marginal adrenocortical function (by increasing metabolism of glucocorticoid hormones).

Delayed diagnoses and even preventable deaths still occur in patients with undiagnosed adrenocortical failure. As mentioned previously, the presentation is often vague and nonspecific, until an Addisonian crisis ensues. In patients with type 1 diabetes, unexplained hypoglycemia or unexplained improvement in blood glucose control might be a clue to the diagnosis of Addison disease. Improved sensitivity to administered insulin and improved glycemia may represent the loss of counterregulatory activity associated with glucocorticoid deficiency. All patients with prolonged or unexplained chronic mucocutaneous candidiasis, or hypoparathyroidism, and females with premature ovarian failure should be evaluated for APS I, especially in the context of potential Addison disease.

Assessment of end-organ function in any patient with positive autoantibodies is recommended annually (see Fig. 22.6). Fasting and/or 2-hour postprandial blood glucose testing, calcium, phosphate plus PTH, and TSH levels can effectively assess islet, parathyroid and thyroid function, respectively, in asymptomatic individuals. Although hemoglobin A1c has a limited sensitivity as a screening tool for type 1 diabetes, publications in the literature have reported that it can predict the clinical onset in those who already have positive islet autoantibodies.¹⁷⁹ Elevated FSH and LH levels with concomitant low sex steroids confirm gonadal failure. Serial assessments of hemoglobin, hematocrit, and red blood cell indices can assess progression to atrophic gastritis in patients with gastric autoimmunity. The findings of a megaloblastic anemia with an elevated mean corpuscular volume suggest vitamin B₁₂ deficiency, whereas a microcytic hypochromic anemia suggests iron deficiency. Vitamin B₁₂ levels should be followed in all patients with PCA because neuropathy can develop without anemia. Before starting therapy, both vitamin B₁₂ level and an iron profile should be obtained. Methylmalonic acid levels are not routinely needed in patients with gastric autoimmunity but may be helpful if vitamin B₁₂ levels are borderline low.¹⁸⁰ Liver function tests and antimitochondrial autoantibodies should be obtained in patients with APS I. Patients with tissue transglutaminase or endomysial autoantibodies are frequently asymptomatic and should be referred to a gastroenterologist to discuss the need for an intestinal biopsy, and potential confirmation of celiac disease.

Low early morning cortisol levels, electrolyte abnormalities (hyponatremia/hyperkalemia), and hypoglycemia represent late changes occurring at or just before the onset of adrenal insufficiency. Just as the natural history of pretype 1 diabetes is well characterized (see Chapter 21), a similar pattern is seen before the development of adrenocortical insufficiency. Four stages have been described (Table 22.3) subsequent to the detection of adrenal autoantibodies: Stage 1: increased plasma renin activity with normal to low aldosterone; Stage 2: decreased cortisol response after parenteral ACTH administration; Stage 3: elevated basal ACTH; and Stage 4: low basal

TABLE 22.3 Stages in the Development of Autoimmune Addison Disease

Stage	Renin	Aldosterone	Basal Cortisol	Cortisol Post ACTH	Basal ACTH
1	Elevated	N or Low	N	N	N
2	Elevated	N or Low	N	Low	N
3	Elevated	N or Low	N	Low	Elevated
4	Elevated	Low	Low ^a	Low	Elevated

N = normal.

^aClinical Addison disease.

ACTH, Adrenocorticotropic hormone; N, normal.

cortisol.^{181,182} In individuals with adrenocortical autoantibodies, screening with a measurement of the morning cortisol concentration and ACTH should be done. A morning cortisol of less than 5 µg/dL with a concomitant plasma ACTH more than twofold above the reference range is highly diagnostic of primary adrenal insufficiency.¹⁸³ If these values are normal but the clinical suspicion is high, a confirmatory 250-µg ACTH stimulation test can be performed. Basal elevated renin values are also very suggestive of adrenocortical failure, and an ACTH stimulation test should be carried out for confirmation.

TREATMENT

Hormone or other replacement therapies for the component disorders of APS I and APS II, such as adrenal insufficiency, hypothyroidism, type 1 diabetes, iron-deficiency anemia, and pernicious anemia are similar whether the ailments occur in isolation or in association with other conditions. Specific endocrine disorder-related therapies are described in the individual chapters. Multidisciplinary teams must be involved in the evaluation and management of patients with APS I or II, given the multitude of pathologies that can manifest in the same individual. In addition, patients with APS I have been reported to have asplenia or functional hyposplenism,¹⁸⁴ and these patients are prone to fulminant infections. Timely immunizations and, if required, prophylactic antibiotics must be part of the management of these patients.

AUTOANTIBODIES IN AUTOIMMUNE POLYGLANDULAR SYNDROMES

Autoantibodies in APS are predominantly IgG antibodies that bind to self-antigens. Autoantibodies may be pathogenic, as observed in Graves disease or myasthenia gravis. In Graves disease, agonistic autoantibodies directed to the TSH receptor stimulate overproduction of thyroid hormone causing hyperthyroidism.¹⁸⁵ In myasthenia gravis, autoantibodies directed against the motor end plate acetylcholine receptor located on myocytes stimulate internalization of the acetylcholine receptor, producing muscular weakness. However, autoantibodies usually serve solely as serologic markers of autoimmunity, such as in type 1 diabetes where ICAs, IAA, GADA, IA-2A, and autoantibodies to the ZnT8A are indicators of ongoing autoimmunity.^{186,187} Interestingly, in patients with APS I, GADA are not as predictive of the subsequent development of type 1 diabetes.⁷⁵

As previously stated, detection of autoantibodies in APS serves several important functions. First, detection of autoantibodies allows for a specific autoimmune diagnosis to be established.¹⁸⁸ Secondly, autoantibody detection in asymptomatic individuals indicates an increased risk for the later development of clinical disease and patients are monitored more closely.¹⁴¹ Thirdly, the presence of an autoantibody or one autoimmune disease in an individual may suggest increased risk for other associated autoimmune diseases.

Nonorgan-Specific Autoantibodies

1) Antiinterferon Autoantibodies

Autoantibodies to interferons (α and ω) are highly specific and sensitive for APS I,¹⁶⁹ and some authors have advocated the use of these antibodies as screening assays rather than evaluating for AIRE mutations. These autoantibodies were first used clinically as indicators of risk for myasthenia gravis and thymoma, and high-titer AIA were unexpectedly found in 60/60 Finnish APS I patients and 16/16 Norwegian APS I patients.¹⁶⁸ Notably, AIA were detected in stored serum samples in known APS I patients before the onset of organ-specific autoantibodies. In

fact, several APS I patients developed AIA before the onset of mucocutaneous candidiasis or other organ manifestations. As further evidence of the specificity of AIA, subjects with AIRE mutations but lacking classic features of APS I (mucocutaneous candidiasis, hypoparathyroidism, or adrenal insufficiency) have also demonstrated high titers to AIA. As such, AIA are likely to become the primary screening test for APS I; AIA can be detected by a competitive immunoassay.¹⁷¹ Recent animal studies have also demonstrated that IFN- α autoantibodies have neutralizing effects in vitro and in vivo, with the potential to ameliorate autoimmune disease processes in APS I.¹⁸⁹ The authors of the same study also questioned if the lower rate of development of type 1 diabetes in APS I patients (10%–20%), despite many having GAD autoantibodies, may be associated with the presence of these neutralizing IFN- α autoantibodies.

2) Autoantibodies to IL-17A, IL-17F, and IL-22

Mucocutaneous candidiasis is often the presenting clinical abnormality in APS I. Although AIA can predict APS I, they do not predict the order or severity of the known APS I components. Disease or organ-specific autoantibodies are therefore needed to provide patients and physicians with optimal anticipatory guidance. Several groups have reported that autoantibodies to cytokines, such as IL-17A, IL-17F, and IL-22, may both predict and explain the mucocutaneous candidiasis associated with APS I. Under normal circumstances, these pro-inflammatory cytokines with antifungal and microbicidal properties are produced by Th17 cells.

In one study, 33/33 patients with APS I and mucocutaneous candidiasis demonstrated high-titer autoantibodies to IL-17A, IL-17F, and IL-22, whereas 0/37 controls, and 0/103 patients with isolated autoimmune diseases were positive for these antibodies.^{123,176,177} These observations have been confirmed in subsequent cohort studies.^{123,190,191} However, these autoantibodies are only used in the research setting currently.

Organ-Specific Autoantibodies

Adrenal Cytoplasmic Autoantibodies

Adrenal cytoplasmic autoantibodies (ACA) were first detected using a complement-fixation technique with saline extracts of adrenal tissue and soon afterward by indirect immunofluorescence.¹⁹² Usually all layers of the adrenal cortex (but not the medulla) fluoresce. The microsomal localization of autoantigens has been confirmed using ultracentrifuged cellular components.¹⁹³ Other assays for adrenal autoantibodies include solid-phase radioimmunoassays and nonradioactive enzyme-linked immunoabsorbent assays.^{182,194}

ACA are detected in all forms of autoimmune Addison disease, be it isolated Addison disease or as part of APS I or APS II. Approximately 50% of asymptomatic ACA-positive individuals develop Addison disease within 3 years, and up to 75% to 100% of those with recent onset or close to clinical onset of Addison disease exhibit ACA.^{181,194} In a follow-up of 20 ACA-positive children followed for up to 11 years, the cumulative risk for developing Addison disease was 100%.¹⁸¹ ACA are also predictive of the subsequent development of Addison disease in adults, although less frequently than in children. Higher titers of ACA and complement fixing ACA have been associated with an increased risk of progressing to clinical disease.¹⁹⁵

Autoantibodies to the surface of adrenal cortical cells have been described, and almost 90% of individuals with Addison disease were reported to exhibit these autoantibodies.¹⁹⁶ However, these assays are rarely done because of the difficulty in obtaining fresh human or animal adrenal tissue that are used in these assays.

Adrenal Enzyme Autoantibodies

Typical of many organ-specific autoimmune diseases, major autoantigens that serve as targets of autoantibodies in APS are enzymes. Examples of various autoimmune diseases where enzymes are the target of the immune system are illustrated in Table 22.2. A discussion of adrenal hormone synthesis can be found in Chapter 14.

21-Hydroxylase (P450c21) is a major autoantigen recognized by autoantibodies in sera from patients with Addison disease.¹⁹⁷ There is a strong correlation between positivity for ACA and P450c21 (21-hydroxylase) autoantibodies, and P450c21 autoantibodies appear to be an even more sensitive indicator of disease.¹⁹⁸ Nearly 75% of APS I and APS II patients have positive P450c21 autoantibodies.¹⁸⁰ Other enzymes have been identified as autoantigens in patients with either isolated autoimmune Addison disease or APS, including P450 cholesterol side-chain cleavage enzyme (P450scc), 17 α -hydroxylase (P450c17), and 3 β -hydroxysteroid dehydrogenase.¹⁹⁸

The autoantigenic epitopes of the P450c21 enzyme are located in the C-terminal end and in a central region of the enzyme.¹⁹⁹ It has been reported that two of four epitopes recognized by P450c17 autoantibodies cross-react with P450c21, indicating that reactivity to one of these autoantigens could actually reflect molecular mimicry between such epitopes.²⁰⁰ Except for the N-terminal amino acids 1–40 and the C-terminal amino acids 456–521, immunoreactive epitopes have been described throughout P450scc.²⁰¹

High titers of both ACA and P450c21 autoantibodies are associated with greater impairment of adrenocortical function and subsequent development of Addison disease.¹⁷⁹ As with many autoimmune diseases, an inverse correlation exists between autoantibody titer and duration of disease in patients with Addison disease.²⁰² This is consistent with the concept that once an autoantigen is completely destroyed, the immune system is no longer stimulated to produce autoantibodies.

When clinical or preclinical Addison disease is present, there is no unique combination of adrenal or gonadal antibodies that separate APS I from APS II.^{187,203,204} The differentiation of APS I from APS II is made either on clinical grounds or, in asymptomatic patients, by detecting concomitant autoantibodies associated with APS II. Furthermore, there are no unique epitopes recognized by P450c21 autoantibodies that allow differentiation of isolated Addison disease versus APS I or APS II.²⁰⁵ However, APS I can be definitely distinguished from APS II via molecular diagnostic tests for mutation in the *AIRE* gene and inferred by the presence (APS I) or absence (APS II) of interferon antibodies.

Steroidal Cell/Gonadal Autoantibodies

Some individuals with ACA have sera that cross-react with reproductive-steroid producing tissues, including the theca interna of the Graafian follicle, Leydig cells of the testis, and/or syncytiotrophoblastic layer of the placenta.^{206,207} Sera that recognize antigens in both adrenal and reproductive-steroid producing tissues whose immunoreactivity cannot be absorbed with adrenal extracts are termed *steroidal cell/gonadal antibodies* (SCA). Such variability in immunoreactivity likely represents differences in autoantigen density or epitope availability differences among the tissues.

In asymptomatic patients, SCA are associated with an increased risk of developing primary autoimmune gonadal failure. In women, this usually manifests as either primary amenorrhea or premature menopause; men are usually asymptomatic. SCA, depending on the assay used are found in 4% to 87% of women with premature ovarian failure.^{206,208,209} SCA have also been reported to be predictive

of gonadal failure in women with APS who had normal menses at the time of initial study. In a small cohort study of 11 APS I female patients, 100% (11 of 11) who were positive for SCA developed primary ovarian failure during a follow-up period of 12 years.²¹⁰ When ACA are present in the absence of SCA, gonadal failure is rare.²⁰⁷ However, approximately one-third of individuals with ACA have SCA (using ovary, testis, or placenta as the antigen source). A small study of five women with SCA who also had ACA, consistent with an APS I manifested ovarian failure.²¹⁰ SCA are more common in APS I than in APS II patients, and it is estimated that 60% of subjects with APS I and Addison disease have SCA, compared with 30% of patients with APS II.

Premature ovarian failure independent of APS may also occur as a consequence of autoimmunity.²¹¹ However, Addison disease coexists in approximately 2% to 10% of women with autoimmune premature ovarian failure, and thus should be sought.²¹² Like other organ-specific autoimmune diseases, autoimmune ovarian failure is characterized histologically by ovarian infiltration with inflammatory cells.²¹³

The nature of the ovarian autoantigens in premature ovarian failure is controversial. Autoantibodies reacting strongly to pig zona pellucida, as determined by indirect immunofluorescence, were observed in six of 22 women with infertility.²¹⁴ Because the adrenal cortex and gonad share several synthetic pathways common to cells producing both adrenocortical and sex steroids, humoral autoimmunity to shared autoantigens might be expected in patients with both Addison disease and gonadal failure. The synthesis of sex and adrenal steroids requires P450scc, 3 β -hydroxysteroid dehydrogenase, and P450c17. Data are, however, contradictory. In one study, SCA activity was removed by preabsorption with recombinant human 3 β -hydroxysteroid dehydrogenase, suggesting that this enzyme was a major autoantigen detected by SCA positive sera.²¹⁵ Another study suggested that SCA correlated best with reactivity to P450scc and a 51-KDa autoantigen that binds to the aromatic L-amino acid decarboxylase present in granulosa cells, placenta, liver, and pancreatic beta cells.²¹⁶

Autoantibodies in Hypoparathyroidism

Autoimmune hypoparathyroidism is unique to APS I. In the original report of parathyroid autoantibodies detected using indirect immunofluorescence, nearly 40% of patients with autoimmune hypoparathyroidism were positive for parathyroid cytoplasmic autoantibodies versus 6% of controls.^{217,218} However, other laboratories did not confirm the initial reports of the existence of such parathyroid cytoplasmic autoantibodies.^{219,220} It was shown that autoantibodies detected by indirect immunofluorescence directed against the parathyroid gland could be preabsorbed with human mitochondria, indicating that such autoantibodies were not tissue specific.²²⁰

Since then, several different antiparathyroid autoantibodies have been reported in patients with hypoparathyroidism. Screening of human parathyroid complementary DNA libraries with sera from patients with known APS I and hypoparathyroidism have identified NALP5 as an important parathyroid autoantigen. Autoantibodies were detected in 49% of patients with APS I and hypoparathyroidism, but were absent in all 293 controls.¹⁷¹ Although NALP5 autoantibodies are rarely (0.69%) seen in patients with idiopathic hypoparathyroidism,²²¹ their specificity for diagnosing APS I-associated hypoparathyroidism is only around 50% and sensitivity 26%.¹⁷² Activating autoantibodies to CaSR are also seen in patients with autoimmune hypoparathyroidism. The specificity of CaSR autoantibodies for diagnosing autoimmune hypoparathyroidism is 83%, which is better than that of NALP autoantibodies. However, the sensitivity of CaSR autoantibodies at 39% remains low.¹⁷²

Other Autoantibodies in APS I and II

In addition to the autoantibodies discussed earlier, autoantibodies to tyrosine hydroxylase have been reported in around 40% of patients with APS I and appear to correlate with alopecia areata,²²² whereas autoantibodies to tryptophan hydroxylase correlate with intestinal malabsorption.²²³ Antibodies to liver/kidney microsome type 1 (LKM1) are found in nearly 100% of patients with type 2 autoimmune hepatitis.²²⁴ Some 40% of such patients have associated autoimmune disease commonly seen in APS I. The hepatic autoantigens P450IA2 and P450A6 seem to be the targets of these antibodies. Autoantibodies to aromatic L-amino acid decarboxylase have also been recognized in APS I patients, and is associated with autoimmune hepatitis and vitiligo.²²⁵ Antibodies to human pituitary enolase are associated with autoimmune hypophysitis.¹⁷⁴ Finally, autoantibodies against bactericidal/permeability-increasing fold-containing B1 (BPIFB1) and the potassium channel regulator KCNRC have been associated with development of pneumonitis. BPIFB1 has been reported to be highly sensitive, whereas KCNRC has a low sensitivity, but 100% specificity for pneumonitis.¹²²

SUMMARY

The APS result from a loss of tolerance to self-antigens. APS I, an autosomal recessive disorder mapped to the *AIRE* gene, is defined by the presence of at least two of the following three findings: (1) adrenocortical autoimmunity, (2) hypoparathyroidism, or (3) mucocutaneous candidiasis. APS II is defined by the coexistence of autoimmune adrenocortical insufficiency or serologic evidence of adrenalitis with autoimmune thyroiditis and/or type 1 diabetes mellitus. The occurrence of any one component disease of an APS may be linked to the occurrence of others through shared autoimmunity background genes that are involved with loss of tolerance. As such, a high index of suspicion should be maintained whenever one autoimmune disorder is diagnosed. For example, in type 1 diabetes, it is routine to screen for thyroid autoimmunity and celiac disease.

Treatment of APS should be aimed at optimal management of the specific underlying diseases. Screening for the presence of associated autoimmune disorders should be performed regularly. In conclusion, an improved understanding of the interaction between susceptibility genes, environmental triggers, individualized genomic background and its relation to the development of impaired immune tolerance, new immunological applications (such as generation of thymic epithelial cells from stem cells or use of engineered Tregs) should prove to be the best path to improved diagnostic and therapeutic modalities in the care of patients with APS.

Autoimmune type 1 diabetes mellitus and other immune endocrinopathies before the age of 6 months of life, associated with enteropathy in a male, should raise consideration of the IPEX syndrome and mutational analysis of the *FOXP3* gene; without evidence of autoimmunity, for example, antiislet antibodies, a form of neonatal diabetes mellitus, should be considered because specific therapies for the latter include effective oral medications and may not require insulin (see Chapter 10).

The increasing use of immune checkpoint inhibitors is associated with various adverse reactions, including endocrinopathies, such as hypophysitis, leading to secondary adrenal insufficiency, or primary adrenal insufficiency, thyroiditis, and occasionally type 1 diabetes mellitus. These may occur in 10% to 20% of adult patients weeks to months after the use of the drugs, though the frequency in childhood and adolescence is not yet defined. However, physicians should be aware of these entities and treat with appropriate hormone replacement as indicated.^{226,227}

REFERENCES

- Ballotti S, Chiarelli F, de Martino M. Autoimmunity: basic mechanisms and implications in endocrine diseases. *Part II Horm Res.* 2006;66(3):142–152.
- Ballotti S, Chiarelli F, de Martino M. Autoimmunity: basic mechanisms and implications in endocrine diseases. *Part I Horm Res.* 2006;66(3):132–141.
- Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the Aire protein. *Science (New York, NY).* 2002;298(5597):1395–1401.
- Mathis D, Benoist C. A decade of Aire. *Nat Rev Immunol.* 2007;7(8):645–650.
- Turvey SE, Broide DH. Innate immunity. *J Allergy Clin Immunol.* 2010;125:S24–S32.
- Bonilla FA, Oettgen HC. Adaptive immunity. *J Allergy Clin Immunol.* 2010;125:S33–S40.
- Delves PJ, Roitt IM. The immune system. Second of two parts. *N Engl J Med.* 2000;343(2):108–117.
- Delves PJ, Roitt IM. The immune system. First of two parts. *N Engl J Med.* 2000;343(1):37–49.
- Feero WG, Green ED. Genomics education for health care professionals in the 21st century. *JAMA.* 2011;306:989–990.
- Unanue ER. Perspective on antigen processing and presentation. *Immunol Rev.* 2002;185:86–102.
- Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol Rev.* 2005;207:145–157.
- Holling TM, Schooten E, van Den Elsen PJ. Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men. *Hum Immunol.* 2004;65(4):282–290.
- Neeffes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol.* 2011;11(12):823–836.
- Anderson MS, Su MA. Aire expands: new roles in immune tolerance and beyond. *Nat Rev Immunol.* 2016;16:247–258.
- Ruan QG, Tung K, Eisenman D, Setiady Y, Eckenrode S, Yi B, et al. The autoimmune regulator directly controls the expression of genes critical for thymic epithelial function. *J Immunol (Baltimore, Md: 1950).* 2007;178(11):7173–7180.
- Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D. The cellular mechanism of Aire control of T cell tolerance. *Immunity.* 2005;23(2):227–239.
- Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, et al. Positional cloning of the APECED gene. *Nat Genet.* 1997;17(4):393–398.
- Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet.* 1997;17(4):399–403.
- Nishino M, Ashiku SK, Kocher ON, Thurer RL, Boisselle PM, Hatabu H. The thymus: a comprehensive review. *Radiographics.* 2006;26:335–348.
- Edelmann SL, Marconi P, Brocker T. Peripheral T cells re-enter the thymus and interfere with central tolerance induction. *J Immunol (Baltimore, Md: 1950).* 2011;186(10):5612–5619.
- von Boehmer H, Aifantis I, Gounari F, Azogui O, Haughn L, Apostolou I, et al. Thymic selection revisited: how essential is it? *Immunol Rev.* 2003;191:62–78.
- von Boehmer H, Kisielow P. Self-nonself discrimination by T cells. *Science (New York, NY).* 1990;248(4961):1369–1373.
- Ramsdell F, Fowlkes BJ. Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. *Science (New York, NY).* 1990;248(4961):1342–1348.
- Danzl NM, Donlin LT, Alexandropoulos K. Regulation of medullary thymic epithelial cell differentiation and function by the signaling protein Sin. *J Exp Med.* 2010;207:999–1013.
- Mathis D, Benoist C. Back to central tolerance. *Immunity.* 2004;20(5):509–516.
- Passos GA, Speck-Hernandez CA, Assis AF, Mendes-da-Cruz DA. Update on Aire and thymic negative selection. *Immunology.* 2018;153:10–20.
- Malchow S, Leventhal DS, Lee V, Nishi S, Socci ND, Savage PA. Aire enforces immune tolerance by directing autoreactive t cells into the regulatory t cell lineage. *Immunity.* 2016;44:1102–1113.
- Gardner JM, Metzger TC, McMahon EJ, Au-Yeung BB, Krawisz AK, Lu W, et al. Extrathymic Aire-expressing cells are a distinct bone

- marrow-derived population that induce functional inactivation of CD4⁺ T cells. *Immunity*. 2013;39:560–572.
29. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol*. 2013;13:227–242.
 30. Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. *Nat Immunol*. 2009;10:1185–1192.
 31. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*. 2009;206:3015–3029.
 32. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature*. 1996;383(6603):787–793.
 33. O’Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4⁺ T cells. *Science (New York, NY)*. 2010;327(5969):1098–1102.
 34. Ria F, Penna G, Adorini L. Th1 cells induce and Th2 inhibit antigen-dependent IL-12 secretion by dendritic cells. *Eur J Immunol*. 1998;28:2003–2016.
 35. Romagnani S. Th1/Th2 cells. *Inflamm Bowel Dis*. 1999;5:285–294.
 36. Lan RY, Ansari AA, Lian ZX, Gershwin ME. Regulatory T cells: development, function and role in autoimmunity. *Autoimmun Rev*. 2005;4(6):351–363.
 37. Ziegler SF. FOXP3: of mice and men. *Annu Rev Immunol*. 2006;24:209–226.
 38. Kyewski B, Klein L. A central role for central tolerance. *Annu Rev Immunol*. 2006;24:571–606.
 39. Fuchizawa T, Adachi Y, Ito Y, Higashiyama H, Kanegane H, Futatani T, et al. Developmental changes of FOXP3-expressing CD4⁺CD25⁺ regulatory T cells and their impairment in patients with FOXP3 gene mutations. *Clin Immunol*. 2007;125:237–246.
 40. Bacchetta R, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, et al. Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest*. 2006;116(6):1713–1722.
 41. Le Bras S, Geha RS. IPEX and the role of Foxp3 in the development and function of human Tregs. *J Clin Invest*. 2006;116(6):1473–1475.
 42. Healy JI, Goodnow CC. Positive versus negative signaling by lymphocyte antigen receptors. *Annu Rev Immunol*. 1998;16:645–670.
 43. Zhang L, Zhang YJ, Chen J, Huang XL, Fang GS, Yang LJ, et al. The association of HLA-B27 and Klebsiella pneumoniae in ankylosing spondylitis: a systematic review. *Microb Pathog*. 2018;117:49–54.
 44. Kyewski B, Derbinski J. Self-representation in the thymus: an extended view. *Nat Rev Immunol*. 2004;4(9):688–698.
 45. Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, et al. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann Intern Med*. 2003;139(5 Pt 1):346–351.
 46. Pisetsky DS. Fulfilling Koch’s postulates of autoimmunity: anti-NR2 antibodies in mice and men. *Arthritis Rheum*. 2006;54:2349–2352.
 47. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature*. 2005;435(7042):590–597.
 48. Viret C, Sant’Angelo DB, He X, Ramaswamy H, Janeway Jr CA. A role for accessibility to self-peptide-self-MHC complexes in intrathymic negative selection. *J Immunol (Baltimore, Md: 1950)*. 2001;166(7):4429–4437.
 49. Vafiadis P, Ounissi-Benkhalha H, Palumbo M, Grabs R, Rousseau M, Goodyer CG, et al. Class III alleles of the variable number of tandem repeat insulin polymorphism associated with silencing of thymic insulin predispose to type 1 diabetes. *J Clin Endocrinol Metab*. 2001;86(8):3705–3710.
 50. Cohen IR. Antigenic mimicry, clonal selection and autoimmunity. *J Autoimmun*. 2001;16(3):337–340.
 51. Damico FM, Kiss S, Young LH. Sympathetic ophthalmia. *Semin Ophthalmol*. 2005;20(3):191–197.
 52. Chang GC, Young LH. Sympathetic ophthalmia. *Semin Ophthalmol*. 2011;26(4–5):316–320.
 53. Zhang M, Alicot EM, Chiu I, Li J, Verna N, Vorup-Jensen T, et al. Identification of the target self-antigens in reperfusion injury. *J Exp Med*. 2006;203(1):141–152.
 54. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. *Clin Rev Allergy Immunol*. 2012;42(1):102–111.
 55. Soulas P, Woods A, Jaulhac B, Knapp AM, Pasquali JL, Martin T, et al. Autoantigen, innate immunity, and T cells cooperate to break B cell tolerance during bacterial infection. *J Clin Invest*. 2005;115(8):2257–2267.
 56. Olson JK, Croxford JL, Miller SD. Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation, and progression of T-cell-mediated autoimmune disease. *Viral Immunol*. 2001;14(3):227–250.
 57. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA*. 2003;290(13):1721–1728.
 58. Martins TB, Hoffman JL, Augustine NH, Phansalkar AR, Fischetti VA, Zabriskie JB, et al. Comprehensive analysis of antibody responses to streptococcal and tissue antigens in patients with acute rheumatic fever. *Int Immunol*. 2008;20:445–452.
 59. Kirvan CA, Cox CJ, Swedo SE, Cunningham MW. Tubulin is a neuronal target of autoantibodies in Sydenham’s chorea. *J Immunol*. 2007;178:7412–7421.
 60. McKay DM. Bacterial superantigens: provocateurs of gut dysfunction and inflammation? *Trend Immunol*. 2001;22(9):497–501.
 61. Michels AW, Gottlieb PA. Autoimmune polyglandular syndromes. *Nat Rev Endocrinol*. 2010;6(5):270–277.
 62. Owen CJ, Cheetham TD. Diagnosis and management of polyendocrinopathy syndromes. *Endocrinol Metab Clin North Am*. 2009;38(2):419–436. x.
 63. Tait KF, Gough SC. The genetics of autoimmune endocrine disease. *Clin Endocrinol*. 2003;59(1):1–11.
 64. Miyazaki Y, Hirayama M, Watanabe H, Usami N, Yokoi K, Watanabe O, et al. Paraneoplastic encephalitis associated with myasthenia gravis and malignant thymoma. *J Clin Neurosci*. 2012;19(2):336–338.
 65. Naoura I, Didelot A, Walker F, Luton D, Koskas M. Anti-N-methyl-D-aspartate receptor encephalitis complicating ovarian teratomas: a case report. *Am J Obstet Gynecol*. 2011;205(4):e6–e8.
 66. Rosin L, DeCamilli P, Butler M, Solimena M, Schmitt HP, Morgenthaler N, et al. Stiff-man syndrome in a woman with breast cancer: an uncommon central nervous system paraneoplastic syndrome. *Neurology*. 1998;50(1):94–98.
 67. June CH, Warshauer JT, Bluestone JA. Is autoimmunity the Achilles’ heel of cancer immunotherapy? *Nat Med*. 2017;23:540–547.
 68. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature*. 2017;545:495–499.
 69. Dong Y, Sun Q, Zhang X. PD-1 and its ligands are important immune checkpoints in cancer. *Oncotarget*. 2017;8:2171–2186.
 70. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol*. 2015;15:486–499.
 71. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, Iyer AK. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *J Pharm Pharmacol*. 2017;8:561.
 72. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139–148.
 73. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*. 2018;359:104–108.
 74. Guo Y, Lei K, Tang L. Neoantigen Vaccine Delivery for Personalized Anticancer Immunotherapy. *Front Immunol*. 2018;9:1499.
 75. Husebye ES, Perheentupa J, Rautema R, Kampe O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J Intern Med*. 2009;265(5):514–529.
 76. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab*. 2006;91(8):2843–2850.
 77. Buzi F, Badolato R, Mazza C, Giliani S, Notarangelo LD, Radetti G, et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome: time to review diagnostic criteria? *J Clin Endocrinol Metab*. 2003;88(7):3146–3148.
 78. Peterson P, Peltonen L. Autoimmune polyendocrinopathy syndrome type 1 (APS1) and AIRE gene: new views on molecular basis of autoimmunity. *J Autoimmun*. 2005;25(Suppl):49–55.

79. Schmidt M. Eine biglandulare Erkrankung (Nebennieren und Schilddrüse) bei Morbus Addisonii. *Verh Dtsch Ges Pathol Ges.* 1926;21:212–221 (in German).
80. Carpenter CC, Solomon N, Silverberg SG, Bledsoe T, Northcutt RC, Klinenberg JR, et al. Schmidt's syndrome (thyroid and adrenal insufficiency). A review of the literature and a report of fifteen new cases including ten instances of coexistent diabetes mellitus. *Medicine.* 1964;43:153–180.
81. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. *N Engl J Med.* 2004;350(20):2068–2079.
82. Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev.* 2002;23(3):327–364.
83. Sperling M, Yau M. Autoimmune Polyglandular Syndromes. [Updated 2017 Oct 29]. In: De Groot LJ, Chrousos G, Dungan K, et al. eds. *Endotext [Internet]. South Dartmouth (MA): MDText.com: Inc.; 2000:2000.*
84. Amerio P, Tracanna M, De Remigis P, Betterle C, Vianale L, Marra ME, et al. Vitiligo associated with other autoimmune diseases: polyglandular autoimmune syndrome types 3B+C and 4. *Clin Exp Dermatol.* 2006;31(5):746–749.
85. Ahonen P. Autoimmune polyendocrinopathy–candidosis–ectodermal dystrophy (APECED): autosomal recessive inheritance. *Clin Genet.* 1985;27:535–542.
86. Perniola R, Musco G. The biophysical and biochemical properties of the autoimmune regulator (AIRE) protein. *Biochim Biophys Acta.* 2014;1842:326–337.
87. Waterfield M, Khan IS, Cortez JT, Fan U, Metzger T, Greer A, et al. The transcriptional regulator Aire coopts the repressive ATF7ip-MBD1 complex for the induction of immunotolerance. *Nat Immunol.* 2014;15:258–265.
88. Gaetani M, Matafora V, Saare M, Spiliotopoulos D, Mollica L, Quilici G, et al. AIRE-PHD fingers are structural hubs to maintain the integrity of chromatin-associated interactome. *Nucleic Acids Res.* 2012;40:11756–11768.
89. Trebusak PK, Bratanic N, Krziszak C, Battelino T. Autoimmune regulator-1 messenger ribonucleic acid analysis in a novel intronic mutation and two additional novel AIRE gene mutations in a cohort of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *J Clin Endocrinol Metab.* 2005;90:4930–4935.
90. Meloni A, Fiorilli E, Corda D, Perniola R, Cao A, Rosatelli MC. Two novel mutations of the AIRE protein affecting its homodimerization properties. *Hum Mutat.* 2005;25:319.
91. Ulinksi T, Perrin L, Morris M, Houang M, Cabrol S, Grapin C, et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome with renal failure: impact of posttransplant immunosuppression on disease activity. *J Clin Endocrinol Metab.* 2006;91:192–195.
92. Cetani F, Barbesino G, Borsari S, Pardi E, Cianferotti L, Pinchera A, Marocchi C. A novel mutation of the autoimmune regulator gene in an Italian kindred with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, acting in a dominant fashion and strongly cosegregating with hypothyroid autoimmune thyroiditis. *J Clin Endocrinol Metab.* 2001;86:4747–4752.
93. Anderson MS, Casanova JL. More than meets the eye: monogenic autoimmunity strikes again. *Immunity.* 2015;42:986–988.
94. Oftedal BE, Hellesen A, Erichsen MM, Bratland E, Vardi A, Perheentupa J, et al. Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune diseases. *Immunity.* 2015;42:1185–1196.
95. Maclaren NK, Riley WJ. Inherited susceptibility to autoimmune Addison's disease is linked to human leukocyte antigens-DR3 and/or DR4, except when associated with type 1 autoimmune polyglandular syndrome. *J Clin Endocrinol Metab.* 1986;62:455–459.
96. Weinstock C, Matheis N, Barkia S, Haager MC, Janson A, Marković A, et al. Autoimmune polyglandular syndrome type 2 shows the same HLA class II pattern as type 1 diabetes. *Tissue Antigens.* 2011;77:317–324.
97. Husebye ES, Anderson MS, Kämpe O. Autoimmune polyendocrine syndromes. *N Engl J Med.* 2018;378:2543–2544.
98. Hunt PJ, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JAH, Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol.* 2001;55:491–499.
99. Levin L, Ban Y, Concepcion E, Davies TF, Greenberg DA, Tomer Y. Analysis of HLA genes in families with autoimmune diabetes and thyroiditis. *Hum Immunol.* 2004;65:640–647.
100. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature.* 2003;423:506–511.
101. Grant SF, Qu HQ, Bradfield JP, Marchand L, Kim CE, Glessner JT, et al. Follow-up analysis of genome-wide association data identifies novel loci for type 1 diabetes. *Diabetes.* 2009;58:290–295.
102. Eriksson D, Bianchi M, Landegren N, Nordin J, Dalin F, Mathioudaki A, et al. Extended exome sequencing identifies BACH2 as a novel major risk locus for Addison's disease. *J Intern Med.* 2016;280:595–608.
103. Pazderska A, Oftedal BE, Napier CM, Ainsworth HF, Husebye ES, Cordell HJ, et al. A variant in the BACH2 gene is associated with susceptibility to autoimmune Addison's disease in humans. *J Clin Endocrinol Metab.* 2016;101:3865–3869.
104. Bottini N, Musumeci L, Alonso A, Rahmouni S, Nika K, Rostamkhani M, et al. A functional variant of lymphoid tyrosine phosphatase is associated with type 1 diabetes. *Nat Genet.* 2004;36:337–338.
105. Kumar PG, Laloraya M, Wang CY, Ruan QG, Davoodi-Semiromi A, Kao KJ, et al. The autoimmune regulator (AIRE) is a DNA-binding protein. *J Biol Chem.* 2001;276(44):41357–41364.
106. Wolff AS, Erichsen MM, Meager A, Magitta NE, Myhre AG, Bollerslev J, et al. Autoimmune polyendocrine syndrome type 1 in Norway: phenotypic variation, autoantibodies, and novel mutations in the autoimmune regulator gene. *J Clin Endocrinol Metab.* 2007;92(2):595–603.
107. Rautemaa R, Hietanen J, Niissalo S, Pirinen S, Perheentupa J. Oral and oesophageal squamous cell carcinoma—a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). *Oral Oncol.* 2007;43(6):607–613.
108. Siikala E, Richardson M, Pfaller MA, Diekema DJ, Messer SA, Perheentupa J, et al. Candida albicans isolates from APECED patients show decreased susceptibility to miconazole. *Int J Antimicrob Agents.* 2009;34(6):607–609.
109. Rautemaa R, Richardson M, Pfaller M, Perheentupa J, Saxen H. Reduction of fluconazole susceptibility of Candida albicans in APECED patients due to long-term use of ketoconazole and miconazole. *Scand J Infect Dis.* 2008;40(11–12):904–907.
110. Loose DS, Kan PB, Hirst MA, Marcus RA, Feldman D. Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes. *J Clin Invest.* 1983;71:1495–1499.
111. Sonino N. The use of ketoconazole as an inhibitor of steroid production. *N Engl J Med.* 1987;317:812–818.
112. Albert SG, DeLeon MJ, Silverberg AB. Possible association between high-dose fluconazole and adrenal insufficiency in critically ill patients. *Crit Care Med.* 2001;29:668–670.
113. Miller A, Brooks LK, Poola-Kella S, Malek R. (2018). Posaconazole-induced adrenal insufficiency in a case of chronic myelomonocytic leukemia. *Case Rep Endocrinol.* 2018;2170484.
114. Winer KK, Sinaii N, Peterson D, Sainz Jr B, Cutler Jr GB. Effects of once versus twice-daily parathyroid hormone 1-34 therapy in children with hypoparathyroidism. *J Clin Endocrinol Metab.* 2008;93(9):3389–3395.
115. Brandi ML, Bilezikian JP, Shoback D, Bouillon R, Clarke BL, Thakker RV, et al. Management of Hypoparathyroidism: Summary Statement and Guidelines. *J Clin Endocrinol Metab.* 2016;101:2273–2283.
116. Vokes TJ, Mannstadt M, Levine MA, Clarke BL, Lakatos P, Chen K, et al. Recombinant human parathyroid hormone effect on health-related quality of life in adults with chronic hypoparathyroidism. *J Clin Endocrinol Metab.* 2018;103:722–731.
117. Maclaren N, Chen QY, Kukreja A, Marker J, Zhang CH, Sun ZS. (2001). Autoimmune hypogonadism as part of an autoimmune polyglandular syndrome. *J Soc Gynecol Invest.* 2001;8(1 Suppl Proceedings):S52–S54.
118. Soderbergh A, Myhre AG, Ekwall O, Gebre-Medhin G, Hedstrand H, Landgren E, et al. Prevalence and clinical

- associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab.* 2004;89(2): 557–562.
119. Ahonen P, Myllarniemi S, Sipila I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med.* 1990;322(26):1829–1836.
 120. Gazulla Abio J, Benavente Aguilar I, Ricoy Campo JR, Madero Barrajon P. Myopathy with trabecular fibers associated with familiar autoimmune polyglandular syndrome type 1. *Neurologia (Barcelona, Spain).* 2005;20(10):702–708.
 121. De Luca F, Valenzise M, Alaggio R, Arrigo T, Crisafulli G, Salzano G, et al. Sicilian family with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and lethal lung disease in one of the affected brothers. *Eur J Pediatr.* 2008;167(11):1283–1288.
 122. Orlova EM, Sozaeva LS, Kareva MA, Oftedal BE, Wolff ASB, Breivik L, et al. Expanding the phenotypic and genotypic landscape of autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab.* 2017;102:3546–3556.
 123. Ferre EM, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, Niemela JE, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight.* 2016;1.
 124. Doniach D, Roitt IM. Auto-immunity in Hashimoto's disease and its implications. *J Clin Endocrinol Metab.* 1957;17(11):1293–3104.
 125. Betterle C, Lazzarotto F, Presotto F. Autoimmune polyglandular syndrome Type 2: the tip of an iceberg? *Clin Exp Immunol.* 2004;137(2):225–233.
 126. Hugle B, Dollmann R, Keller E, Kiess W. (2004). Addison's crisis in adolescent patients with previously diagnosed diabetes mellitus as manifestation of polyglandular autoimmune syndrome type II—report of two patients. *J Pediatr Endocrinol Metab.* 2004;17(1):93–97.
 127. Dittmar M, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. *J Clin Endocrinol Metab.* 2003;88:2983–2992.
 128. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.* 2002;87(2):489–499.
 129. Jaeger C, Hatzigelaki E, Petzoldt R, Bretzel RG. Comparative analysis of organ-specific autoantibodies and celiac disease-associated antibodies in type 1 diabetic patients, their first-degree relatives, and healthy control subjects. *Diabetes Care.* 2001;24(1): 27–32.
 130. Kordonouri O, Klinghammer A, Lang EB, Gruters-Kieslich A, Grabert M, Holl RW. Thyroid autoimmunity in children and adolescents with type 1 diabetes: a multicenter survey. *Diabetes Care.* 2002;25(8):1346–1350.
 131. Alkhateb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res.* 2003;16(3): 208–214.
 132. Ruggeri RM, Galletti M, Mandolino MG, Aragona P, Bartolone S, Giorgianni G, et al. Thyroid hormone autoantibodies in primary Sjogren syndrome and rheumatoid arthritis are more prevalent than in autoimmune thyroid disease, becoming progressively more frequent in these diseases. *J Endocrinol Invest.* 2002;25(5):447–454.
 133. Tsao CY, Mendell JR, Lo WD, Luquette M, Rennebohm R. Myasthenia gravis and associated autoimmune diseases in children. *J Child Neurol.* 2000;15(11):767–769.
 134. Zettinig G, Tanew A, Fischer G, Mayr W, Dudczak R, Weissel M. Autoimmune diseases in vitiligo: do anti-nuclear antibodies decrease thyroid volume? *Clin Exp Immunol.* 2003;131(2):347–354.
 135. Kakourou T, Kanaka-Gantenbein C, Papadopoulou A, Kaloumenou E, Chrousos GP. (2005). Increased prevalence of chronic autoimmune (Hashimoto's) thyroiditis in children and adolescents with vitiligo. *J Am Acad Dermatol.* 2005;53(2): 220–223.
 136. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. *J Am Acad Dermatol.* 1996;35(5 Pt 1):671–674.
 137. Tanwani LK, Lohano V, Ewart R, Broadstone VL, Mokshagundam SP. Myasthenia gravis in conjunction with Graves' disease: a diagnostic challenge. *Endocr Pract.* 2001;7(4):275–278.
 138. Weetman AP. Non-thyroid autoantibodies in autoimmune thyroid disease. *Best Pract Res Clin Endocrinol Metab.* 2005;19(1):17–32.
 139. Karvonen M, Viik-Kajander M, Moltchanova E, Libman I, LaPorte R, Tuomilehto J. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. *Diabetes Care.* 2000;23(10):1516–1526.
 140. Stenstrom G, Gottsater A, Bakhtadze E, Berger B, Sundkvist G. Latent autoimmune diabetes in adults: definition, prevalence, beta-cell function, and treatment. *Diabetes.* 2005;54(Suppl 2): S68–S72.
 141. Barker JM. Type 1 diabetes-associated autoimmunity: natural history, genetic associations, and screening. *J Clin Endocrinol Metab.* 2006;91:1210–1217.
 142. Barker JM, Ide A, Hostetler C, Yu L, Miao D, Fain PR, et al. Endocrine and immunogenetic testing in individuals with type 1 diabetes and 21-hydroxylase autoantibodies: Addison's disease in a high-risk population. *J Clin Endocrinol Metab.* 2005;90: 128–134.
 143. Hansson T, Dahlbom I, Rogberg S, Danaeus A, Hopfl P, Gut H, et al. Recombinant human tissue transglutaminase for diagnosis and follow-up of childhood coeliac disease. *Pediatr Res.* 2002;51:700–705.
 144. Smith CM, Clarke CF, Porteous LE, Elson H, Cameron DJ. Prevalence of coeliac disease and longitudinal follow-up of antigliadin antibody status in children and adolescents with type 1 diabetes mellitus. *Pediatr Diabet.* 2000;1:199–203.
 145. Not T, Tommasini A, Tonini G, Buratti E, Pocecco M, Tortul C, et al. Undiagnosed coeliac disease and risk of autoimmune disorders in subjects with Type I diabetes mellitus. *Diabetologia.* 2001;44:151–155.
 146. Alonso N, Granada ML, Salinas I, Lucas AM, Reverter JL, Junca J, et al. Serum pepsinogen I: an early marker of pernicious anemia in patients with type 1 diabetes. *J Clin Endocrinol Metab.* 2005;90(9): 5254–5258.
 147. Segni M, Borrelli O, Pucarelli I, Delle Fave G, Pasquino AM, Annibale B. Early manifestations of gastric autoimmunity in patients with juvenile autoimmune thyroid diseases. *J Clin Endocrinol Metab.* 2004;89(10):4944–4948.
 148. Welt CK, Falorni A, Taylor AE, Martin KA, Hall JE. Selective theca cell dysfunction in autoimmune oophoritis results in multifollicular development, decreased estradiol, and elevated inhibin B levels. *J Clin Endocrinol Metab.* 2005;90(5):3069–3076.
 149. Cemeroglu AP, Bober E, Dundar B, Buyukgebiz A. Autoimmune polyglandular endocrinopathy and anterior hypophysitis in a 14 year-old girl presenting with delayed puberty. *J Pediatr Endocrinol Metab.* 2001;14(7):909–914.
 150. Zung A, Andrews-Murray G, Winqvist O, Chalew SA. Growth hormone deficiency in autoimmune polyglandular syndrome. *J Pediatr Endocrinol Metab.* 1997;10(1):69–72.
 151. Govindarajan R, Galpin OP. Coexistence of Addison's disease, ulcerative colitis, hypothyroidism and pernicious anemia. *J Clin Gastroenterol.* 1992;15(1):82–83.
 152. Ko GT, Szeto CC, Yeung VT, Chow CC, Chan H, Cockram CS. Autoimmune polyglandular syndrome and primary biliary cirrhosis. *Br J Clin Pract.* 1996;50(6):344–346.
 153. Papadopoulos KI, Hornblad Y, Liljebladh H, Hallengren B. (1996). High frequency of endocrine autoimmunity in patients with sarcoidosis. *Eur J Endocrinol.* 1996;134(3):331–336.
 154. Watson JP, Lewis RA. Schmidt's syndrome associated with sarcoidosis. *Postgrad Med J.* 1996;72(849):435–436.
 155. Fritzen R, Bornstein SR, Scherbaum WA. Megaesophagus in a patient with autoimmune polyglandular syndrome type II. *Clin Endocrinol.* 1996;45(4):493–498.
 156. Heuss D, Engelhardt A, Gobel H, Neundorfer B. Myopathological findings in interstitial myositis in type II polyendocrine autoimmune syndrome (Schmidt's syndrome). *Neurol Res.* 1995;17(3):233–237.
 157. Watkins PJ, Gayle C, Alsanjari N, Scaravilli F, Zanone M, Thomas PK. Severe sensory-autonomic neuropathy and endocrinopathy in insulin-dependent diabetes. *QJM.* 1995;88(11): 795–804.
 158. Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. *Ann N Y Acad Sci.* 2018;1417:5–22.
 159. Wildin RS, Freitas A. IPEX and FOXP3: clinical and research perspectives. *J Autoimmun.* 2005;25(Suppl):56–62.

160. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet.* 2001;27(1):20–21.
161. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet.* 2001;27(1):18–20.
162. Zavattari P, Deidda E, Pitzalis M, Zoa B, Moi L, Lampis R, et al. No association between variation of the FOXP3 gene and common type 1 diabetes in the Sardinian population. *Diabetes.* 2004;53(7):1911–1914.
163. Hori S, Nomura T, Sakaguchi S. Pillars article: control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2017;2003(299):1057–1061.
164. Hori S, Takahashi T, Sakaguchi S. Control of autoimmunity by naturally arising regulatory CD4⁺ T cells. *Adv Immunol.* 2003;81:331–371.
165. Passerini L, Rossi Mel E, Sartirana C, Foustieri G, Bondanza A, Naldini L, et al. CD4⁺ T cells from IPEX patients convert into functional and stable regulatory T cells by FOXP3 gene transfer. *Sci Transl Med.* 2013;5:215ra174.
166. Marshall GP, Cserney J, Perry DJ, Yeh W-I, Seay HR, Elsayed AG, et al. Clinical applications of regulatory t cells in adoptive cell therapies. *Cell Gene Ther Insights.* 2018;4:405–429.
167. Goodwin M, Santoni de Sio F, Dever D, Porteus M, Roncarolo MG, Bacchetta R. Gene editing as a therapeutic approach to treat IPEX syndrome. *Mol Ther.* 2016;24.
168. Meager A, Visvalingam K, Peterson P, Moll K, Murumagi A, Krohn K, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med.* 2006;3(7):e289.
169. Meloni A, Furcas M, Cetani F, Marcocci C, Falorni A, Perniola R, et al. Autoantibodies against type I interferons as an additional diagnostic criterion for autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab.* 2008;93(11):4389–4397.
170. Oftedal BE, Wolff AS, Bratland E, Kampe O, Perheentupa J, Myhre AG, et al. Radioimmunoassay for autoantibodies against interferon omega; its use in the diagnosis of autoimmune polyendocrine syndrome type I. *Clin Immunol(Orlando, Fla).* 2008;129(1):163–169.
171. Zhang L, Barker JM, Babu S, Su M, Stenerson M, Cheng M, et al. A robust immunoassay for anti-interferon autoantibodies that is highly specific for patients with autoimmune polyglandular syndrome type 1. *Clin Immunol(Orlando, Fla).* 2007;125(2):131–137. 2007.
172. Alimohammadi M, Bjorklund P, Hallgren A, Pontynen N, Szinnai G, Shikama N, et al. Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N Engl J Med.* 2008;358(10):1018–1028.
173. Gavalas NG, Kemp EH, Krohn KJ, Brown EM, Watson PF, Weetman AP. The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab.* 2007;92(6):2107–2114.
174. Kemp EH, Habibullah M, Kluger N, Ranki A, Sandhu HK, et al. Prevalence and clinical associations of calcium-sensing receptor and NALP5 autoantibodies in Finnish APECED patients. *J Clin Endocrinol Metab.* 2014;99:1064–1071.
175. O'Dwyer DT, McElduff P, Peterson P, Perheentupa J, Crock PA. Pituitary autoantibodies in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *Acta bio-medica : Atenei Parmensis.* 2007;78(Suppl 1):248–254.
176. Puel A, Doffinger N, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med.* 2010;207(2):291–297.
177. Association AD. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. *Diabetes Care.* 2018;41:S13–S27.
178. Bright GM, Blizzard RM, Kaiser DL, Clarke WL. Organ-specific autoantibodies in children with common endocrine diseases. *J Pediatr.* 1982;100(1):8–14.
179. Helminen O, Aspholm S, Pokka T, Hautakangas MR, Haatanen N, Lempainen J, et al. HbA1c predicts time to diagnosis of type 1 diabetes in children at risk. *Diabetes.* 2015;64:1719–1727.
180. Elin RJ, Winter WE. Methylmalonic acid: a test whose time has come? *Arch Pathol Lab Med.* 2001;125(6):824–827.
181. Coco G, Dal Pra C, Presotto F, Albergoni MP, Canova C, Pedini B, et al. Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. *J Clin Endocrinol Metab.* 2006;91(5):1637–1645.
182. Betterle C, Coco G, Zanchetta R. Adrenal cortex autoantibodies in subjects with normal adrenal function. *Best Pract Res Clin Endocrinol Metab.* 2005;19(1):85–99.
183. Bornstein SR, Alolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, et al. Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2016;101:364–389.
184. Pollak U, Bar-Sever Z, Hoffer V, Marcus N, Scheuerman O, Garty BZ. Asplenia and functional hyposplenism in autoimmune polyglandular syndrome type 1. *Eur J Pediatr.* 2009;168:233–235.
185. Schott M, Minich WB, Willenberg HS, Papewalis C, Seissler J, Feldkamp J, et al. Relevance of TSH receptor stimulating and blocking autoantibody measurement for the prediction of relapse in Graves' disease. *Horm Metab Res.* 2005;37(12):741–744.
186. Winter WE, Harris N, Schatz D. Immunological markers in the diagnosis and prediction of autoimmune type 1a diabetes. *Clin Diabetes.* 2002;20:183–191.
187. Winter WE, Harris N, Schatz D. Type 1 diabetes islet autoantibody markers. *Diabetes Technol Ther.* 2002;4(6):817–839.
188. Soderbergh A, Myhre AG, Ekwall O, Gebre-Medhin G, Hedstrand H, Landgren E, et al. Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab.* 2004;89:557–562.
189. Meyer S, Woodward M, Hertel C, Vlaicu P, Haque Y, Karner J, et al. AIRE-deficient patients harbor unique high-affinity disease-ameliorating autoantibodies. *Cell.* 2016;166:582–595.
190. Ng, W.F., von Delwig, A., Carmichael, A.J., Arkwright, P.D., Abinun, M., Cant, A.J., et al. Impaired T(H)17 responses in patients with chronic mucocutaneous candidiasis with and without autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Allergy Clin Immunol.* 2010; 126(5): 1006–1015, 15.e1–e4.
191. Browne SK, Holland SM. Immunodeficiency secondary to anticytokine autoantibodies. *Curr Opin Allergy Clin Immunol.* 2010;10(6):534–541.
192. Blizzard RM, Kyle M. Studies of the adrenal antigens and antibodies in Addison's disease. *J Clin Invest.* 1963;42:1653–1660.
193. Drexhage HA, Bottazzo GF, Bitensky L, Chayen J, Doniach D. Thyroid growth-blocking antibodies in primary myxoedema. *Nature.* 1981;289(5798):594–596.
194. Betterle C, Volpato M, Pedini B, Chen S, Smith BR, Furmaniak J. Adrenal-cortex autoantibodies and steroid-producing cells autoantibodies in patients with Addison's disease: comparison of immunofluorescence and immunoprecipitation assays. *J Clin Endocrinol Metab.* 1999;84(2):618–622.
195. Betterle C, Volpato M, Rees Smith B, Furmaniak J, Chen S, Greggio NA, et al. I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. *J Clin Endocrinol Metab.* 1997;82(3):932–938.
196. Khoury EL, Hammond L, Bottazzo GF, Doniach D. Surface-reactive antibodies to human adrenal cells in Addison's disease. *Clin Exp Immunol.* 1981;45(1):48–55.
197. Husebye ES, Bratland E, Bredholt G, Fridkin M, Dayan M, Mozes E. The substrate-binding domain of 21-hydroxylase, the main autoantigen in autoimmune Addison's disease, is an immunodominant T cell epitope. *Endocrinology.* 2006;147(5): 2411–2416.
198. de Carmo Silva R, Kater CE, Dib SA, Laureti S, Forini F, Cosentino A, et al. Autoantibodies against recombinant human steroidogenic enzymes 21-hydroxylase, side-chain cleavage and 17alpha-hydroxylase in Addison's disease and autoimmune polyendocrine syndrome type III. *Eur J Endocrinol.* 2000;142(2):187–194.
199. Nikoshkov A, Falorni A, Lajic S, Laureti S, Wedell A, Lernmark K, et al. A conformation-dependent epitope in Addison's disease and other endocrinological autoimmune diseases maps to a carboxyl-terminal functional domain of human steroid 21-hydroxylase. *J Immunol (Baltimore, Md: 1950).* 1999;162(4): 2422–2426.

200. Peterson P, Krohn KJ. Mapping of B cell epitopes on steroid 17 alpha-hydroxylase, an autoantigen in autoimmune polyglandular syndrome type I. *Clin Exp Immunol.* 1994;98(1):104–109.
201. Liiv I, Teesalu K, Peterson P, Clemente MG, Perheentupa J, Uiibo R. Epitope mapping of cytochrome P450 cholesterol side-chain cleavage enzyme by sera from patients with autoimmune polyglandular syndrome type I. *Eur J Endocrinol.* 2002;146(1):113–119.
202. Ross I, Boulle A, Soule S, Levitt N, Pirie F, Karlsson A, et al. Autoimmunity predominates in a large South African cohort with Addison's disease of mainly European descent despite long-standing disease and is associated with HLA DQB*0201. *Clin Endocrinol.* 2010;73(3):291–298.
203. Falorni A, Laureti S, Santeusano F. Autoantibodies in autoimmune polyendocrine syndrome type II. *Endocrinol Metab Clin North Am.* 2002;31(2):369–389. vii.
204. Betterle C, Lazzarotto F, Presotto F. Autoimmune polyglandular syndrome Type 2: the tip of an iceberg? *Clin Exp Immunol.* 2004;137:225–233.
205. Nikoshkov A, Falorni A, Lajic S, Laureti S, Wedell A, Lernmark A, Luthman H. A conformation-dependent epitope in Addison's disease and other endocrinological autoimmune diseases maps to a carboxyl-terminal functional domain of human steroid 21-hydroxylase. *J Immunol.* 1999;162:2422–2426.
206. Dal Pra C, Chen S, Furmaniak J, Smith BR, Pedini B, Moscon A, et al. Autoantibodies to steroidogenic enzymes in patients with premature ovarian failure with and without Addison's disease. *Eur J Endocrinol.* 2003;148(5):565–570.
207. Myhre AG, Undlien DE, Lovas K, Uhlving S, Nedrebo BG, Fougner KJ, et al. Autoimmune adrenocortical failure in norway autoantibodies and human leukocyte antigen Class II associations related to clinical features. *J Clin Endocrinol Metab.* 2002;87:618–623.
208. Falorni A, Laureti S, Candeloro P, Perrino S, Coronella C, Bizzarro A, et al. Steroid-cell autoantibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. *Fertil Steril.* 2002;78(2):270–279.
209. Reimand K, Peterson P, Hyoty H, Uiibo R, Cooke I, Weetman AP, et al. 3beta-hydroxysteroid dehydrogenase autoantibodies are rare in premature ovarian failure. *J Clin Endocrinol Metab.* 2000;85(6):2324–2326.
210. Ahonen P, Miettinen A, Perheentupa J. Adrenal and steroidal cell antibodies in patients with autoimmune polyglandular disease type I and risk of adrenocortical and ovarian failure. *J Clin Endocrinol Metab.* 1987;64(3):494–500.
211. Goswami D, Conway GS. Premature ovarian failure. *Hum Reprod Update.* 2005;11(4):391–410.
212. Bakalov VK, Vanderhoof VH, Bondy CA, Nelson LM. Adrenal antibodies detect asymptomatic auto-immune adrenal insufficiency in young women with spontaneous premature ovarian failure. *Hum Reprod (Oxford, England).* 2002;17(8):2096–2100.
213. Santoro N. Research on the mechanisms of premature ovarian failure. *J Soc Gynecol Invest.* 2001;8(1 Suppl Proceedings):S10–S12.
214. Shivers CA, Dunbar BS. Autoantibodies to zona pellucida: a possible cause for infertility in women. *Science (New York, NY).* 1977;197(4308):1082–1084.
215. Uiibo R, Aavik E, Peterson P, Perheentupa J, Aranko S, Pelkonen R, et al. Autoantibodies to cytochrome P450 enzymes P450_{sc}, P450_{c17}, and P450_{c21} in autoimmune polyglandular disease types I and II and in isolated Addison's disease. *J Clin Endocrinol Metab.* 1994;78(2):323–328.
216. Soderbergh A, Rorsman F, Halonen M, Ekwall O, Bjorses P, Kampe O, et al. Autoantibodies against aromatic L-amino acid decarboxylase identifies a subgroup of patients with Addison's disease. *J Clin Endocrinol Metab.* 2000;85(1):460–463.
217. Blizzard RM, Chee D, Davis W. The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. *Clin Exp Immunol.* 1966;1(2):119–128.
218. Irvine WJ, Scarth L. Antibody to the oxyphil cells of the human parathyroid in idiopathic hypoparathyroidism. *Clin Exp Immunol.* 1969;4(5):505–510.
219. Chapman CK, Bradwell AR, Dykks PW. Do parathyroid and adrenal autoantibodies coexist? *J Clin Pathol.* 1986;39(7):813–814.
220. Betterle C, Caretto A, Zeviani M, Pedini B, Salviati C. Demonstration and characterization of anti-human mitochondria autoantibodies in idiopathic hypoparathyroidism and in other conditions. *Clin Exp Immunol.* 1985;62(2):353–360.
221. Tomar N, Kaushal E, Das M, Gupta N, Betterle C, Goswami R. Prevalence and significance of NALP5 autoantibodies in patients with idiopathic hypoparathyroidism. *J Clin Endocrinol Metab.* 2012;97(4):1219–1226.
222. Hedstrand H, Ekwall O, Haavik J, Landgren E, Betterle C, Perheentupa J, et al. Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem Biophys Res Commun.* 2000;267(1):456–461.
223. Ekwall O, Hedstrand H, Haavik J, Perheentupa J, Betterle C, Gustafsson J, et al. Pteridin-dependent hydroxylases as autoantigens in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab.* 2000;85(8):2944–2950.
224. Diamantis I, Boumpas DT. Autoimmune hepatitis: evolving concepts. *Autoimmun Rev.* 2004;3(3):207–214.
225. Soderbergh A, Rorsman F, Halonen M, Ekwall O, Bjorses P, Kampe O, et al. Autoantibodies against aromatic L-amino acid decarboxylase identifies a subgroup of patients with Addison's disease. *J Clin Endocrinol Metab.* 2000;85(1):460–463.
226. Postow MA, Sidlow R, Hellmann MD. Immune related adverse events associated with the use of immune checkpoint blockade. *New Engl J Med.* 2018;378:158–168.
227. Johnson DB, Chandra S, Sosman JA. Immune checkpoint inhibitor toxicity in 2018. *JAMA.* 2018;320(16):1702–1703.

CHAPTER OUTLINE

INTRODUCTION 904**PHYSIOLOGIC DEVELOPMENT OF GLUCOSE METABOLISM DURING INFANCY AND CHILDHOOD 904**

Glucose Production and Utilization 904

Adaptation to Longer Feeding Intervals 905

SYMPTOMS, SIGNS, AND EFFECTS OF HYPOGLYCEMIA 906**DEFINITION OF HYPOGLYCEMIA 908****FASTING SYSTEMS APPROACH TO THE DIAGNOSIS OF HYPOGLYCEMIA 908****MAJOR CAUSES OF HYPOGLYCEMIA IN THE TODDLER AND CHILD 910**

Hyperinsulinism 910

Glycogen Storage Diseases 916

Hormone Deficiency 923

Genetic Disorders of Gluconeogenesis 924

Hypoglycemia Induced by Exogenous Agents 925

Hypoglycin A and Jamaican Vomiting Sickness 926

Hypoglycemia in Fasting, Starvation, Illness, and Stress 926

Reactive Hypoglycemia and “Spells” 927

Artifactual Hypoglycemia 927

EMERGENCY TREATMENT OF HYPOGLYCEMIA 928**APPENDIX: USEFUL TESTS FOR DIAGNOSIS OF HYPOGLYCEMIA DISORDERS 929**

References 929

INTRODUCTION

Glucose is an obligate fuel for the brain under physiological conditions. The brain can neither synthesize glucose nor store more than approximately a 20-minute supply of glycogen; therefore brain survival requires a continuous supply of glucose.^{1,2} The brain can use alternative fuels from the circulation, provided their concentrations rise high enough to enter the brain in sufficient amounts; for example, elevated ketones during prolonged fasting³ or lactate during vigorous exercise⁴ and in untreated type 1 glycogen storage disease (GSD).⁵ Blood-to-brain glucose transport mediated by glucose transporter 1 (GLUT-1) is a direct function of the arterial plasma glucose concentration, and physiologic mechanisms normally maintain plasma glucose at levels that ensure adequate glucose delivery to the brain. At physiological (70–100 mg/dL¹) plasma glucose concentrations, the rate of blood-to-brain glucose transport exceeds the rate of brain glucose metabolism. At plasma glucose concentrations lower than 54 mg/dL,² however, the cerebral metabolic rate of glucose decreases,^{6,7} and at even lower plasma glucose concentrations, functional brain failure occurs, and profound and prolonged hypoglycemia causes permanent brain injury and eventually brain death.²

Beyond the newborn period and early infancy, hypoglycemia is uncommon and is usually caused by an acquired disorder of the endocrine system, prolonged fasting in susceptible individuals (e.g., an intercurrent gastrointestinal illness), congenital abnormalities, such as hyperinsulinism (HI) or inborn errors of metabolism, or accidental and rarely deliberate exposure to medication or toxins. As the interval between feedings increases in the growing infant, physiologic endocrine and metabolic processes normally ensure the maintenance of normoglycemia; however, hypoglycemia can first manifest later in infancy or early childhood when there are mild congenital defects of these systems. In contrast, hypoglycemia presenting in older children and adults is typically caused by an acquired disorder.

This chapter describes an approach to diagnosis based on identifying the specific cause of failure to maintain normal

glucose homeostasis. Key diagnostic information is often best derived from blood and urine specimens (referred to as *critical samples*) obtained at the time of hypoglycemia and immediately before reversing hypoglycemia.

PHYSIOLOGIC DEVELOPMENT OF GLUCOSE METABOLISM DURING INFANCY AND CHILDHOOD

Glucose Production and Utilization

Rates of glucose flux into and out of the circulation are normally tightly regulated and systemic glucose balance is maintained, while ensuring a continuous supply of glucose to the brain. After a typical carbohydrate-containing meal, increased insulin secretion (together with inhibition of counterregulatory hormone secretion) leads to rapid disposal of ingested glucose, either for immediate energy needs or storage via deposition of glycogen and conversion to fat, resulting in restoration of plasma glucose concentrations to basal levels within 2 to 3 hours. Bier et al. showed that in children, ranging from premature infants to age 6 years, mean glucose production rates are 5 to 8 mg/kg/min; thereafter, glucose production as a function of body weight decreases toward the mean adult value of 2.3 mg/kg/min.⁸ Furthermore, the rate of glucose production is linearly correlated with estimated brain weight at all ages.⁸ Because the brain accounts for the bulk of daily glucose utilization, it is not surprising that adult glucose production rates are reached by mid-childhood (body weight ~30 kg), when the child's brain weighs about 90% of the adult brain (Fig. 23.1).

During fasting, the brain initially uses glucose almost exclusively provided from a combination of glycogenolysis and gluconeogenesis in the liver. As liver glycogen stores diminish, adipose tissue lipolysis is activated to increase availability of free fatty acids (FFA) as fuel for peripheral tissues, such as muscle, and for ketogenesis in the liver, which makes ketones available as a brain fuel and partly replaces glucose utilization. Note that the time to reach the fasting hyperketonemia stage is markedly shorter in younger infants and children than in adults,

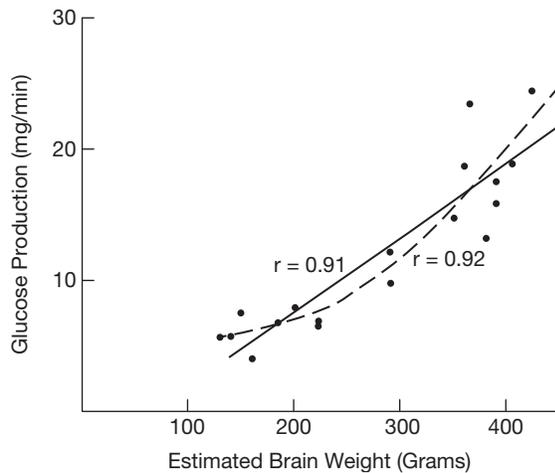
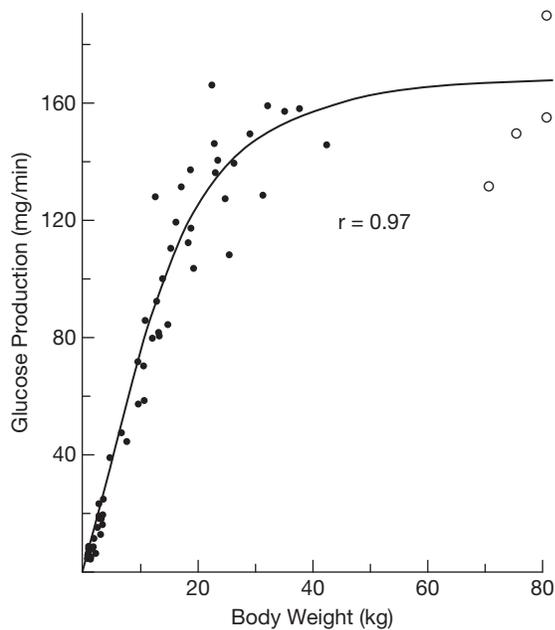


Fig. 23.1 Glucose production as a function of body weight (*top*) and estimated brain weight (*bottom*). Note the change in slope at approximately 30 to 40 kg of body weight, when brain growth is complete. (From Bier, D.M., Leake, R.D., Haymond, M.W., Arnold, K.J., Gruenke, L.D., Sperling, M.A., et al. (1977). Measurement of “true” glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes*, 26, 1016–1023.)⁸

because of their larger ratio of brain relative to muscle mass. As shown in Fig. 23.2, the change in sources of fuel for the brain is reflected in the changes in plasma concentrations of the major metabolic fuels. The substantially smaller muscle mass of infants and young children relative to brain mass limits their ability to tolerate prolonged fasting.⁹ Because brain growth is nearly complete by 10 to 12 years of age, plasma glucose can be maintained above 70 mg/dL for progressively longer fasting durations in adolescents and adults. Nonetheless, most full-term infants, from about 1 week to 1 year of age, are able to tolerate fasting for 15 to 18 hours before plasma glucose decreases below 70 mg/dL,^{10–12} and by 1 year of age, most normal children are able to fast for up to 24 hours.^{12–15} By 5 years of age, a fast of up to 36 hours may be tolerated, and most adults can maintain fasting plasma glucose above 70 mg/dL for 48 to 72 hours (Fig. 23.3).¹⁶ When hypoglycemia is induced by fasting for a shorter duration than would be expected for the child’s

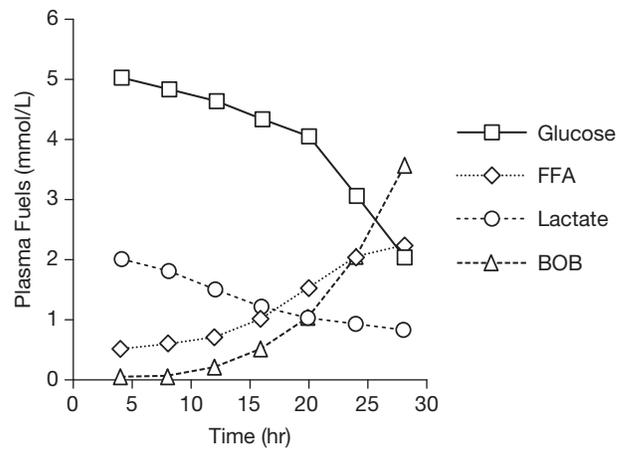


Fig. 23.2 Changes in plasma concentrations of glucose and major metabolic fuels during a fast in a normal child. Note that plasma glucose concentrations decline toward hypoglycemic values by 24 hours, as hepatic glycogen reserves are depleted. The level of lactate, a representative gluconeogenic substrate, declines gradually during fasting. Late in fasting, levels of plasma free fatty acids increase as lipolysis is activated—followed by an increase in beta-hydroxybutyrate as rates of hepatic fatty acid oxidation and ketogenesis increase. BOB, beta-hydroxybutyrate; FFA, free fatty acid.

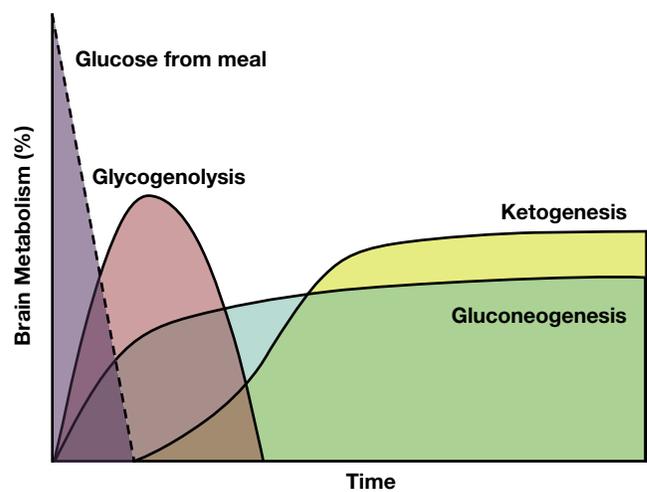


Fig. 23.3 Change in fuel sources during fasting. 1 month old—14- to 18-hour fast; 1 to 5 years old—16- to 24-hour fast; 6 to 12 years old—24- to 36-hour fast, adult—30- to 48-hour fast

age, the possibility of an underlying hypoglycemia disorder should be considered.^{12,17}

Adaptation to Longer Feeding Intervals

With feeding, plasma insulin concentrations in normal-weight children rise from basal levels of 3 to 10 μ U/mL to peak levels of 20 to 50 μ U/mL, which serves to stimulate hepatic glycogen synthesis, inhibit hepatic glycogenolysis and gluconeogenesis, and enhance peripheral (muscle and adipose tissue) glucose uptake and utilization (Table 23.1). Simultaneously, triglyceride synthesis is activated and lipolysis and ketogenesis are suppressed. In the postabsorptive state, a characteristic sequence of physiological responses are involved in preventing or correcting hypoglycemia. The earliest response to decreasing plasma glucose concentrations is a decrease in insulin secretion, as

TABLE 23.1 Hormonal Regulation of Fuel Metabolism

	Insulin	Glucagon	Epinephrine ^a	Cortisol	Growth hormone
Glucose uptake	+		-	-	-
Glycogenolysis	-	+	+	^b	^b
Gluconeogenesis	-	+	+	+	+
Lipolysis	-		+	+	+
Ketogenesis	-	+	+	+	

+ denotes stimulates, - inhibits; ^aincludes sympathetic nervous system activation ^bcortisol stimulates glycogen synthesis, and both cortisol and growth hormone exert permissive effects on the gluconeogenic and glycogenolytic effects of glucagon and epinephrine.

plasma glucose declines within the physiological range (80–85 mg/dL) followed by increased secretion of glucose counterregulatory hormones (glucagon, epinephrine, cortisol, and growth hormone [GH]), when plasma glucose falls just below the postabsorptive physiological range (65–70 mg/dL), and sympathetic neural activation occurs at 55 mg/dL (Fig. 23.4). The combined effects of suppressed insulin secretion, increased levels of counterregulatory hormones, and sympathetic neural activation mobilize stored fuels and reduce glucose utilization, thereby preventing hypoglycemia.^{18–20} With insulin levels suppressed, elevated glucagon secretion and sympathetic neural activation trigger glycogenolysis, the first phase in the metabolic defense against hypoglycemia. In infants, liver glycogen stores may provide glucose for up to 4 hours (see Fig. 23.3). As the child grows, hepatic glycogen content relative to brain glucose utilization is greater and may be able to provide glucose for up to 8 hours of fasting. Isolated glucagon deficiency is extremely rare and, with the exception of children on beta-blocker drugs, deficiency of sympathetic nervous activity is equally unusual. Therefore hypoglycemia occurring within 2 to 4 hours of a feed suggests either a primary disorder of glycogenolysis or excess (dysregulated) insulin secretion. As glycogen stores become depleted, maintenance of plasma glucose levels relies on gluconeogenesis and reduced tissue glucose utilization (resulting from increased oxidation of FFA and ketones) (see Fig. 23.3). The main gluconeogenic precursors are amino acids, especially alanine and glutamine, from skeletal muscle, and glycerol from adipose tissue lipolysis (Fig. 23.5). FFA become the body's major fuel source, as the duration of fasting becomes more prolonged.

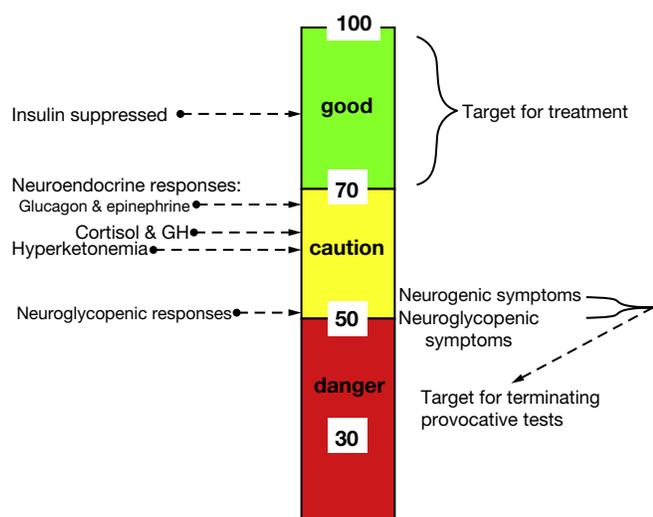


Fig. 23.4 Interpreting plasma glucose levels (mg/dL) and plasma glucose targets for treatment of hypoglycemia and for terminating provocative tests. *GH*, growth hormone. (Based on data from Cryer PE. *Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. Diabetes. 1994;43(11):1378-89.*³⁹⁰)

Mitochondrial fatty acid oxidation in the liver produces ketone bodies (acetoacetate and β -hydroxybutyrate [BOHB]), which are used for energy production by the brain and skeletal muscle, including cardiac muscle (see Fig. 23.2).^{12,14,21,22} Oxidation of FFA and ketone utilization decreases peripheral glucose utilization, which helps to ensure an adequate supply of glucose to the brain and tissues that can only use glucose as fuel (e.g., red blood cells and renal medullae), while preventing excessive breakdown of muscle protein. Lipolysis of triglycerides in adipose tissue is triggered by low levels of insulin together with increased secretion of the counterregulatory hormones (especially GH²³) and sympathetic neural activation. In infants, elevation of plasma ketone concentrations begins within 12 to 18 hours of fasting; whereas, in older children, substantial ketonemia may not appear until 18 to 24 hours of fasting.^{12,24,25} Cortisol, secreted in response to stress, accelerates gluconeogenesis and lipolysis and decreases glucose utilization.²⁶

Defects in gluconeogenesis (e.g., fructose-1,6-bisphosphatase deficiency; see Fig. 23.5) usually become manifest only after glycogen stores have been depleted; accordingly, hypoglycemia typically does not occur in the recently fed state. Disorders of fatty acid oxidation also typically manifest after more prolonged fasting. In the first few months of life, the interval between feedings in the on-demand breastfed infant gradually increase from about 2 to 3 hours to 4 hours or more, and eventually to 8 to 12 hours as nighttime feedings are omitted. Therefore disorders of gluconeogenesis and fatty acid oxidation seldom present as hypoglycemia in the newborn period when feeding is frequent; rather, they present later in infancy as the interval between feeds becomes more prolonged. They may, however, present immediately after birth before lactation is established. Congenital or acquired deficiencies of cortisol and GH may also cause hypoglycemia either in the newborn period, if congenital and severe, or later in infancy when longer periods of fasting typically occur. Combined deficiency of cortisol (adrenocorticotropic hormone [ACTH]) and GH in hypopituitarism may cause earlier onset and more severe hypoglycemia than occurs with isolated deficiencies of either GH or cortisol.

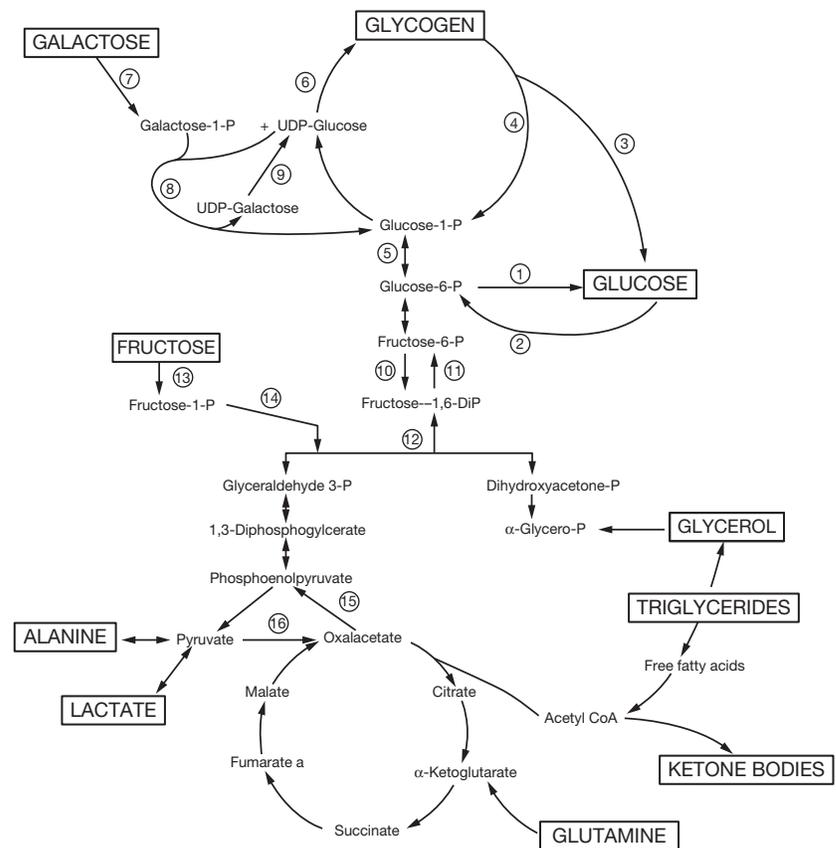
SYMPTOMS, SIGNS, AND EFFECTS OF HYPOGLYCEMIA

Control of the counterregulatory responses is at least partially local: a low glucose concentration and a decrease in intraislet β -cell insulin secretion stimulate glucagon secretion.^{27,28} Low plasma glucose concentrations are sensed in the hepatic portal vein,²⁹ as well as in the gut, carotid body, and oral cavity (and are transmitted to the brain) and also are directly sensed in the hindbrain and hypothalamus,³⁰ which initiates an increase in sympathoadrenal activity.

The trigger for counterregulatory responses is the plasma glucose level itself. The rate at which glucose decreases and the absolute level of insulin have little effect.^{20,31} The symptoms of hypoglycemia, which reflect the brain's response to glucose

Fig. 23.5 Key metabolic pathways of intermediary metabolism.

Disruption of the elements of these pathways may be pathogenetic in the development of hypoglycemia. Not shown is the hormonal control of these pathways. Indicated are: (1) glucose 6-phosphatase, (2) glucokinase, (3) amylo-1,6-glucosidase, (4) phosphorylase, (5) phosphoglucomutase, (6) glycogen synthetase, (7) galactokinase, (8) galactose 1-phosphate uridyl transferase, (9) uridine diphosphogalactose-4-epimerase, (10) phosphofruktokinase, (11) fructose 1,6- diphosphatase, (12) fructose 1,6-diphosphate aldolase, (13) fructokinase, (14) fructose 1-phosphate aldolase, (15) phosphoenolpyruvate carboxykinase, and (16) pyruvate carboxylase. *UDP*, Uridine diphosphate. (From Pagliara, A. S., Karl, I. E., Haymond, M., & Kipnis, D. M. (1973). Hypoglycemia in infancy and childhood. *J Pediatr*, 82, 365–379, 558–577.)



BOX 23.1 Symptoms of Hypoglycemia

NEUROGENIC SYMPTOMS CAUSED BY ACTIVATION OF AUTONOMIC NERVOUS SYSTEM

- Sweating
- Shakiness, trembling
- Tachycardia
- Anxiety, nervousness
- Weakness
- Hunger
- Nausea, vomiting
- Pallor
- Hypothermia

NEUROGLYCOPENIC SYMPTOMS CAUSED BY DECREASED CEREBRAL GLUCOSE USE

- Headache
- Visual disturbances
- Lethargy, lassitude
- Restlessness, irritability
- Difficulty with speech and thinking, inability to concentrate

- Mental confusion
- Somnolence, stupor, prolonged sleep
- Loss of consciousness, coma
- Hypothermia
- Twitching, convulsions, “epilepsy”
- Bizarre neurologic signs
- Motor disturbances
- Sensory disturbances
- Loss of intellectual ability
- Personality changes
- Bizarre behavior
- Outburst of temper
- Psychologic disintegration
- Manic behavior
- Depression
- Psychoses
- Permanent mental or neurologic damage

deprivation, have been extensively studied and well characterized in adults (Box 23.1).^{32,33} Autonomic (neurogenic) symptoms primarily arise from perception of the physiological changes caused by sympathetic (not adrenomedullary) neural activation and include both adrenergic (tremor, palpitations, anxiety/nervousness) and cholinergic responses (sweating, hunger, paresthesias).³³ Awareness of hypoglycemia chiefly depends on perception of the central and peripheral effects of these

neurogenic (in contrast to neuroglycopenic) responses to hypoglycemia. Neurogenic symptoms are perceived at a plasma glucose of less than 54 mg/dL, the threshold at which brain glucose utilization becomes limiting.^{6,7} In older children and adults, the search for food or assistance is a crucially important defense against more severe hypoglycemia. Plasma glucose below 50 mg/dL causes neuroglycopenic manifestations (the result of an insufficient supply of glucose to maintain brain energy), such

as impaired cognition, loss of motor coordination, confusion, coma, and seizures. Hypothermia often occurs with prolonged hypoglycemia in older children and adults and is thought to be the result of a neurogenic mechanism.^{34,35} It is noteworthy that young children (6–11 years old) with type 1 diabetes have poor ability to detect low blood glucose levels.³⁶

Activation thresholds for the neuroendocrine responses have been best established for healthy young adults and vary only slightly by sex,³⁷ age,³⁸ exercise,³⁹ sleep,⁴⁰ and nutritional status.⁴¹ There is no evidence that the definition of hypoglycemia should be lower in infants and young children. Indeed, both epinephrine and GH are released at a higher plasma glucose level in children than in adults, suggesting that the threshold for neuroendocrine responses to decreasing plasma glucose levels in children is not lower and may actually be higher than in adults. At comparable plasma glucose thresholds, epinephrine levels are also markedly increased in children as compared with adults.³⁸ Drugs alter the neuroendocrine responses to hypoglycemia, for example, beta-blockers, dampen the response, whereas caffeine amplifies the response.⁴² Prior exposure to hypoglycemia and hyperglycemia cause clinically important alterations of neuroendocrine response thresholds. Even a single episode of moderately severe hypoglycemia can blunt or lower the activation threshold for 24 hours or more;^{43,44} after prolonged or recurrent hypoglycemia, autonomic responses may be sufficiently attenuated that neuroglycopenic effects may be the sole clinical manifestation of severe hypoglycemia, a condition referred to as *hypoglycemia-associated autonomic failure (HAAF)*.⁴⁵ HAAF has been demonstrated in adults and children with type 1 diabetes, in nondiabetic adults with an insulinoma,⁴⁶ and in infants with recurrent hypoglycemia caused by congenital HI.⁴⁷ Conversely, chronic hyperglycemia is associated with higher glucose thresholds for counterregulatory responses.^{48,49}

The effects on cognition, behavior, and level of consciousness are typically completely reversed when the glucose level is raised, although subtle neuropsychological impairment may be measurable days later.⁵⁰ Severe and prolonged neuroglycopenia, however, causes brain injury and neuronal death.^{2,51} In experiments with primates, plasma glucose below 20 mg/dL for 5 to 6 hours reliably produces severe injury.² In both infants and adults, magnetic resonance imaging (MRI) shows characteristic changes caused by hypoglycemia-induced brain injury.^{52,53} Pathologic changes are observed particularly in cortical tissue and white matter; whereas, the cerebellum and brainstem tend to be spared.⁵⁴ Permanent cognitive impairment is common both in children and adults after recurrent, severe hypoglycemia.^{55–62}

DEFINITION OF HYPOGLYCEMIA

Clinical hypoglycemia is defined as a plasma glucose concentration low enough to elicit defensive neuroendocrine responses, which cause symptoms and/or signs, or impair brain function.⁶³ Because the signs and symptoms are nonspecific, hypoglycemia may be difficult to recognize, and a single low plasma glucose concentration may be an artifact. For example, when blood is drawn but plasma not immediately separated from the cellular elements, glycolysis in red cells causes glucose levels to decline at a rate of 6 mg/dL/h. This is a common cause of artifactually low glucose levels reported in metabolic panels measured at commercial laboratories. Point-of-care glucose meters, originally designed for diabetes management, are useful for screening purposes, but their accuracy is limited to approximately ± 10 to 15 mg/dL in the hypoglycemia range.⁶⁴ Whole blood glucose levels are approximately 15% lower than plasma concentrations, and it is preferable consistently to refer to plasma glucose concentrations. Before establishing a

diagnosis of hypoglycemia and undertaking a diagnostic evaluation, it is essential to confirm a low plasma glucose concentration using a clinical laboratory method. For these reasons, guidelines emphasize the value of Whipple's triad for confirming hypoglycemia^{63,65,66}: (1) symptoms and/or signs consistent with hypoglycemia, (2) a documented low plasma glucose concentration, and (3) relief of symptoms and signs when plasma glucose is restored to normal. Because infants and young children cannot dependably recognize or communicate their symptoms, recognition of hypoglycemia may require confirmation by repeated measurements of plasma glucose concentration and formal provocative testing.

It is important to appreciate that hypoglycemia cannot be defined as a single specific plasma glucose concentration because thresholds for specific brain responses to hypoglycemia occur across a range of plasma glucose concentrations, and these thresholds can be altered by the presence of alternative fuels, such as ketones, and also by recent antecedent hypoglycemia. It is also not possible to identify a single plasma glucose value that causes brain injury, and the extent of injury is influenced by other factors, such as the duration and degree of hypoglycemia, availability of alternative fuels, and potential artifacts and technical factors that lead to inaccuracies in glucose measurements.

Despite the frequency with which hypoglycemia is not accompanied by obvious symptoms in infants, evidence of injury to the brain from prolonged or recurrent hypoglycemia suggests the same clinical thresholds and treatment goals are applicable irrespective of age beyond the immediate (48–72 hours) newborn period. Two different hypoglycemia thresholds are recommended for use in clinical practice. To determine the etiology of hypoglycemia and terminate a diagnostic provocative fasting test, obtain critical samples when plasma glucose is less than 55 mg/dL. For therapeutic purposes, however, the lower limit of the plasma glucose range should be 70 mg/dL (see Fig. 23.4). This goal is important to avoid periods of low glucose that may blunt the neuroendocrine and symptomatic responses to hypoglycemia and lead to greater susceptibility to subsequent more severe episodes of hypoglycemia.

As noted earlier, the effects of specific plasma glucose levels on the central nervous system may vary among patients, especially after experiencing previous episodes of hypoglycemia.^{43,46–48,67–70} Because there is no exact correspondence between the risk of harm (including brain injury) and either severity of symptoms or specific plasma glucose levels, hypoglycemia should always be treated urgently.

FASTING SYSTEMS APPROACH TO THE DIAGNOSIS OF HYPOGLYCEMIA

To provide appropriate treatment for a hypoglycemia disorder, it is essential to establish a specific diagnosis of the etiology. Because most hypoglycemia disorders, especially in infants and children, involve a disturbance of fasting adaptation, the best approach to diagnosis is a closely monitored fasting test, which both reproduces the hypoglycemia and surveys the integrity of the major metabolic and endocrine systems shown in Figs. 23.3 and 23.5 and in Table 23.1. These "fasting systems" include the four metabolic systems (hepatic glycogenolysis, hepatic gluconeogenesis, adipose tissue lipolysis, and hepatic ketogenesis) and their regulation by the endocrine system. The endocrine system includes: (1) insulin, which suppresses activity of all four metabolic systems; and (2) the four counterregulatory hormones that oppose insulin action in specific tissues: (a) glucagon acts acutely on liver to stimulate glycogenolysis and gluconeogenesis and facilitate ketogenesis;

(b) adrenergic nervous system activation (reflected by increases in plasma epinephrine levels) acts acutely on adipose tissue and liver to stimulate lipolysis, ketogenesis, glycogenolysis, and gluconeogenesis; (c) cortisol has longer term effects required for maintaining liver glycogen stores and promoting gluconeogenesis; and (d) GH, which has longer-term effects on the capacity for adipose tissue lipolysis (see Table 23.1).

The operation of these metabolic and endocrine systems is reflected by changes in major circulating metabolic fuels during normal fasting, as shown in Fig. 23.2. As liver glycogen reserves become depleted, plasma glucose falls toward hypoglycemic levels, plasma lactate declines by 25% to 50% (a marker of enhanced gluconeogenesis), plasma FFA rise 4- to 6-fold (a marker of lipolysis), and plasma ketones rise 20- to 40-fold (a marker of ketogenesis, usually measured as BOHB the major ketone). Measurement of these fuels at the time of hypoglycemia (the critical samples) provides crucial information about which component of the fasting systems is responsible for causing hypoglycemia (Fig. 23.6). For example, abnormal elevation of plasma lactate suggests a defect in gluconeogenesis; abnormal suppression of BOHB, with a normal or excessive rise in FFA, suggests a defect in fatty acid oxidation; abnormal suppression of both FFA and BOHB suggests excessive insulin action; and a normal fall in plasma lactate accompanied by a rapid rise in FFA and ketone concentrations occurring after a relatively brief period of fasting (i.e., ketotic hypoglycemia) suggests a defect in glycogenolysis. Cortisol or GH deficiency usually manifest with ketotic hypoglycemia; however, in the neonatal period, combined pituitary hormone deficiencies, especially, may present as hypoketotic hypoglycemia, mimicking HI. Deficiency of sympathetic nervous activity (e.g., secondary to beta-blocker drugs) may present with hypoketotic hypoglycemia because of impaired lipolysis. Deficiency of

glucagon as a primary disease has not been described, whereas secondary deficiency is common in patients with recurrent hypoglycemia, such as congenital HI or type 1 diabetes (HAAF).

Additional tests may be done separately to further assess for specific disorders or can be conveniently added at the end of the fasting test by using extra blood obtained with the critical sample. Serum insulin levels are often not elevated enough for diagnosis of HI disorders; therefore insulin levels, within the normal range, do not exclude the possibility of HI; plasma C-peptide levels may more accurately reflect pancreatic insulin secretion because insulin delivered by the portal vein is variably extracted by the liver, whereas C-peptide passes through the liver without hepatic extraction.⁷¹ HI can be conveniently confirmed with a glucagon stimulation test: in normal children, there is little or no response at the time of hypoglycemia, because liver glycogen stores have been depleted; whereas in HI, there is an abnormally large glycemic response caused by inhibition of glycogenolysis.⁷¹ Plasma levels of cortisol or GH sometimes increase to values sufficiently high during hypoglycemia to exclude deficiency of either or both; however, before making a diagnosis of cortisol or GH deficiency, low values must be confirmed by additional specific provocative tests of these hormones.⁷² Plasma free and total carnitine levels, together with the profile of plasma acylcarnitines, are useful for the diagnosis of most, but not all, of the fatty acid oxidation defects.

Other tests may be considered if specific disorders are suspected. Plasma ammonia concentrations are elevated 3- to 5-fold in patients with the HI hyperammonemia syndrome caused by activating mutations of *GLUD1*.⁷³ Plasma C-peptide suppression in the face of elevated insulin levels may be helpful in demonstrating exogenous insulin administration. Plasma

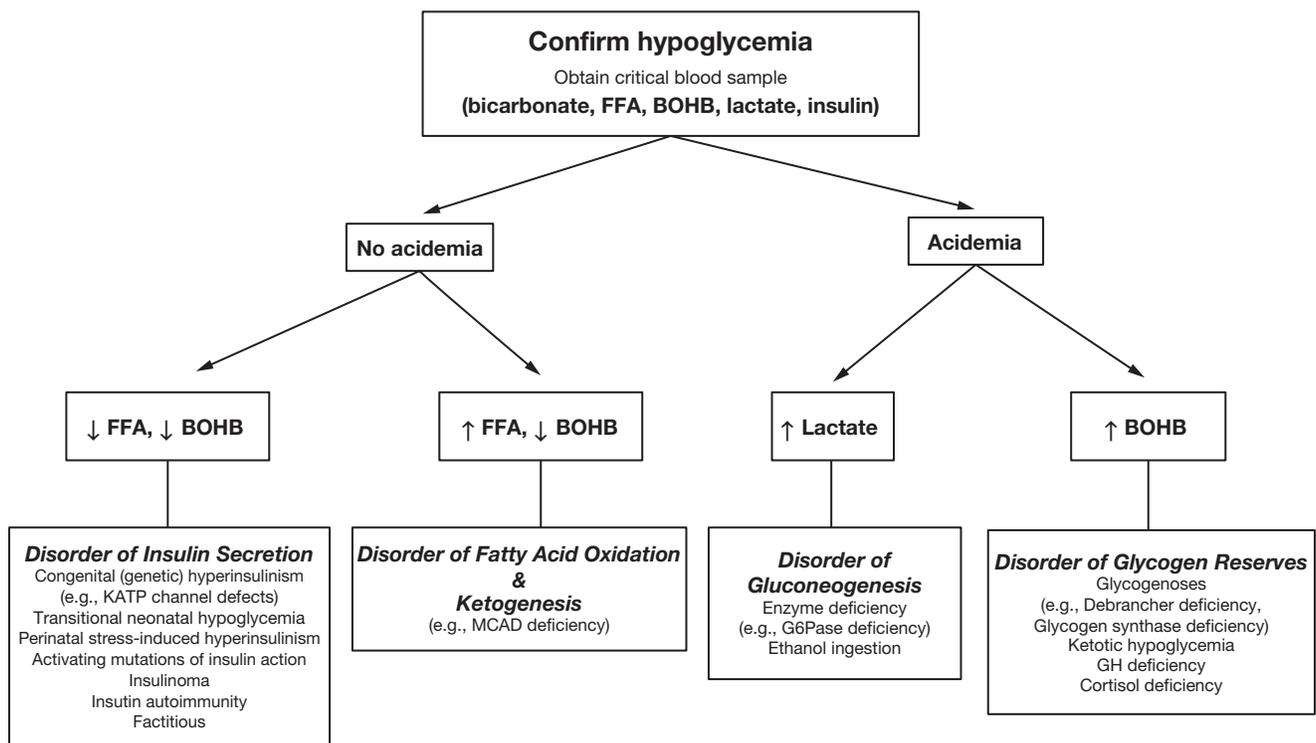


Fig. 23.6 An algorithmic approach to hypoglycemia. *BOHB*, Beta-hydroxybutyrate; *FFA*, free fatty acids; *G-6-Pase*, glucose 6-phosphatase; *GH*, growth hormone; *MCAD*, medium chain acylCoA dehydrogenase. (Modified from Thornton, P.S., Stanley, C.A., De Leon, D.D., Harris, D., Haymond, M.W., Hussain, K., et al. (2015). Recommendations from the Pediatric Endocrine Society for evaluation and management of persistent hypoglycemia in neonates, infants, and children. *J Pediatr*, 167 (2), 238–245.)⁶⁶

insulin-like growth factor binding protein-1 (IGFBP-1) concentration usually rises 5- to 10-fold as insulin levels decline during fasting, so that low IGFBP-1 values support a diagnosis of HI.^{71,74} Specimens to test for drugs or toxins may be considered in cases of suspected ingestion of oral hypoglycemic drugs, plant toxins (unripe ackee-ackee fruit, litchi fruit),^{75,76} or surreptitious insulin administration (factitious hypoglycemia or Munchausen by proxy).

Detailed methods for performing fasting tests and other tests useful for diagnosis of hypoglycemia are provided at the end of this chapter.

MAJOR CAUSES OF HYPOGLYCEMIA IN THE TODDLER AND CHILD

Hyperinsulinism

HI is the most common and the most severe form of hypoglycemia in neonates and young infants, and is also one of the most common causes of hypoglycemia in toddlers and older children.^{77–80} Hypoglycemia caused by HI is particularly dangerous to the brain, because glucose deficiency cannot be compensated for by increased production of ketones to support brain metabolism.⁷¹ Although many neonates with severe congenital HI present in the first week of life, milder genetic forms of HI continue to be an important cause of hypoglycemia throughout infancy. Older children and adolescents with HI may have acquired insulin-secreting pancreatic adenomas.

Congenital HI may present beyond the first months of life as feeding intervals lengthen and overnight feedings are omitted. Such infants may have early morning lethargy or seizures and may have a history of previous seizures and unexplained developmental delay, or of hypoglycemia in the newborn period that was not fully evaluated or treated. In dominant forms of HI, there may be a history of hypoglycemic symptoms in a parent or other relatives; however, carriers may often not recognize symptoms.⁸¹ As outlined in Chapter 7, all of the genetic causes of HI may initially present in the neonatal period.

The diagnosis of HI is suggested when hypoglycemia is not accompanied by hyperketonemia and can be confirmed when injection of glucagon causes a large increase (>30–40 mg/dL relative to baseline) in plasma glucose concentration. HI may be suggested by increased rates of glucose utilization (glucose infusion rate [GIR] >8 mg/kg/min), but this is less consistently present in older children than in neonates. A critical specimen obtained during hypoglycemia, which demonstrates increased levels of insulin or C-peptide can be conclusive; however, it is often not possible to demonstrate clearly elevated insulin levels.^{71,82} Table 23.2 shows the diagnostic criteria for hyperinsulinemic hypoglycemia. In cases of surreptitious insulin administration, standard insulin immunoassays may not

detect exogenously injected insulin analogs, such as lispro, aspart, glargine, etc.⁸³ In suspected cases, specific assays must be requested.

Adenosine Triphosphate Sensitive Potassium Channel Hyperinsulinism

Mutations in the adenosine triphosphate-sensitive potassium (KATP) channel genes are the most commonly identified genetic defects in patients with congenital HI.⁸⁴ The *ABCC8* and *KCNJ11* genes on chromosome 11p encode the two subunits, SUR1 and Kir6.2, of the KATP channel in the plasma membrane of the beta-cell. Potassium efflux through this channel hyperpolarizes the beta-cell plasma membrane and is a key negative regulator of insulin secretion; genetic defects which impair KATP channel activity and preclude channel opening cause persistent depolarization of the plasma membrane and uncontrolled insulin secretion.⁸⁰ As described in Chapter 7, there are three distinct forms of KATP-HI:

- Biallelic inheritance of two recessive KATP mutations causes diffuse HI with severe neonatal onset that is unresponsive to diazoxide.^{85–88}
- Recessive KATP channel mutations also can cause focal congenital HI by a two-hit mechanism, involving a paternally transmitted recessive KATP mutation combined with an embryonic somatic mutation in the corresponding maternal gene, resulting in effective paternal 11p uniparental isodisomy for the region containing both KATP and the Beckwith-Wiedemann imprinted genes. The resulting focal area of islet cell adenomatosis contains beta cells with defective KATP channels, resulting in HI.^{89–91}
- Dominantly inherited KATP channel mutations act in dominant-negative fashion to produce diffuse HI with KATP channels that have varying degrees of impaired channel function. Most dominant KATP mutations are diazoxide responsive, but diazoxide-unresponsive mutations do occur.^{81,84,92}

The diffuse and focal forms of KATP-HI account for more than 90% of diazoxide-unresponsive cases of congenital HI in early infancy, and are similar in their clinical manifestations.⁸⁴ Both may require surgery to maintain safe glucose levels.

Beyond the neonatal period, dominant KATP mutations are more common and are often diazoxide-responsive.⁸⁴

Diazoxide (5–15 mg/kg/day) is the first-line drug for treatment of HI, but is often ineffective for KATP-HI, because it acts as a KATP channel opener. Octreotide may be given by subcutaneous injection at 5 to 15 mcg/kg/day.^{80,93} Longer acting somatostatin analogs, such as lanreotide, have recently been introduced, with some success in older infants.⁹⁴ Calcium-channel blockers are not effective.⁹⁵ If medical management cannot maintain the plasma glucose concentration greater than 70 mg/dL, with a normal feeding schedule, surgery may be necessary. Surgical treatment for focal KATP-HI can be curative, whereas for diffuse disease even a 95% to 99% pancreatectomy may not cure the hypoglycemia, and insulin-dependent diabetes is a frequent consequence.^{96,97} 18F-fluorodopa positron-emission tomography (¹⁸F-L-DOPA PET) scans have been accurate in preoperative localization of a focal lesion.^{91,98,99}

Glutamate Dehydrogenase Hyperinsulinism

HI can be caused by dominant activating mutations in *GLUD1* on 10q, which encodes glutamate dehydrogenase (GDH).¹⁰⁰ This disorder is also known as the *HI hyperammonemia syndrome*, because the increased GDH enzymatic activity in kidney

TABLE 23.2 Diagnostic Criteria for Hyperinsulinemic Hypoglycemia

- Glucose infusion rate >8 mg/kg/min
- Laboratory plasma glucose ≤50 mg/dL
- Detectable serum insulin
- C-peptide ≥0.5 ng/mL
- BOHB <1.8 mmol/L^a
- FFA <1.7 mmol/L
- Response to glucagon ≥30 mg/dL
- IGFBP-1 ≤110 ng/mL

^a100% sensitive.

BOHB, Beta-hydroxybutyrate; FFA, free fatty acids; IGFBP-1, insulin-like growth factor binding protein 1.

(Modified from Ferrara, C., Patel, P., Becker, S., Stanley, C.A., Kelly, A. (2016). Biomarkers of insulin for the diagnosis of hyperinsulinemic hypoglycemia in infants and children. *J Pediatr*, 168, 212–219.)⁷¹

tubules results in increased renal ammoniogenesis.¹⁰¹ Hypoglycemia in GDH-HI is usually milder than in KATP-HI, and less likely to present in the newborn period.^{102,103} As is common with dominant genetic disorders, up to 80% of cases with GDH-HI have a de novo mutation. The hyperammonemia in GDH-HI appears to be asymptomatic. However, affected children frequently have brain manifestations, possibly because of increased GDH activity in neurons and altered levels of glutamate or other neurotransmitters: these include absence seizures (generalized epilepsy), behavior disorders, and mild to moderate developmental delay.¹⁰⁴

In GDH-HI, activating mutations of GDH in the beta cell amplify leucine-triggered production of ATP, leading to closure of KATP channels and insulin release, independent of glucose levels. In affected children, ingestion of high-protein feedings can induce hypoglycemia (leucine-sensitive or protein-sensitive hypoglycemia).^{105,106} In the kidney, the same mutations increase oxidation of glutamate to alpha-ketoglutarate with the release of ammonia.¹⁰⁷

The diagnosis of GDH-HI is similar to that of other forms of HI; however, persistently elevated plasma ammonia levels (typically 80–120 $\mu\text{mol/L}$) are specific for this disorder.¹⁰⁵ Because the hyperammonemia is of renal origin, plasma ammonia levels are unrelated to either insulin secretion or plasma glucose levels and are also not related to protein feeding. Thus elevated ammonia levels can be readily demonstrated in casual (random) specimens of plasma. Most patients respond well to diazoxide and do not need surgery.^{102,108}

Glucokinase Hyperinsulinism

Glucokinase (encoded by *GCK*) is the enzyme that serves as the glucose sensor in the beta cells of the pancreas.¹⁰⁹ Gain-of-function glucokinase mutations result in a lower glucose threshold for insulin secretion, leading to persistent hypoglycemia (in contrast, *GCK* loss-of-function mutations produce a higher glucose threshold for insulin release, causing a common form of mild monogenic diabetes (MODY2)).¹¹⁰ At least 15 different dominantly expressed *GCK*-HI mutations have been reported.¹¹¹ Alterations in the enzyme kinetics of individual mutations predict widely different degrees of lowering of glucose threshold, but do not correlate with clinical severity and course.¹¹¹ In some cases, *GCK*-HI mutations present with neonatal hypoglycemia, severe enough to require pancreatectomy to control hypoglycemia; however, some mutation carriers have escaped recognition even into adulthood.

GCK-HI may be especially challenging to diagnose, because fasting plasma glucose levels tend to stabilize at a glucose threshold between 50 and 65 mg/dL. During fasting, if plasma glucose remains at this threshold for an extended period, levels of FFA and BOHB can rise, suggesting a form of ketotic hypoglycemia rather than HI.¹¹² Plasma ammonia levels are normal in *GCK*-HI patients and they do not have protein-sensitive hypoglycemia. The response to diazoxide has been only partial and transient in most patients with *GCK*-HI and some have required pancreatectomy.¹¹³ One report has described good results using a ketogenic diet to prevent symptomatic hypoglycemia;¹¹⁴ the long-acting somatostatin analog, lanreotide, might be useful in older patients, but experience is limited.

Hexokinase 1 Hyperinsulinism

Hexokinase 1 (*HK1*) is closely related to glucokinase and carries out the first step in glucose oxidation by phosphorylating glucose to glucose-6-phosphate in most tissues of the body, except for beta cells. In contrast to glucokinase, which has a low affinity for glucose ($K_m \sim 5 \text{ mM}$) that sets the threshold for glucose stimulation of insulin secretion at around 4 to 4.5 mM (70–80 mg/dL), *HK1* has a very high affinity for glucose

($K_m \sim 1 \text{ mM}$), and its expression in beta cells is therefore normally disallowed.¹¹⁵ Two reports of congenital HI possibly because of beta-cell expression of *HK1* have recently been published. The first report described a large, dominant pedigree with diazoxide-responsive congenital HI, in which linkage analysis identified a shared haplotype on chromosome 10 that included 3 noncoding mutations in the *HK1* locus.¹¹⁶ This family was in one of the original descriptions of congenital HI by McQuarrie in 1953.¹¹⁷ It was suggested that one or more of these mutations caused *HK1* to be expressed in beta cells and to reduce the glucose threshold for insulin secretion to around 55 to 60 mg/dL. In the second report,¹¹⁸ increased beta-cell expression of *HK1* was detected by immunohistochemical staining of islets from several infants who underwent surgery for diazoxide-unresponsive congenital HI; these infants were also suggested to have HI because of a failure of *HK1* silencing in beta cells. As described later, failure to silence expression of a “disallowed” beta-cell gene has also been suggested as the mechanism of exercise-induced HI: expression of the pyruvate plasma membrane transporter, MCT1.^{119,120}

Short Chain 3-Hydroxyacyl-Coa Dehydrogenase Hyperinsulinism

Short chain 3-hydroxyacyl-CoA dehydrogenase ([SCHAD], encoded by *HADH* on chromosome 4 at q22-26) is a mitochondrial fatty acid oxidation enzyme that catalyzes oxidation of short chain 3-hydroxyacyl-CoAs.¹²¹ Levels of SCHAD protein are relatively high in pancreatic beta cells, where it appears to negatively regulate the activity of GDH, the target of the gain-of-function mutations responsible for GDH-HI (the hyperammonemia HI syndrome).¹²² Homozygous inactivating mutations of the *HADH* gene are associated with increased beta-cell GDH activity and cause a form of HI similar to GDH-HI, but without hyperammonemia or specific brain defects. The systemic defect in fatty acid oxidation in SCHAD deficiency leads to accumulation of upstream substrates resulting in diagnostic elevations of urinary organic acids (3-hydroxyglutarate) and plasma acylcarnitines (3-hydroxybutyryl-carnitine). Patients with HI caused by SCHAD deficiency have responded well to treatment with diazoxide.

Hepatocyte Nuclear Factor 4a Hyperinsulinism

Hepatocyte nuclear factor 4a (HNF4A) is a transcription factor important for pancreatic beta-cell development and insulin secretion. Heterozygous inactivating mutations of *HNF4A* are a well-recognized cause of monogenic diabetes (MODY1). Recently, it was recognized that these same mutations also cause excessive insulin secretion in early life, as manifested by fetal macrosomia and persistent diazoxide-responsive neonatal hyperinsulinemic hypoglycemia. The HI is usually transient, lasting only a few weeks to a few months after birth; however, it may persist for several years into childhood.¹²³ In the second and third decades, these children may develop the MODY1 form of diabetes, which is responsive to treatment with sulfonylureas. In most cases of *HNF4A* HI, birthweight has been above average, hypoglycemia occurred within the first days of life, and one parent had a history of MODY-type diabetes.^{124,125} Children with a specific *HNF4A* mutation, p.Arg76Trp, have additional manifestations, including renal Fanconi syndrome and hepatomegaly and elevated transaminases, suggesting that expression of the GLUT2 transporter in kidney tubules and in liver was impaired by the defect in *HNF4A*.¹²⁶

Hepatocyte Nuclear Factor 1A Hyperinsulinism

Hepatocyte nuclear factor 1a (HNF1A) is another transcription factor important for beta-cell development. Heterozygous

inactivating mutations of *HNF1A* cause the most common form of monogenic diabetes, MODY3. Several cases of *HNF1A* mutations causing congenital HI have been reported,^{126–128} several of which also were associated with MODY diabetes in older patients. An interesting, unexplained discrepancy between *HNF4A* and *HNF1A* HI is that large for dates birthweight has only been associated with *HNF4A*, but not *HNF1A* mutations.

Uncoupling Protein 2 Hyperinsulinism

Uncoupling protein 2 (UCP2) transfers Krebs cycle intermediates out of the mitochondria and favors oxidation of amino acids versus glucose in beta cells, liver, and other tissues. Loss-of-function mutations of UCP2 may enhance ATP generation from glucose and consequently amplify insulin responses to glucose stimulation, sufficiently as to cause hyperinsulinemic hypoglycemia. Several cases have been reported, which have generally responded to treatment with diazoxide.¹²⁹

Exercise-Induced Hyperinsulinism

A total of 13 patients belonging to three families have been found with dominantly inherited exercise-induced hyperinsulinemic (EIHI) hypoglycemia following periods of intense anaerobic exercise.¹²⁰ Clinical presentations have varied, with some cases having severe hypoglycemic episodes from infancy; some presenting with hypoglycemic syncope after exercise, as adolescents; and some only mildly affected, as adults. Most patients maintained normal glucose levels during prolonged fasting. Provocative testing, with brief, intense bicycle exercise, induced the expected rise in plasma lactate and pyruvate levels, but markedly increased plasma insulin concentrations for about 10 minutes after the exercise, causing hypoglycemia over the next 45 minutes.¹³⁰ Intravenous infusion of pyruvate stimulated a similar rise in insulin, suggesting that the defect reflected an abnormal beta-cell responsiveness to pyruvate. Recurrent hypoglycemia was only partially preventable with diazoxide treatment.

Excessive insulin secretion in EIHI occurs because of mutations upstream of the promoter region *SLC16A1* that allows the MCT1 monocarboxylate transporter to be expressed on the beta-cell plasma membrane. Normally, expression of MCT1 is “disallowed” in beta cells to prevent pyruvate and lactate from acting as insulin secretagogues.

Syndromic Forms of Congenital Hyperinsulinism

Beckwith-Wiedemann Syndrome. Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome caused by embryonic mosaic mutations of an imprinted region on 11p, which is close to the location of the beta-cell KATP channel genes, *SUR1* and *Kir6.2*. The BWS locus includes growth-inhibiting genes expressed on the maternal chromosome (H19, P57) and growth-promoting genes (*IGF2*) expressed on the paternal chromosome; expression of these genes is controlled by two imprinting control sites. Although BWS has been associated with HI for many years, recently it has been found that most cases of BWS with persistent HI are associated with paternal isodisomy for the 11p region (11pUPD overgrowth), which is the cause of around 20% of BWS cases.¹³¹ The histopathology in these cases shows islet cell overgrowth (adenomatosis), which may involve nearly the entire pancreas. Some cases are responsive to diazoxide, but hypoglycemia in some may not be controllable with diazoxide and may require more intensive medical management with octreotide and continuous feedings or pancreatectomy. Some cases of 11pUPD BWS also have a paternally transmitted recessive KATP channel mutation; these cases can have severe HI and unusually high glucose requirements. The possibility of 11pUPD BWS should be suspected

in infants with HI who have features of BWS, such as hemihypertrophy, macroglossia, or umbilical hernia.

Kabuki Syndrome. Kabuki syndrome is associated with recognizable dysmorphic features including facial features resembling a Kabuki actor mask and caused by somatic mosaic mutations in either *MLL2* (*KMT2D*) or *KDM6A*, two genes which regulate chromatin methylation.¹³² A proportion of infants with Kabuki syndrome have persistent hypoglycemia caused by HI, which may either be responsive or nonresponsive to treatment with diazoxide.

Turner Syndrome. Turner syndrome has been reported to be associated with congenital HI for several decades. A recent study found that the frequency of Turner syndrome in a large series of children with HI was increased ~50-fold, indicating that the risk of HI in Turner syndrome was around 1 in 1000 compared with the risk of HI in the general population of 1 in 40,000.¹³³ Some of the Turner syndrome cases were diazoxide responsive, but others failed to respond to diazoxide and required pancreatectomy. The only consistent chromosomal anomaly in Turner syndrome girls with HI was a 45 XO karyotype, suggesting that loss of a gene on the X-chromosome was the cause of the HI. A possible candidate gene is *KDM6A*, a pseudoautosomal X-chromosome gene, because inactivating mutations of *KDM6A* are one of the causes of Kabuki syndrome in which there is also an increased frequency of HI.

Carbohydrate-Deficient Glycoprotein Hyperinsulinism

Congenital disorders of glycosylation (CDG) are caused by recessively inherited loss-of-function mutations in genes that regulate glycosylation of proteins.^{134,135} Most produce syndromic diseases affecting multiple organ systems, including brain, skeleton, liver, and kidney. Hypoglycemia resulting from HI has been reported in children with three of the identified defects of N-glycosylation: CDG Ia, Ib, and Id (OMIM IDs: 601785, 602579, and 601110). Most cases of CDG-HI have been identified in the newborn period, but some have been diagnosed later in infancy or early childhood. CDG-HI is usually suspected in a child with a dysmorphic facies and manifestations affecting multiple other organ systems, including—especially the brain, liver, gut, and skeleton. In all three types, cases have been reported in which hyperinsulinemic hypoglycemia was the presenting or dominant problem.^{136–138} The mechanism of excessive insulin production has not been determined.

Phosphomannomutase 2 (PMM2)-CDG (CDG-Ia) is the most common type of CDG and involves deficient activity of phosphomannomutase 2 resulting from mutations of *PMM2*. Most patients have severe developmental delay, cerebellar hypoplasia, hypotonia, and seizures. Protein-losing enteropathy and liver disease contribute to failure to thrive. Deficient levels of antithrombin III can cause thromboses. Dysmorphic features are common and may be subtle or obvious, including unusual fat distribution and inverted nipples. Hyperinsulinemic hypoglycemia occurs in only a minority of patients, but can be mild or severe enough to warrant pancreatectomy.^{136,139}

Phosphomannose isomerase (MPI)-CDG (CDG-Ib) involves deficient activity of phosphomannose isomerase resulting from mutations of *MPI*. Unlike CDG-Ia, the central nervous system is spared and hyperinsulinemic hypoglycemia is a common feature, presenting in the first days of life or later in the first year.^{137,140} Liver disease and protein-losing enteropathy are usually the dominant clinical problems.¹⁴¹ Some children have had cyclic vomiting. Clinical severity is variable, and mildly affected adults have been diagnosed. Oral mannose in doses of up to 150 mg/kg/day can correct most of the clinical

abnormalities, making MPI-CDG the only CGD with a specific treatment.¹⁴²

ALD3-CDG (CDG-Id) involves deficient activity of mannosyltransferase 6 resulting from mutations of *ALD3*. Clinical features resemble those of PMM2-CDG, with severe central nervous system damage. One case with severe neonatal hyperinsulinemic hypoglycemia has been reported, but whether it was a direct result of the CDG or a consequence of perinatal stress is unknown.

PMG-1 reversibly converts glucose-6-P to glucose-1-P and is required both for glycogen formation and degradation. Recessive inactivating mutations of *PGM1* cause a syndrome of dysmorphic features, short stature, and fasting hyperketonemic hypoglycemia.¹⁴³ In addition to abnormalities in protein glycosylation (hence the designation of CDG-It), hyperinsulinemic hypoketonemic hypoglycemia occurs when patients are fed. Deficiency of *PGM1* impairs both the synthesis and breakdown of liver glycogen (hence the fasting hyperketonemic hypoglycemia and the labeling of the disorder as *GSD type 14*). However, deficiency of *PGM1* in the beta cell appears to cause an exaggerated insulin response to a glucose load, leading to postprandial hyperinsulinemic hypoglycemia. The diagnosis can be made by screening tests for abnormalities in transferrin glycosylation. Treatment with diazoxide has not been effective in controlling postprandial hyperinsulinemic hypoglycemia, but low glycemic index feedings, uncooked cornstarch (UCS), or continuous low-dose glucose infusions have been more successful. Because the defect potentially impairs synthesis of galactose, treatment with galactose supplements have been tried with some success in correcting abnormalities of transferrin glycosylation.¹⁴⁴

AKT2 Hypoglycemia

Three children with hypoglycemia caused by gain-of-function mutations of *AKT2* have been described.¹⁴⁵ *AKT2* is a serine/threonine kinase that is one of the downstream steps in post-insulin receptor signaling; loss of *AKT2* activity is associated with insulin resistance, whereas activation of *AKT2* causes an increase of insulin sensitivity. Beginning around 6 months of age, all of the reported cases had episodes of severe, symptomatic, hypoketotic hypoglycemia resembling HI, but associated with low levels of insulin, proinsulin, and FFA. Hypoglycemia was primarily postprandial and could be controlled with low dose continuous dextrose feedings. Other clinical features included large birth weight and postnatal asymmetric overgrowth of body or face. Each of the three unrelated patients had the same *de novo* mutation of *AKT2*. The mutations were postzygotic and mosaic, which probably explains the localized nature of the overgrowth.

Phosphatidylinositol-3-Kinase Hypoglycemia

Phosphatidylinositol-3-Kinase (PI3K) is an enzyme step in the pathway of postinsulin receptor signaling upstream from *AKT2*. Postzygotic gain-of-function mutations of two PI3-kinase-related enzymes, *PIK3CA* and *PIK3R2*, were recently reported to cause a syndromic form of hypoketotic hypoglycemia with low insulin concentrations, similar to gain-of-function mutations of *AKT2* described earlier. Features included asymmetric overgrowth, megalencephaly, and developmental delay. Diazoxide was ineffective and treatment with continuous dextrose at a low glucose infusion rate was needed to control the hypoglycemia. The hypoglycemia appears to be caused primarily by increased postinsulin receptor activity in the liver.¹⁴⁶

Hyperinsulinemic Hypoglycemia Associated With Insulin Receptor Defects

Inactivating mutations of the insulin receptor (*INSR*), which cause insulin resistance and diabetes mellitus, can also be associated with postprandial hyperinsulinemic hypoglycemia of varying severity. Severe defects in the *INSR* present in early infancy and childhood with dysmorphic features because of marked deficiency of adipose tissue (lipodystrophy) and, in order of severity, include Donohue syndrome (leprechaunism) (OMIM ID: 246200) and Rabson-Mendenhall syndrome (OMIM ID: 262190); milder defects may present later in childhood or early adulthood as type A insulin resistance (OMIM ID: 610549).¹⁴⁷ More severe forms of insulin resistance may be caused by biallelic *INSR* mutations, and monoallelic defects may be associated with milder forms of insulin resistance; mutations in the *INSR* beta subunit tend to be milder than mutations in the alpha subunit. Plasma insulin levels are increased 10 to 100 times normal. Hypoglycemia in these patients is usually postprandial and has been suggested to be caused by delayed insulin clearance¹⁴⁸ (a similar delay in insulin clearance has also been suggested to be responsible for postprandial hypoglycemia in autoimmune hypoglycemia caused by antiinsulin antibodies). Fasting hypoglycemia has been associated with the more severe insulin resistance seen in neonates and infants with Donohue syndrome and has been suggested to be caused by the very high insulin levels, having an insulin-like action by cross-reaction with the insulin-like growth factor-1 (IGF-1) receptor, although an earlier report demonstrated a pattern of accelerated hyperketonemic fasting adaptation, suggesting a possible impairment in hepatic glycogen storage.^{149–151} Infants with Donohue syndrome present in the newborn period and most die of infection in the first years of life. Features include intrauterine growth retardation, markedly diminished subcutaneous fat, typical facial appearance reminiscent of a leprechaun (“leprechaunism”), enlargement of breasts and clitoris or phallus, hypertrichosis, acanthosis nigricans, and postprandial hyperglycemia. Hypoglycemia, a few hours after meals, may be noted within the first days after birth and persists throughout life. Frequent feedings have been the mainstay of management for the hypoglycemia; diazoxide has generally not been effective.^{152,153}

Children with Rabson-Mendenhall syndrome are somewhat less severely affected than those with Donohue syndrome, but share many of the features, including reduced adipose tissue, acanthosis nigricans, hypertrichosis, and enlargement of the genitals in both males and females. Many of these children also have postprandial hypoglycemia. Diabetes tends to be progressive and insulin resistance can be severe. Adolescent females may have evidence of hyperandrogenism similar to polycystic ovarian syndrome. Death in childhood from diabetic ketoacidosis is common. Treatment with leptin (metreleptin) has been reported to modestly improve glucose control in some patients with Rabson-Mendenhall syndrome.¹⁵⁴

Postprandial (reactive) hypoglycemia has also been reported in adolescents with type A insulin resistance in which diabetes mellitus develops more gradually, sometimes as the adolescent reaches adulthood.¹⁵⁵ Features may include reduced adipose tissue, acanthosis nigricans, and elevated triglyceride levels. Insulin resistance may lead to eventual beta-cell failure and insulin insufficiency.

Reactive hypoglycemia has also been reported in insulin-resistant conditions because of postreceptor disorders,¹⁵⁶ and should be considered in other insulin resistance syndromes, such as congenital lipodystrophy (Berardinelli-Seip syndrome), because of recessive defects in *AGPAT2*, *BSCL2*, or *PTRF*.

Factitious Hyperinsulinism

Instances of factitious HI have been reported in infants and children, typically the result of a parent or other caregiver administering insulin or an insulin secretagogue.¹⁵⁷ This form of child abuse is referred to as *Munchausen by proxy*¹⁵⁸ and may cause needless pancreatic surgery and can be lethal.¹⁵⁹ In most reported cases, the parent (often the mother) was a nurse or other medical professional or had access to insulin or sulfonylureas being used at home for treatment of diabetes. Typically, classic symptoms of HI are present, but can occur irregularly. As in other forms of HI, hypoglycemia is accompanied by suppression of plasma ketones and FFA and an abnormal positive response to glucagon. Fasting tests in the absence of the caregivers will be normal; however, extreme caution is required to ensure no interference by the suspected agent. An extremely high plasma insulin concentration (e.g., >100 $\mu\text{U}/\text{mL}$) is sometimes a clue (especially likely to be found after administration of short- or intermediate-acting insulin, such as regular or isophane (NPH); in contrast, plasma insulin levels are usually only mildly elevated in HI from endogenous causes. Insulin administration has become increasingly difficult to detect by standard insulin assays because these can vary in their ability to detect insulin analogues (lispro, aspart, glargine, glulisine, detemir, degludec).^{83,160,161}

The most conclusive evidence of exogenous insulin is a discrepancy between elevated plasma insulin, but very low plasma C-peptide concentration, indicating that endogenous insulin production has been suppressed.¹⁶² Insulinomas may be associated with elevated plasma insulin, but values are rarely greater than 100 $\mu\text{U}/\text{mL}$ and levels of C-peptide and proinsulin should be correspondingly elevated.

Surreptitious (or, more commonly, accidental) administration of long-acting oral hypoglycemic agents that induce insulin secretion, such as glyburide or other sulfonylureas, cause elevation of both insulin and C-peptide and may cause persistent hypoglycemia for 24 hours or longer. Routine toxicology screens of blood and urine may not detect sulfonylureas, but specific testing can be arranged if a specific drug is suspected.

If surreptitious insulin or oral hypoglycemic drug administration is suspected, specimens of plasma/serum, from the time of hypoglycemia, should be obtained for testing of plasma insulin and C-peptide concentrations and preserved for other tests that may become necessary to document the involvement of an exogenous agent. Consultation with the laboratory is helpful in interpreting and selecting appropriate tests. Treatment of the hypoglycemia with intravenous glucose is usually sufficient, but continuous intravenous glucagon may be needed in severe cases and diazoxide or octreotide may be helpful in cases of prolonged insulin hypersecretion.

Autoimmune Hypoglycemia

Two types of autoimmune hypoglycemia have been reported: one mediated by antibodies to insulin and the other by antibodies to the insulin receptor. Hypoglycemia caused by antiinsulin antibodies has been reported most often from Japan ("Hirata disease"),¹⁶³ but cases have been described in both sexes, all ages, and from many regions of the world.¹⁶⁴ Nearly all autoimmune hypoglycemia in infants and children have been of this type.^{165–168} The presumed mechanism of hypoglycemia is slow dissociation of insulin from antibodies during the postprandial or postabsorptive periods. The hypoglycemia is most often postprandial, but may be fasting, and has sometimes been severe enough to cause seizures or coma.¹⁶⁹ Metabolic features are those of HI, with low plasma ketone and FFA levels. Assays of plasma insulin can give very high values that remain constantly high over

time because of the interference by endogenous insulin binding antibodies (this may vary, depending on the assay method); in contrast, plasma C-peptide values may be suppressed, suggesting the possibility of surreptitious insulin or sulfonylurea administration. The disorder may be triggered by infections with common viruses or by exposure to drugs containing sulfhydryl or thiol groups, such as methimazole or lipoic acid, and appears to be associated with other autoimmune endocrine disorders, such as hyperthyroidism. In Japan, an association with the HLA allele HLA-DRB1*04:06 has been reported; a high incidence of HLA-DRB1*04:03 has been described in Europeans with the disorder. Among various treatments reported to improve the hypoglycemia have been courses of glucocorticoids, plasmapheresis, and intravenous immunoglobulin infusions. The disorder often resolves over time as autoantibody titers wane.

The second form of autoimmune hypoglycemia is caused by antibodies that bind to and activate the insulin receptor (analogous to thyroid-stimulating immunoglobulins in Graves disease). Childhood onset is rare, but cases as young as 8 months old have been seen.¹⁶⁸ In children, there may be severe fasting hypoglycemia with very elevated rates of glucose utilization that is unresponsive to diazoxide or octreotide. In adults, the disorder has been associated with type B (autoimmune) insulin resistance, or a malignancy or severe inflammatory disorder. Some patients have had both antiinsulin and antireceptor antibodies.¹⁷⁰ C-peptide and insulin levels are typically undetectable and diazoxide and octreotide are ineffective in controlling hypoglycemia. Treatment with glucocorticoids, plasmapheresis, or intravenous immunoglobulin may be considered.

Insulinoma

Insulinomas are the most common cause of acquired persistent hypoglycemia in adults and older children and may rarely occur in children as young as 2 years of age.¹⁷¹ Most often, insulinomas are solitary, small tumors, 0.5 to 1 cm in size, which are slow-growing and rarely become malignant or metastasize; multiple tumors and recurrent insulinomas may occur, especially in patients with multiple endocrine neoplasia type 1 (MEN1) syndrome because of dominant inactivating mutations in *MEN1* on 11q. Children with insulinomas present with recurrent episodes of symptomatic hypoglycemia, which are often initially mild and may go unrecognized for several months before severe manifestations, such as a seizure, occurs. The diagnosis of HI is most easily made with a fasting test to demonstrate hypoketonemic hypoglycemia, together with an inappropriately large glycemic response to glucagon. In younger children, especially, it may not be possible to distinguish clinically between an insulinoma and a genetic form of diffuse HI.

The majority of pediatric insulinomas appear to be associated with paternal 11p uniparental isodisomy, although this appears not to be so common in adult insulinomas.¹⁷¹ The frequency of genetic mutations in *MEN1* appears to be higher in children (42% of childhood insulinomas at Children's Hospital of Philadelphia) than in adults with insulinomas (~8%). This is important, because the presence of multiple tumors at first surgery and recurrence of tumors is more likely in patients with MEN1 than in cases of sporadic insulinomas.

Initially, diazoxide may provide good control of hypoglycemia in patients with an insulinoma, especially in early stages, while the tumor is small; but diazoxide may become ineffective as the tumor grows. Surgical resection is the treatment of choice; therefore careful preoperative imaging to locate the lesion (or lesions) before surgery is important. In a series of eight pediatric cases, imaging was successful localizing the

insulinoma in 40% by MRI, 40% by endoscopic ultrasound, 30% by computed tomography, and only 20% by abdominal ultrasound.¹⁷² Octreotide scan and F-DOPA PET scans occasionally provide additional diagnostic utility. At surgery, palpation and inspection and intraoperative ultrasound were generally successful in identifying and resecting the insulinoma. Patients with MEN1, caused by dominant mutations of *MEN1*, are at risk for other endocrine tumors, including functioning or nonfunctioning adenomas of the parathyroids, pituitary, and pancreas and require regular monitoring, as well as testing to identify affected family members.

Postprandial Hypoglycemia After Gastrointestinal Surgery (“Late Dumping Syndrome”)

Gastrointestinal hormones and signals—the enteroinsular axis—play an important role in regulating insulin secretion in response to meals. Disruption of this system by gastric surgery can result in hypoglycemia from excessive postprandial insulin release.¹⁷³ In children, the most common cause is gastric surgery for gastroesophageal reflux (e.g., Nissen fundoplication); in adults, the most common cause is gastric bypass bariatric surgery for severe obesity. This “alimentary hypoglycemia” is exclusively postprandial (i.e., reactive), rather than fasting, and is caused by an amplified release of glucagon-like peptide 1 from the small intestine.^{174–177} The disorder is sometimes called *late dumping syndrome* to contrast it with *early dumping syndrome*, which is caused by rapid gastric emptying that induces osmotic shifts and symptoms, such as hypotension, sweating, and diarrhea, immediately after a feeding.

In young children, postprandial hypoglycemia after Nissen fundoplication may be accompanied by bowel symptoms of early dumping syndrome, but more often presents as isolated neuroglycopenic and autonomic symptoms 1 to 3 hours after meals.^{174,178} Hypoglycemia may be confirmed in association with symptoms after a regular feeding or can be elicited by a formal mixed meal or oral glucose tolerance test. Although the glucose tolerance is somewhat unphysiologic, and normal individuals may develop hypoglycemia 3 to 4 hours after a glucose load, the glucose tolerance test is preferred by some as a more standardized test. Patients with late dumping syndrome hypoglycemia have an unusually large rise in plasma insulin to as high as 200 to 300 $\mu\text{U}/\text{mL}$ in the first 30 to 60 minutes after ingesting glucose, which is followed by hypoglycemia at 3 to 4 hours. There is often also a marked hyperglycemic spike shortly after glucose ingestion because of rapid gastric emptying; however, this does not always occur and is not the cause of the hypoglycemia.

Treatment of late dumping syndrome hypoglycemia may include dietary measures that slow gastric emptying and the release of free glucose into the small bowel, such as frequent small feedings of complex carbohydrates, small snacks between meals, avoidance of simple sugars, and providing fat and protein along with carbohydrates.¹⁷⁹ In more severe cases, acarbose, an alpha-glucosidase inhibitor that slows digestion of starch and other complex carbohydrates, may be effective using doses between 25 and 100 mg, with each feeding.¹⁸⁰ In some cases, continuous intragastric or intrajejunal tube feedings have been required.¹⁸¹

Hyperinsulinemic Hypoglycemia as a Prodrome to the Onset of Diabetes Mellitus. Instances of fasting, but more commonly postprandial hypoglycemia, have occasionally been described before the onset of type 1 diabetes or during the “honeymoon” remission phase when insulin requirements are low.^{182,183} In the Diabetes Prevention Trial-Type 1, closely observed first- and second-degree relatives of patients with type 1 diabetes who were at increased risk of diabetes (i.e.,

individuals with prediabetes) were found to have chemical hypoglycemia detected by quarterly glucose profiles at a rate of 7.5 episodes per 100 person-years. Episodes of hypoglycemia, with documented blood glucose lower than 50 mg/dL, occurred at a rate of 2.6 episodes per 100-person years. There were no reported episodes of severe hypoglycemia.¹⁸⁴ The mechanism most likely involves excessive second-phase insulin release as a consequence of a defective first-phase insulin response; other potential mechanisms have also been proposed, including delayed action of insulin, because of antiinsulin antibodies or insulin release secondary to inflammatory destruction of beta cells. A relationship between reactive hypoglycemia and onset of type 2 diabetes (“dysinsulinism”) was postulated as early as 1930,¹⁸⁵ and widely recognized among diabetologists in the middle of the 20th century.^{186–189} Early type 2 diabetes is also often characterized by loss of the first-phase insulin response to food, resulting in higher glucose excursions, followed by lower glucose nadirs. In most cases of prodromal hypoglycemia in both type 1 and type 2 diabetes, plasma glucose does not reach levels low enough to cause neuroglycopenic symptoms. If necessary, treatment to avoid excessive postprandial hyperglycemia, such as low glycemic index meals, may be effective. In type 1 diabetes during the remission phase, raising the dose of insulin to reduce postprandial hyperglycemia may be helpful.

Hypoglycemia in Cystic Fibrosis Related Diabetes. In cystic fibrosis (CF) first-phase insulin secretion is impaired and glucagon secretion decreases with worsening glucose tolerance.¹⁹⁰ Both fasting and reactive hypoglycemia during a routine glucose tolerance test is relatively common before the onset of cystic fibrosis-related diabetes (CFRD);^{191–193} however, the frequency of reactive hypoglycemia may not be more common than in individuals without CF.¹⁹⁰ In one study, the occurrence of hypoglycemia following an oral glucose tolerance test (OGTT) was associated with a lower 10-year risk of progression to CFRD, as compared with those without hypoglycemia.¹⁹³

Nonislet Tumor Hypoglycemia

Certain noninsulin-secreting tumors are occasionally associated with paraneoplastic hypoglycemia.¹⁹⁴ Most cases have involved large, malignant tumors of mesenchymal, epithelial, or hematopoietic origin. Cases in children are rare, but have been reported with neuroblastoma and Wilms tumor.¹⁹⁵ The hypoglycemic episodes typically occur with fasting and are associated with inappropriately low plasma ketones and FFA, and with increased glucose utilization, which mimic insulin excess. Plasma levels of insulin, proinsulin, and C-peptide are low, and drugs which suppress insulin secretion (diazoxide, somatostatin) are ineffective, suggesting activation of insulin receptors by other circulating factors.¹⁹⁶ In most cases, levels of IGF-2 have been found to be elevated and are postulated to cause hypoglycemia by cross-reaction with both insulin and IGF-1 receptors.¹⁹⁷ IGF-2 is normally produced in the liver and limited amounts are secreted into the circulation bound to IGFBP-3 and the acid-labile subunit (ALS). Tumor-produced IGF-2 may be more active because of incomplete processing and less efficient binding to IGFBP-3 and ALS or may be structurally abnormal—a large molecular form called *big IGF-2*. In addition to stimulation of the insulin receptor, stimulation of the IGF-2 receptor may downregulate GH secretion, resulting in low levels of IGFBP-3.¹⁹⁸

The demonstration of elevated plasma IGF-2 with suppression of both IGF-1 and insulin levels confirms tumor hypoglycemia of this variety. If the tumor cannot be completely removed, a possibly effective intervention is treatment with GH to raise IGFBP-3 levels and reduce the free IGF pool, thereby ameliorating hypoglycemia.¹⁹⁵

It should be noted that not all paraneoplastic hypoglycemia is mediated by IGF-2. Hypoglycemia associated with lactic acidemia, attributed to increased anaerobic glucose metabolism, has been reported in lymphomas and leukemias.^{199,200}

Glycogen Storage Diseases

The GSDs or glycogenoses comprise several inherited diseases caused by deficiencies of enzymes that regulate the synthesis or degradation of glycogen, resulting in increased storage of glycogen in several tissues, especially liver and muscle. Glycogen is the storage form of carbohydrate in humans: hepatic glycogen serves as a crucial glucose reservoir in the intervals between meals; glycogen in muscle and other tissues cannot be released as free glucose, but is used for endogenous needs. Hepatic glycogenolysis provides a large fraction of the glucose that enters the circulation, beginning within a few hours after eating, and continuing until the store of glycogen is depleted.

Glycogen is a highly branched polymer of glucose residues, most of which form straight chains linked by α -1,4-glycosidic bonds. Branches are created by α -1,6-glycosidic bonds, which occur on the average of once every 10 residues. After carbohydrates have been ingested, plasma glucose and insulin levels rise and new glycogen is synthesized.

Glycogen synthesis and degradation in the liver follow distinct pathways that begin and end with glucose 1-phosphate (see Fig. 23.5). The liver is freely permeable to glucose, which is first converted to glucose 6-phosphate before it can enter one of several metabolic pathways. Glucose 6-phosphate can be reversibly converted to glucose-1-phosphate, which is the starting point for glycogen synthesis. Alternatively, glucose-6-phosphate can be hydrolyzed to glucose by glucose-6-phosphatase (G6Pase), or it can be metabolized via the glycolytic pathway to pyruvate and lactate, or via the pentose phosphate pathway, to ribose-5-phosphate, a precursor of nucleotide synthesis. Glycogen synthase catalyzes the formation of α -1,4-linkages. A branching enzyme forms the α -1,6-linkages that make glycogen a branched polymer.

Glycogen breakdown (glycogenolysis) requires the sequential interaction of several enzymes. First, hepatic phosphorylase successively cleaves the 1,4 links to within four glucosyl units of the branch point. Then 4- α -glucanotransferase exposes the 1,6-linked branch points by transferring three glucosyl residues to elsewhere on the glycogen molecule. Amylo-1,6-glucosidase, the debranching enzyme, then splits the 1,6-linked glucosyl units. Thus the sequential actions of phosphorylase and debrancher enzyme liberate the stored glucose units; the action of phosphorylase yields glucose 1-phosphate and the debrancher enzyme liberates free glucose. During fasting, the debrancher

TABLE 23.3 Hepatic Glycogen Storage Diseases

Disorder	Affected Tissue	Enzyme	Inheritance	Gene	Chromosome	Fasting Hypoglycemia
Type 0 GSD	Liver	Glycogen synthase	AR	<i>GYS2</i>	12p12.2	Mild to moderate
Type Ia GSD	Liver, kidney, intestine	Glucose 6-phosphatase	AR	<i>G6PC</i>	17q21	Severe
Type Ib GSD	Liver	Glucose 6-phosphate transporter (T1)	AR	<i>SLC37A</i>	11q23	Severe
Type IIIa GSD	Liver, muscle, heart	Glycogen debranching enzyme	AR	<i>AGL</i>	1p21	Mild to moderate
Type IIIb GSD	Liver	Glycogen debranching enzyme	AR	<i>AGL</i>	1p21	Mild to moderate
Type IV GSD	Liver	Branching enzyme	AR	<i>GBE</i>	3p12.2	With liver failure
Type VI GSD	Liver	Glycogen phosphorylase	AR	<i>PYGL</i>	14q21–22	Mild
Type IXa GSD	Liver, erythrocytes, leukocytes	Liver isoform of α subunit of phosphorylase kinase	X-linked	<i>PHKA2</i>	Xp22.1–p22.2	Mild to moderate
Type IXb GSD	Liver muscle, erythrocytes, leukocytes	β subunit of liver and muscle phosphorylase kinase	AR	<i>PHKB</i>	16q12–q13	Mild to moderate
Type IXc GSD	Liver	Testis/liver isoform of γ subunit of phosphorylase kinase	AR	<i>PHKG2</i>	16p11–p12	Mild
Type XI GSD	Liver, pancreas, intestine, and kidney	Glucose transporter 2 (GLUT2)	AR	<i>SCL2A2</i>	3q26.1–q26.3	Mild

AR, Autosomal recessive; GSD, glycogen storage disease.

TABLE 23.4 Biochemical Characteristics of the Most Common Hepatic Glycogenoses

Type	At Time of Hypoglycemia			Response to Oral Glucose		Response to Glucagon 4–8 Hours After a Meal ^a		Response to Glucagon 2 Hours After a Meal ^b	
	Triglyceride	Uric acid	Lactate	Glucose	Lactate	Glucose	Lactate	Glucose	Lactate
GSD-0	N	N	N	↑↑	↑↑	0-↑	0	↑	↓
GSD-I	↑↑↑	↑↑	↑↑↑	↑	↓↓	0	↑↑↑	0	↑↑
GSD-III	↑	N	N	↑	↑	0	0	↑	0
GSD-VI, IX	0-↑	N	N	↑	↑	0-↑	0	↑	0

0, No increase; 0-↑, variable increase; ↑, mild increase; ↑↑, moderate increase; ↑↑↑, marked increase; ↓, mild decrease; ↓↓, moderate decrease.

GSD, glycogen storage disease; N, normal.

Subjects with suspected GSD-I should not be permitted to fast for more than 4 hours.

^aAfter a glucose-containing meal.

^bSee text to explain why there is a mild glycemic response early after a meal in the GSD types listed.

enzyme mobilizes approximately 8% of hepatic glycogen as free glucose; the remainder requires activity of hepatic G6Pase.

The hallmark of the hepatic glycogenoses is fasting hypoglycemia. The types of hepatic glycogenoses, their specific enzyme deficiencies, affected tissues, their modes of inheritance, and the chromosomal localization of the relevant genes are shown in Table 23.3. Table 23.4 summarizes the major biochemical characteristics of the hepatic glycogenoses (types 0, I, III, VI, and IX) that typically cause hypoglycemia.

Glycogen Synthase Deficiency (Type 0 GSD)

Mutations in the glycogen synthase gene (*GYS2*) on chromosome 12p12.2 cause a rare autosomal recessive disorder characterized by inability to store hepatic glycogen, resulting in markedly reduced hepatic glycogen content 4 to 6 hours after a meal (~0.5 g/100 g wet weight of liver as compared with 5 g/100 g in a normal child), but normal muscle glycogen content. Ingested glucose is preferentially converted to lactate. Symptoms of morning hypoglycemia appear when nocturnal feeding ceases.²⁰¹ Type 0 GSD has a characteristic biochemical phenotype: fasting hypoglycemia and hyperketonemia alternating with transient hyperglycemia with glucosuria and hyperlacticacidemia during the daytime, especially after high-carbohydrate meals.²⁰² During fasting hypoglycemia, plasma levels of the counterregulatory hormones are appropriately elevated, insulin levels are appropriately low, and ketones are elevated.

Unlike the other hepatic GSDs, the liver is not enlarged; therefore referral to glycogen synthase deficiency as a storage disease is a misnomer. Despite its rarity, the disorder should be considered in the differential diagnosis of "ketotic hypoglycemia."^{203,204} After an overnight fast, oral glucose (1.75 g/kg) causes hyperglycemia and hyperlacticacidemia, whereas glucagon (0.03 mg/kg intramuscularly [IM]) typically has no appreciable effect on the plasma glucose level. Genetic testing for mutations in *GYS2* is commercially available.

The goal of treatment is to prevent hypoglycemia and ketonemia during the night, and hyperglycemia and hyperlacticacidemia during the day. Fasting hypoglycemia and ketosis may be prevented by bedtime feedings of UCS, 1 to 1.5 g/kg.²⁰¹ During illness, administration of a similar dose of cornstarch every 6 hours can be used to prevent hypoglycemia. During the day, patients are fed frequently (e.g., every 4 hours); because gluconeogenesis is intact, the diet should contain an increased amount of protein to provide substrate for gluconeogenesis and a decreased amount of carbohydrate (predominantly complex, low glycemic index carbohydrates) to minimize postprandial hyperglycemia and hyperlacticacidemia. This dietary regimen relieves symptoms, reverses the biochemical abnormalities, and improves growth.

Glucose-6-Phosphatase Deficiency (Type I GSD, Von Gierke Disease, Hepatorenal Glycogenosis)

First described in 1929, type I GSD is an autosomal recessive disorder caused by lack of activity of the hepatic enzyme G6Pase, either because of a deficiency of G6Pase itself or a deficiency of the transporter enzyme G6P translocase (*G6PT*). G6Pase catalyzes the final step in the production of glucose from glucose-6-phosphate. Deficiency of this enzyme impairs glucose production both from glycogenolysis and gluconeogenesis (see Fig. 23.5). Decreased production of glucose causes hypoglycemia in the intervals between meals, and increased production of lactate, uric acid, and triglycerides. Glycogen and triglycerides accumulate in the liver, resulting in marked

hepatomegaly; glycogen also accumulates in the kidney and intestinal mucosa.

The G6Pase enzyme system is located in the endoplasmic reticulum (ER) membrane and consists of several subunits. The catalytic subunit, which converts glucose-6-phosphate to glucose, faces into the ER. Three transport systems transport the substrate, Glucose-6-phosphate, and the products, phosphate, inorganic orthophosphate, and glucose, across the ER membrane. glucose-6-phosphate transporter transports glucose-6-phosphate into and phosphate out of the ER; GLUT2 transports glucose out of the ER.²⁰⁵

Approximately 80% of patients with GSD-I have deficient catalytic activity of the G6Pase system leading to type Ia GSD (GSD-Ia). Approximately 100 different mutations have been found in the G6Pase gene, *G6PC*, located on chromosome 17q21. Patients with GSD type Ib, which is caused by failure to transport glucose-6-phosphate into the lumen of the ER, have mutations (about 80 mutations have been described) in the *G6PT* gene, *SLC37A4*, on chromosome 11q23.²⁰⁵

The estimated incidence of GSD-I is one in 100,000 births in the general population; its prevalence is one in 20,000 in Ashkenazi Jews. The presenting symptoms vary according to age. Symptomatic hypoglycemia may appear soon after birth; however, most patients are asymptomatic, as long as they receive frequent feedings that contain sufficient glucose to prevent hypoglycemia. Symptoms of hypoglycemia typically appear only when the interval between feedings increases, such as when the infant starts to sleep through the night or when an intercurrent illness disrupts normal feeding patterns. The condition may not be recognized until the child is several months old and an enlarged liver and protuberant abdomen are noted during a routine physical examination. Patients may present with hyperpnea (from lactic acidosis) and a low-grade fever without a demonstrable infection. Untreated patients may have a cushingoid appearance (a round cherubic or "doll-like" facies), growth failure, and delayed motor development. Social and cognitive development typically are not affected unless the infant suffers brain injury from recurrent severe hypoglycemia.

During infancy, the blood glucose concentration typically drops to less than 50 mg/dL within 3 to 4 hours of a feeding. Longer intervals of fasting cause even more severe hypoglycemia accompanied by hyperlacticacidemia and metabolic acidosis. Despite plasma glucose values in the range of 20 to 50 mg/dL, patients experience few autonomic or neuroglycopenic symptoms of hypoglycemia reflecting the brain's ability to use lactate as an alternative fuel and the downregulation of autonomic responses after recurrent prolonged hypoglycemia.⁵ Serum insulin is appropriately suppressed and the counterregulatory hormones are elevated in a futile attempt to stimulate glycogenolysis and gluconeogenesis, resulting instead in lactic acidosis and exaggerated lipolysis, which causes marked hyperfattyacidemia. Fructose-1-6-bisphosphatase deficiency in infancy may resemble GSD I, with recurrent hypoglycemia and lactic acidosis (see Fig. 23.6); however, in contrast to GSD-I, glucagon administration elicits a brisk glycemic response in the recently fed state and genetic testing for *FBP1* confirms the diagnosis (see Table 23.4).

The serum of untreated patients may be lipemic, with extremely high triglyceride concentrations (typically >500 mg/dL) and moderately increased levels of phospholipids and total and low-density lipoprotein-cholesterol; the high-density lipoprotein-cholesterol concentration is low. High triglyceride levels are caused by increased hepatic synthesis (mobilization of FFA from adipose tissue in response to hypoglycemia), impaired ketogenesis, and reduced clearance of triglycerides, secondary to decreased lipoprotein lipase activity.²⁰⁶ Eruptive xanthomata may appear on the extensor surfaces of the

extremities and on the buttocks and severe hypertriglyceridemia (>1000 mg/dL) may cause acute pancreatitis.

Untreated or poorly controlled GSD I is associated with marked elevations of plasma uric acid. The hyperuricemia is caused by increased degradation of adenosine monophosphate (AMP) to inosine and uric acid because of de/inhibition of AMP deaminase as a consequence of accumulation of glucose-6-phosphate and lowering of both ATP and inorganic phosphate in hepatocytes. A similar mechanism causes hyperuricemia in fructose-1,6-bisphosphatase deficiency (see later section on Genetic Disorders of Gluconeogenesis) because of accumulation of fructose-1,6-phosphate and is the reason fructose is not used in intravenous fluids.

Although symptomatic hypoglycemia becomes less severe with increasing age, nonetheless, without adequate therapy growth is stunted, and puberty is delayed. However, when continuous glucose therapy is started early in life and long-term good metabolic control is maintained, patients can grow and develop normally.²⁰⁷

Impaired platelet function causes a bleeding tendency manifested as recurrent epistaxes or oozing after dental or other surgery. Reduced platelet adhesiveness, abnormal platelet aggregation, and impaired release of adenosine diphosphate (ADP) in response to collagen and epinephrine have been observed. The defects in platelet function are secondary to the systemic metabolic abnormalities and are corrected by improving the metabolic state.

Anemia is common in children with GSD I. The etiology of the anemia is multifactorial and includes iron and other nutritional deficiencies, chronic lactic acidosis, blood loss from menorrhagia, end-stage renal disease (ESRD), and enterocolitis (in patients with GSD Ib). Patients with liver adenomas may have iron refractory anemia secondary to aberrant hepcidin expression.²⁰⁸ Intestinal symptoms are usually not a prominent feature but can include diarrhea.

Kidney enlargement is readily demonstrated by ultrasonography. Deficiency of G6Pase leads to glycogen deposition in the kidneys and disturbs the metabolism of renal tubular cells, resulting in a relative energy deficiency.²⁰⁹ Increased renal blood flow and glomerular filtration rate (GFR) may be a compensatory mechanism for the intracellular energy deficit. Renal manifestations include proximal and distal renal tubular dysfunction, as well as glomerular injury that can lead to ESRD.²¹⁰ Proximal tubular dysfunction (glucosuria, phosphaturia, hypokalemia, and a generalized aminoaciduria) is reversible when biochemical control of the disease improves.²¹¹ Some patients have a distal renal tubular acidification defect associated with hypocitraturia and hypercalciuria, which predisposes to renal calculi and nephrocalcinosis. Increased urinary albumin excretion may be observed in adolescents. Progressive severe renal injury with proteinuria, hypertension, and decreased creatinine clearance because of focal segmental glomerulosclerosis and interstitial fibrosis, may be seen in young adults. Patients with persistently elevated blood lactate, serum lipid, and uric acid concentrations appear to be at increased risk of developing nephropathy. Normalization of metabolic parameters decreases proteinuria, and optimal therapy instituted at or before age 1 year may delay, prevent, or slow the progression of renal disease.^{212,213}

Development of hepatic adenomas is a common complication, occurring in up to 75% of patients by the time they reach adulthood. Although adenomas are usually first observed in the second and third decades of life, they may appear before puberty. Adenomas may hemorrhage or become malignant hepatocellular carcinomas.²¹⁴ Ultrasonography is the preferred method of screening for hepatic adenomas. When malignancy is suspected, MRI and measurement of serum alpha-fetoprotein should be performed.

Radiographic studies have demonstrated osteopenia, and pathologic studies have shown osteoporosis, without evidence of abnormalities in calcium, phosphate, parathyroid, or vitamin D metabolism. Bone mineral content is decreased compared with age-matched normal children. Endocrine and metabolic disturbances, including hypercortisolemia, resistance to GH, delayed puberty, and lactic acidosis, may account for decreased bone mineralization.

Menstrual irregularities and hirsutism caused by hyperandrogenism are uncommon; however, in all types of hepatic GSDs, ultrasonography has shown a high prevalence of morphologically polycystic ovaries (even in prepubertal children), the clinical significance of which is still unclear.

Although the heart itself is not affected in GSD I (unlike GSD III), hypertension may develop in association with renal disease. Pulmonary hypertension presenting in the second or third decade of life and leading to death from progressive heart failure has also been described.

Patients with GSD Ib have symptoms similar to GSD Ia, but with the addition of either constant or cyclic neutropenia of variable severity, ranging from mild to complete agranulocytosis, and is associated with recurrent bacterial infections.²¹⁵ Neutropenia is a consequence of disturbed myeloid maturation and is accompanied by functional defects of circulating neutrophils and monocytes.²⁰⁵ A recent case series suggests that vitamin E supplementation may improve neutropenia and reduce infection rates.²¹⁶ Patients frequently develop an inflammatory bowel disease resembling Crohn disease, which is responsive to treatment with granulocyte colony-stimulating factor (G-CSF). Children with GSD Ib are prone to oral complications (recurrent mucosal ulceration, gingivitis, and rapidly progressive periodontal disease), painful perianal abscesses, and have an increased prevalence of thyroid autoimmunity and primary hypothyroidism.

Diagnostic Studies. During infancy, plasma glucose typically decreases to less than 50 mg/dL within 3 to 4 hours of a feeding and is accompanied by hyperlactacidemia and metabolic acidosis. The serum may be cloudy or milky, with very high triglyceride and moderately increased levels of cholesterol. Serum uric acid, aspartate aminotransferase, and alanine aminotransferase levels are usually increased. The simplest method of determining the probable nature of the enzymatic deficiency in a child suspected of having a GSD I is to obtain serial blood samples for measurement of metabolites (glucose, lactate, FFA, ketones, and uric acid) for up to 6 hours (or until the plasma glucose decreases to ≤ 50 mg/dL), following oral glucose (1.75 g/kg), or 30 minutes after stopping a continuous overnight intravenous or intragastric glucose infusion. Hypoglycemia, together with elevated blood lactate levels, combined with an abnormal fed glucagon stimulation test, clinches the diagnosis. In the normal child, a 2-hour postprandial (fed) glucagon stimulation test causes the plasma glucose to increase by 30 mg/dL or more within 30 minutes without a change in lactate concentration, whereas in GSD I, the glucose response is minimal and there is a marked increase in lactate. Because administration of glucagon can lead to pronounced worsening of metabolic acidosis and decompensation in GSD I, it is no longer recommended in the diagnostic evaluation of suspected GSD I. Mutation analysis of *G6PC* (GSD Ia) and *SLC37A4* (GSD Ib) genes is recommended to confirm type I GSD.^{217,218} Because GSD Ia is more common, unless neutropenia is present, complete *G6PC* sequencing is recommended first. Note that although neutropenia suggests GSD Ib, it has also been occasionally seen in GSD Ia and the neutrophil count can be normal in patients with GSD Ib in the first years of life. A liver biopsy for measurement of G6Pase activity may be necessary in the rare patient who does not have an identifiable gene mutation. In most

individuals with GSD Ia, G6Pase enzyme activity is <10% of normal. G6Pase activity must be assayed in both intact and fully disrupted microsomes, as enzyme activity normalizes when freezing disrupts the integrity of the ER. For this reason, G6Pase enzyme activity on snap-frozen liver biopsy tissue will not detect GSD Ib, and fresh (unfrozen) liver biopsy tissue is needed to assay G6PT activity accurately. The glycogen itself has a normal appearance. Ultrasound of the abdomen shows hepatomegaly and nephromegaly without splenomegaly. Nephromegaly is characteristic of GSD I and is not a feature of the other GSDs.

Treatment consists of providing a continuous dietary source of glucose to prevent the plasma glucose from falling below the threshold for glucose counterregulation.²¹⁸ When hypoglycemia is prevented by providing an appropriate amount of glucose throughout the day and night, the biochemical abnormalities are ameliorated, liver size decreases, the bleeding tendency is reversed, and growth improves. Recommended biochemical targets: plasma glucose greater than 70 to 75 mg/dL, triglycerides less than 500 mg/dL, and uric acid less than 7.5 mg/dL.

Various methods may be used to provide a continuous source of glucose at a rate sufficient to satisfy glucose requirements in the intervals between meals: intravenously, via the gastrointestinal tract by intragastric infusion (either nasogastric or gastrostomy tube), or by use of low glycemic index foods.²¹⁸ Of the latter, UCS has the most suitable properties described to date. The *minimum* amount of glucose required in the basal state may be obtained by using the formula for calculating the basal glucose production rate: $y = 0.0014x^3 - 0.214x^2 + 10.411x - 9.084$, where $y = \text{mg glucose per minute}$ and $x = \text{ideal body weight in kilograms}$.⁸ The amounts and/or schedule of glucose or UCS administered are modified, if necessary, based on the results of clinical and biochemical monitoring.

Infants with GSD I usually require feeds every 2 to 3 hours of a formula that does not contain either lactose or sucrose. The formula must contain a polymer of glucose (corn syrup solids, maltodextrins) that will yield an amount of glucose equal to the calculated glucose production rate.²¹⁹ If nighttime feedings are challenging, continuous overnight feedings, using the same formula, may be given via nasogastric or gastrostomy tube controlled by an infusion pump.

Orally administered UCS acts as an intestinal reservoir of glucose that is slowly absorbed into the circulation. In many centers, UCS has replaced frequent daytime feedings of glucose or glucose polymers and overnight continuous intragastric infusion of glucose. It has been used successfully in infants as young as 8 months of age.²¹⁹ The UCS is given in a slurry of water or an artificially sweetened beverage (e.g., Kool-Aid) or in formula for infants, at 3- to 5-hour intervals during the day, and at 4- to 6-hour intervals overnight. The amount given is determined by multiplying the time interval between feedings by the calculated hourly glucose requirement for ideal body weight. One tablespoon (8 g) of UCS contains 7.3 g of carbohydrate. The optimum schedule and amounts of intermittent UCS feedings for patients of different ages is determined empirically by metabolic monitoring to ensure that the biochemical goals are being achieved.²²⁰

Extended Release Waxy Maize Cornstarch (Glycosade, Vita-flo, International Ltd., Liverpool, England) was approved in England in 2009 for the management of GSD I and was released in the United States as a medical food in 2012. Efficacy studies in children as young as 5 years old have shown prolongation of fasting tolerance from an average of 4.1 to 7.8 hours, using extended release cornstarch as compared with standard UCS.^{221,222} Gastrointestinal intolerance and exacerbation of

inflammatory disease are common side effects of the extended release formulation.

When adequate exogenous glucose is provided, significant hyperuricemia and hyperlipidemia are usually restored to near normal. If severe hyperuricemia persists, allopurinol, a xanthine oxidase inhibitor (5–10 mg/kg/day as a single daily dose or divided q12 hours), effectively lowers serum uric acid to normal levels. Lipid-lowering agents (gemfibrozil or fenofibrate) are indicated when persistent severe hyperlipidemia, despite optimal glucose therapy, poses a significant risk of acute pancreatitis.

Dietary fat should be restricted to less than 20% of the total energy intake, equally distributed among monounsaturated, polyunsaturated, and saturated fats. Dietary cholesterol is restricted to less than 300 mg/day. Carbohydrate typically provides 60% to 65% of the daily calories. Of the total daily calories, 30% to 45% is prescribed (both the amount and schedule) in the form of UCS. Most of the remaining dietary carbohydrate should, ideally, be low glycemic index starches. With the glucose requirements prescribed, the total caloric intake is determined by the child's appetite, as long as the rate of weight gain is not excessive. The dietitian must ensure that the patient is consuming an adequate amount of protein, fat, minerals, and vitamins to support optimal growth. When adequate glucose is prescribed to maintain normoglycemia, milk products and fruit, despite their content of galactose and fructose, respectively, may be used sparingly to supply essential nutrients, minerals, and vitamins.

Renal disease. Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers have been reported to improve GFR in patients with hyperfiltration. Thiazide diuretics and oral citrate can help prevent nephrocalcinosis and renal calculi.²²³ Loop diuretics should be avoided because of the risk of hypercalciuria.

Anemia. Iron, vitamin B₁₂, and folic acid intake should be optimized. Patients with hepatic adenomas may develop iron refractory anemia caused by aberrant tumor expression of hepcidin, a key regulator of iron metabolism.²⁰⁸ Erythropoietin supplementation may be required in patients with ESRD.

Neutropenia. G-CSF is efficacious for treatment of neutropenia and infections in patients with GSD-Ib; monitoring for splenomegaly should be performed because it is the most serious complication of G-CSF therapy in this population.^{224,225}

Monitoring. Frequent plasma glucose monitoring before meals, before cornstarch administration, and before and after exercise is essential to establish good metabolic control. If the cornstarch dose is changed, plasma glucose should also be monitored frequently after cornstarch administration to establish the effective duration of action. Clinical studies of continuous glucose sensors, in both adults and children, show that they can be used safely and reliably in patients with GSD I, and can help reduce duration of hypoglycemia, as well as detect asymptomatic hypoglycemia.²²⁶

Routine monitoring should include: complete blood count with differential white cell count, serum uric acid, triglycerides, and cholesterol. Triglyceride concentrations are considered the most useful parameter of metabolic control, given the rapid fluctuations in the levels of plasma glucose, lactate, and transaminases. Significantly increased adenoma progression has been reported in patients with mean triglyceride greater than 500 mg/dL.

The American College of Medical Genetics and Genomics has provided guidelines for screening patients with GSD I for complications.²¹⁸ These include measurement of serum creatinine, urea, electrolytes, calcium, phosphate, liver function tests, protein, and

albumin every 6 months. Urine microalbumin, protein, creatinine, calcium, and citrate should be checked every year from 0 to 5 years of age and every 6 months if older than 5 years of age. Creatinine clearance (GFR estimate) should be performed yearly for those older than 5 years of age. An abdominal ultrasound examination to evaluate liver, kidneys, spleen, and ovaries should be performed annually if younger than 10 years of age, and every 6 months after age 10 years. Bone densitometry should be obtained every 1 to 2 years for those older than 5 years. Periodic echocardiography (ECHO) and electrocardiogram (ECG) should be performed to screen for pulmonary hypertension starting at 10 years of age and repeated at least every 3 years.

A clinical study to evaluate the efficacy and safety of gene therapy to replace hepatic G6Pase in human subjects, using adeno-associated virus carrying the human *G6PC* gene, is currently in progress.

Amylo-1,6-Glucosidase Deficiency (Type III GSD, Debranching Enzyme Deficiency, Limit Dextrinosis, Cori Disease, Forbes Disease)

Type III GSD is caused by deficient activity of the glycogen debranching enzyme (GDE) (see Fig. 23.5), leading to impaired glycogen degradation, reduced glycogenolysis, and accumulation of abnormal glycogen (limit dextrin). It is an autosomal recessive disease resulting from mutations of the *AGL* gene on chromosome 1p21, which encodes four GDE isoforms. Isoform 1, the most widely distributed and the predominant form in the liver, is defective in GSD-IIIa. Isoforms 2, 3, and 4 are exclusively located in skeletal and cardiac muscle. GDE has two enzymatic functions: a transferase that exposes the 1,6-linkage and amylo-1,6-glucosidase, which produces free glucose. Without GDE, glycogen breakdown cannot proceed past the outermost branch points, resulting in limit dextrin, an abnormal form of glycogen with short side chains. Only the outer 5% to 10% of glucose residues can be released by phosphorylase. In the United States, 80% to 85% of patients with GSD-III lack GDE activity in both liver and muscle (GSD-IIIa), 15% lack GDE activity in the liver alone (GSD-IIIb); rarely, patients lack activity in muscle alone (GSD-IIIc). In contrast, in Israel the majority of patients lack enzyme activity only in the liver (GSD-IIIb).

Clinical and enzymatic variability is a feature of GDE deficiency. During infancy and childhood, the presentation may be indistinguishable from GSD-I. Hepatomegaly, fasting hypoglycemia with ketosis, hyperlipidemia, and growth retardation are the predominant features. About 70% of patients have muscle weakness, but this is usually not clinically significant in childhood.

Because small amounts of glucose can be produced from 1,4-segments beyond the outermost glycogen branch points and from gluconeogenesis, patients with GDE deficiency may be able to tolerate longer periods of fasting, and hypoglycemia usually is less severe than in those with G6Pase deficiency. Infants with GSD-III may be asymptomatic on their usual frequent feeding schedules and typically do not become as severely ill with infections and other stresses that disrupt feeding as do children with GSD-I.

Infants with GSD-III develop fasting hyperketonemia as a result of an accelerated transition to the fasting state. Blood lactate and uric acid levels are normal, because the gluconeogenic pathway is intact and hepatic glycolysis is not increased (see Fig. 23.5). Hyperlipidemia is less severe in GSD-III than in GSD-I. The presenting clinical finding may be hepatomegaly and growth failure. An enlarged spleen may be seen at 4 to 6 years of age in patients who develop hepatic fibrosis. The kidneys are not large and renal dysfunction does not occur.

Untreated infants and children grow slowly and puberty is delayed. Patients who lack GDE in muscle usually have minimal, clinically insignificant muscle weakness during childhood. With the exception of myopathy, symptoms and signs characteristically ameliorate with increasing age. Myopathy, however, usually becomes prominent in the third or fourth decade of life, manifesting as slowly progressive muscle weakness involving the proximal muscles. In some patients, the small muscles of the hands are also affected. Cardiac involvement is common as a result of limit dextrin accumulation in the heart causing a cardiomyopathy that resembles idiopathic hypertrophic cardiomyopathy. Significant concentric left ventricular hypertrophy usually develops after puberty and manifests as ventricular hypertrophy on ECG and increased left ventricular mass and wall thickness on an echocardiogram.²²⁷ Ventricular changes are more commonly seen in patients with GSD-IIIa, but have also been reported in patients with GSD-IIIb. Symptomatic cardiomyopathy may occur and sudden death has been attributed to cardiac arrhythmias; glycogen accumulation in the cardiac conduction system has been observed at autopsy.

The size of the liver tends to decrease during puberty. Biopsy usually shows hepatic fibrosis, and some adult patients develop cirrhosis. Hepatic adenomas have been found in 4% to 25% of patients, compared with 22% to 78% of GSD-I patients. The development of hepatocellular carcinoma is rare, but has been described in patients more than 30 years old.

Typical biochemical features are fasting hypoglycemia with ketosis (i.e., ketotic hypoglycemia), but without elevation of blood lactate or serum uric acid concentrations (see Fig. 23.6). Hypertriglyceridemia and hypercholesterolemia occur in approximately two-thirds and one-third of patients, respectively. Hypertriglyceridemia is most commonly found in younger children (<3 years old) and does not reach levels high enough to cause pancreatitis. Liver transaminases are consistently elevated in children (at least twice the upper limit of normal, and often >500 IU/L), but decline at puberty and may be normal in adults. Creatine kinase (CK) is often markedly elevated in patients with GSD-IIIa.

After an overnight fast, plasma glucose and lactate concentrations do not increase after administration of glucagon (see Table 23.4). However, when the test is performed 2 hours after a high-carbohydrate meal (before the outer branches of glycogen are degraded), a glycemic response can be elicited. Diagnosis of GSD-III is made by mutation analysis of the *AGL* gene or, alternatively, by liver and/or muscle biopsy.²²⁸ Biopsy results show a 3- to 5-fold elevation of structurally abnormal (highly branched) glycogen content in the involved tissue. Definitive subtyping of GSD-III requires a biopsy of both liver and muscle. Although muscle involvement can be inferred from the presence of high levels of serum CK, a normal level does not rule out muscle enzyme deficiency. At the time of diagnosis, muscle biopsy is the only way to predict accurately whether skeletal muscle disease or heart muscle involvement is likely to develop in the future, unless a GSD-IIIb specific mutation is identified.

Because only a limited amount of glucose can be mobilized from glycogen, hypoglycemia develops during an overnight fast in infancy and early childhood. This occurs despite increased gluconeogenesis and enhanced hepatic uptake of gluconeogenic amino acids, which results in low plasma levels of several amino acids, such as alanine. As in GSD-I, continuous provision of an adequate amount of glucose, using UCS, combined with a normal intake of total calories, protein, and other nutrients, corrects the clinical and biochemical disorder and restores normal growth. UCS 1 to 1.75 g/kg at 6-hour intervals (e.g., at midnight, 6 AM, etc.) maintains normoglycemia, increases

growth velocity, and decreases serum aminotransferase concentrations.²²⁸ Infants less than 12 months old may not produce sufficient amylase to digest UCS and experience bloating, flatulence, and diarrhea. If these side effects occur, a slow rate of introduction of UCS or addition of pancrelipase (which contains amylase) may improve tolerance. UCS can be mixed in any beverage, although addition to milk or yogurt is preferable, as it adds protein and fat. Addition of whey protein is recommended to prolong normoglycemia. Excessive UCS should be avoided because it leads to excess glycogen deposition and weight gain. For patients who have significant growth retardation and myopathy, continuous nocturnal feeding of a nutrient mixture composed of glucose, glucose oligosaccharides, and amino acids combined with intermittent feedings during the day of meals with a high protein content may be beneficial. Adolescents and adults with GSD IIIa should aim for a high-protein (25% of total calories) and low complex carbohydrate (<50% of total calories) diet and should avoid simple sugars and fasting. A high-protein diet may improve muscle function by enhancing muscle protein synthesis and reducing glycogen storage. It has also been reported that a high-protein diet (~30% of total calories) may reverse and possibly prevent cardiomyopathy.²²⁹ Patients with GSD-IIIb may be able to transition to a regular well-balanced diet.

Plasma glucose monitoring should be performed before meals or UCS, before bedtime and breakfast, with addition of extra monitoring during times of illness. Liver function tests should be performed every 6 months. As in patients with GSD-I, annual hepatic ultrasound examinations should be performed to screen for hepatic adenomas. MRI of the liver should be performed every 6 to 12 months in older patients and in children, when an ultrasound examination shows an adenoma has increased in size. Routine ECG and ECHO should be performed to evaluate for dysrhythmias, ventricular hypertrophy, and systolic or diastolic dysfunction. Cardiac ECHO should be done every 12 to 24 months in patients with GSD-IIIa and every 5 years in those with GSD-IIIb. ECG should be done every other year in patients with GSD-IIIa. No exercise restrictions are recommended for patients with GSD-III, unless they have significant cardiac disease. Exercise helps prevent worsening myopathy and low bone mineral density. Physical therapy evaluation is recommended every 6 months.

Hepatic Phosphorylase Complex Deficiency (Type VI GSD, Hepatic Phosphorylase Deficiency, Hers Disease; Type IX GSD, Phosphorylase Kinase Deficiency)

Hepatic phosphorylase, the rate-limiting enzyme of glycogenolysis, is activated by a cascade of enzymatic reactions triggered by glucagon and epinephrine. First, adenylate cyclase catalyzes the formation of cyclic adenosine monophosphate (cAMP), which then activates a cAMP-dependent protein kinase. Protein kinase then phosphorylates a phosphorylase kinase (PHK), which converts inactive hepatic phosphorylase to its active form. Active phosphorylase hydrolyzes the α -1,4-linkages and mobilizes glucose from glycogen (see Fig. 23.5).

The GSDs caused by a reduction in liver phosphorylase activity are a heterogeneous group of disorders (see Table 23.3). Hepatic PHK or type IX GSD, which occurs in approximately one in 100,000 births and accounts for about 25% of all cases of GSDs is the most common form; whereas, deficiency of hepatic phosphorylase (type VI GSD) itself is rare.

PHK of liver and muscle is a complex enzyme consisting of four subunits: α , β , γ , δ . The enzyme is regulated by phosphorylation of specific serine residues of the α and β subunits and by calcium through the δ subunit, a member of the calmodulin family. The γ subunit is catalytically active. Mutations in three

different genes of PHK subunits (*PHKA2*, *PHKB*, and *PHKG2*) can result in deficient activity of hepatic phosphorylase. GSD IXa, because of mutations in *PHKA2*, is the most common variant (see Table 23.3).

GSD IXa is an X-linked disorder restricted to boys. Patients seldom have symptomatic hypoglycemia during infancy, unless they fast for a prolonged period.²³⁰ They can develop hyperketonemia similar to, but usually milder than, that seen in type III GSD. Metabolic acidosis is rare. The disorder is usually discovered when an enlarged liver and protuberant abdomen are noted during a physical examination.^{231,232} Physical growth is usually impaired, and motor development may be delayed as a consequence of hypotonia. With increasing age, clinical and biochemical abnormalities gradually ameliorate, and catch-up growth may occur; most adult patients are asymptomatic. GSD IXc, because of *PHKG2* mutations, tends to have a more severe phenotype that includes liver fibrosis and cirrhosis in childhood. Recent reports indicate that liver fibrosis may also occur in GSD VI and IXa. Mild cardiomyopathy has been identified using ECHO on long-term follow-up of patients with GSD VI and IXb.²³³

Hypoglycemia is unusual and blood lactate and uric acid levels are normal. Mild hypertriglyceridemia, hypercholesterolemia, and elevated transaminase levels may be present. Ketonemia occurs with fasting.²⁰⁴ Functional tests are not especially useful in evaluating these patients. After an overnight fast, blood lactate level is normal, and administration of glucagon elicits a brisk glycemic response, without a rise in the blood lactate concentration. The glycemic response to glucagon cannot be used to distinguish between phosphorylase kinase deficiency and lack of phosphorylase itself. Thus the definitive diagnosis of type IX GSD requires genetic testing or determination of phosphorylase kinase activity in erythrocytes or leukocytes. The diagnosis of type VI GSD can also be established by genetic testing or, alternatively, by assaying the activity of phosphorylase in purified blood cell fractions, and usually does not require a liver biopsy. Muscle phosphorylase activity is normal; muscle histology and glycogen content are normal.

In the past, specific treatment other than avoidance of prolonged fasting was considered unnecessary. Recent research, however, suggests that therapy with a high-protein diet and UCS in patients with GSD IX improves linear growth, general wellbeing, decreases hepatomegaly, and may be associated with regression of ultrasound findings of liver fibrosis. UCS, 2 g/kg at bedtime, typically prevents hypoglycemia and ketosis in these patients.²³²

Plasma glucose and ketone monitoring should be done routinely. Growth and pubertal progression should be carefully monitored. Annual liver ultrasound examinations should be performed starting at age 5 years. Because there is increased risk of osteoporosis, bone densitometry is recommended once growth is complete.

Glycogen Branching Enzyme Deficiency (Type IV GSD, Andersen Disease)

There is minimal impairment of glycogenolysis and gluconeogenesis is normal; accordingly, hypoglycemia is rare; however, fasting hypoglycemia may occur when severe cirrhosis develops.²³⁴

Fanconi-Bickel Syndrome (Type XI GSD)

GSD XI is a rare autosomal recessive disorder characterized by hepatorenal glycogen accumulation caused by deficiency of the facilitative GLUT2 that mediates the bidirectional transport of

glucose and galactose in hepatocytes, pancreatic beta cells, enterocytes, and in the proximal tubules of the kidney.²³⁵

Patients usually present in infancy with failure to thrive, chronic diarrhea from carbohydrate malabsorption, hepatomegaly, and fasting hypoglycemia, as the interval between feeds increases. A general renal proximal tubulopathy with severe glucosuria and hypophosphatemic rickets are characteristic features. Older patients have a moon-shaped face, a protuberant abdomen, short stature, and delayed puberty. Kidneys are enlarged (detectable by ultrasound) as a result of glycogen accumulation.²³⁶ Clinical findings are of variable severity.^{237,238}

Fasting ketotic hypoglycemia and postprandial hyperglycemia and hypergalactosemia (caused by impaired hepatic uptake of the two sugars) are characteristic biochemical features. Decreased insulin secretion (because of an impairment of beta-cell glucose sensing) may also contribute to impaired hepatic glucose uptake and postprandial hyperglycemia. Other laboratory findings include hypergalactosemia, glucosuria, renal bicarbonate wasting, proteinuria, phosphaturia, generalized aminoaciduria, and elevated serum alkaline phosphatase level. Mutation analysis of the *GLUT2* gene confirms the diagnosis.

Frequent feeds using slowly absorbed carbohydrates and restriction of galactose are recommended. Supplementation with UCS has a beneficial effect on metabolic control and growth. Fructose metabolism is not affected; therefore this monosaccharide may be used as an alternative carbohydrate source. Water and electrolytes must be replaced. Alkali may be necessary to compensate for renal tubular acidosis, and hypophosphatemic rickets requires supplemental phosphate and vitamin D.

Ketotic Hypoglycemia. Ketotic hypoglycemia has been recognized for nearly a century²³⁹ as the most common type of childhood hypoglycemia between 1 to 6 years of age, with a well-characterized presentation and course, but an incompletely understood etiology.^{240–243} Usually, this condition presents as recurrent episodes of morning hypoglycemia in the second or third year of life—but onset as early as 6 months has been reported. The condition usually remits spontaneously by the age of 8 to 9 years. The classic history is of a child, often a male with a history of low birthweight, who has eaten poorly the previous day or misses an evening meal, is difficult to rouse from sleep the next morning, and displays neuroglycopenic symptoms that may range from lethargy to seizure. Hypoglycemic episodes are especially likely to occur during an illness, when food intake is limited. Treatment to avoid prolonged overnight fasting usually limits recurrent attacks to less than one to two a year.

At the time of hypoglycemia, high levels of ketones are found in plasma and urine, and plasma insulin concentrations are suppressed, indicating that HI is not responsible for the hypoglycemia. It remains unsettled whether these children display an accelerated, but qualitatively normal, metabolic response to fasting (i.e., the lower end of the normal distribution of fasting tolerance, “accelerated starvation”) or whether ketotic hypoglycemia represents a heterogeneous group of disorders of limited substrate availability awaiting further delineation.^{244,245} For example, fasting tolerance is much shorter in young infants because of their larger ratio of brain versus body mass and therefore are more vulnerable to becoming hypoglycemic, when fasted for longer than 12 hours, especially if normal feeding has been impaired by anorexia, vomiting, or diarrhea because of an intercurrent illness. On the other hand, in children previously diagnosed as having ketotic hypoglycemia, an increasing number of specific metabolic defects have been recognized, such as mild forms of glycogen storage disorders (Type IXa GSD)²⁰⁴ or of ketone utilization.²⁴⁶ Thus ketotic

hypoglycemia should be considered a diagnosis of exclusion and further investigation should be considered in any child with ketotic hypoglycemia who has recurrent episodes.

Several studies have shown that ketotic hypoglycemia reflects underproduction rather than overutilization of glucose.^{247,248} Infusions of alanine, fructose, or glycerol produce a rise in plasma glucose concentration, without significant changes in blood lactate or pyruvate levels, indicating that the entire gluconeogenic pathway from the level of pyruvate is intact, and suggesting that a deficiency of substrate rather than a defect in gluconeogenesis is involved.^{247,249}

Glucagon induces a normal glycemic response in affected children during the fed state, but not at the time of hypoglycemia, indicating that glycogenolytic pathways are also intact. Plasma glycerol levels are normal in these children, in both the fed and fasted states. The metabolic response to infusion of BOHB does not differ from that of normal children. Finally, the levels of hormones that counter hypoglycemia are appropriately elevated, whereas insulin levels are appropriately low.^{14,249,250}

Children with ketotic hypoglycemia have plasma alanine concentrations that are reduced in the basal state, after an overnight fast, and fall still farther with prolonged fasting.^{21,251} Alanine is the major amino acid used for gluconeogenesis. Its formation and release from muscle during periods of caloric restriction is enhanced by the presence of a glucose-alanine cycle, as well as by de novo formation from other substrates, such as branched chain amino acids. Hypoalaninemia in ketotic hypoglycemia probably reflects “accelerated starvation,” rather than a specific defect in alanine metabolism. As pointed out in the original description of ketotic hypoglycemia, the children are frequently smaller than age-matched controls and often have a history of transient neonatal hypoglycemia.²⁴⁰ Thus ketotic hypoglycemia may simply reflect the reduced reserve of a small muscle mass at an age when glucose demands per unit of body weight to support brain metabolism are relatively high; children with ketotic hypoglycemia may represent the lower end of the normal range for fasting tolerance.²⁴⁴ Spontaneous remission of ketotic hypoglycemia by age 8 to 9 years might be explained by the increase in muscle bulk relative to brain size, with a resultant increase in the supply of endogenous substrate, and the relative decrease in glucose requirement per unit of body mass, with increasing age.

The diagnosis of ketotic hypoglycemia is confirmed by a supervised fast. Hypoglycemia with elevated plasma FFA, BOHB, and acetoacetate develops within 14 to 24 hours in most of these children, whereas normal children of similar age can withstand fasting without developing hypoglycemia for at least 24 hours.

Episodes of ketotic hypoglycemia can be prevented or minimized by avoidance of prolonged fasting. The overnight fast should be shortened to less than 10 to 12 hours, with a bedtime snack containing carbohydrate and prompt breakfast. When episodes have been triggered by illness, parents may test the child’s urine for ketones or, preferably, plasma BOHB. The appearance of hyperketonemia precedes the hypoglycemia by several hours and indicates a need for high-carbohydrate liquids. If these cannot be tolerated, the child should be taken to the emergency department for intravenous glucose. A letter of explanation and treatment recommendation may expedite the emergency department response.

Ketotic hypoglycemia should be considered only a diagnosis of exclusion, as episodic hypoglycemia with ketosis can occur with deficiencies of several hormones or a variety of defects of gluconeogenesis or glycogen metabolism, especially hypopituitarism and GSDs.²⁰⁴ Recurrent episodes of ketotic hypoglycemia that cannot be explained by intercurrent illness

should trigger reevaluation for a possible underlying disorder that had previously been missed. Among the disorders that deserve special consideration are the milder glycogen storage disorders (e.g., Type IXa GSD caused by X-linked phosphorylase kinase deficiency, *PHKA2*); adrenal insufficiency because of inhaled or intranasal glucocorticoids; or defects in ketone utilization, such as deficiency of MCT1 encoded by *SLC16A1*.²⁴⁶

Hormone Deficiency

GH and cortisol both augment glucose production, decrease glucose utilization, and accelerate lipolysis and ketogenesis.^{23,26} In contrast with glucagon and epinephrine, which raise plasma glucose levels within minutes, the actions of GH and cortisol are delayed for several hours and neither has a primary role in the defense against acute insulin-induced hypoglycemia. They are important, however, in preventing hypoglycemia during prolonged fasting.

Deficiency of Growth Hormone and Hypopituitarism

Deficiency of GH is an important cause of fasting hypoglycemia, especially in infants and young children. Although less common, symptomatic and asymptomatic hypoglycemia, usually precipitated by stress, starvation, or exercise, may also occur in older children and adults with either isolated GH deficiency or multiple pituitary deficiencies.^{252–254}

Hypopituitarism in the newborn period can cause persistent or recurrent severe hypoglycemia,²⁵⁵ and some infants may require a glucose infusion rate comparable to that required to treat congenital HI, in addition to mimicking HI by having suppressed FFA and ketone levels, and a glycemic response to glucagon.²⁵⁶ A microphallus suggests coexistent gonadotropin deficiency.²⁵⁷ Infants with congenital hypopituitarism may have cholestatic jaundice with elevated liver enzymes consistent with neonatal hepatitis.^{258,259} The diagnosis of GH deficiency cannot be based on a single GH value obtained at the time of spontaneous or fasting-induced hypoglycemia. Studies have shown that a single low GH value, at the time of fasting hypoglycemia, has poor specificity for the diagnosis of GH deficiency.^{72,260} Confirmation of the diagnosis should be based on a firm foundation of clinical evidence, including demonstration by imaging of abnormal pituitary gland development or other midline defects.²⁶¹

Isolated GH deficiency in young children may cause morning hypoglycemia with hyperketonemia (“ketotic hypoglycemia”).^{262–264} Plasma insulin levels are appropriately suppressed and the glycemic response to glucagon during spontaneous hypoglycemia is poor. The degree of ketosis at the time of hypoglycemia may be lower than expected in the more common type of idiopathic ketotic hypoglycemia, perhaps reflecting increased insulin sensitivity and impaired lipolysis. It has been suggested that the relative hypoketonemia (i.e., impaired generation of alternative fuels) may contribute to the development of fasting hypoglycemia in young children with GH deficiency.²⁶⁴ After early infancy, slow growth and low IGF-1 levels are more reliable indicators of GH deficiency, and the definitive diagnosis of GH deficiency should be confirmed by demonstrating low GH responses to standard provocative stimuli.

Growth Hormone Resistance and Insulin-like Growth Factor-1 Deficiency

Recurrent fasting hypoglycemia is common in GH resistance because of molecular defects in the GH receptor, leading to

an inability to generate IGF-1, commonly referred to as *Laron dwarfism*.^{265,266} The hypoglycemia tends to improve in adolescence, although still can occur with prolonged fasting. Similar frequencies of hypoglycemia ($\geq 45\%$) have been reported in the two largest populations with this condition, in Israel and Ecuador. The vulnerability to fasting hypoglycemia improves with treatment with synthetic IGF-1; however, one of the insulin-like effects of IGF-1 treatment is hypoglycemia, which can occur within an hour of an injection, if the child has not eaten.

Deficiencies of Cortisol and Adrenocorticotropin

Cortisol is crucially important in supporting glucose production during fasting by increasing glycogen storage and gluconeogenesis, and for mobilizing FFA;²⁶ cortisol also has an indirect role in the defense against hypoglycemia by enabling epinephrine to be synthesized normally in the adrenal medulla. Activity of phenylethanolamine N-methyl transferase, the enzyme that converts norepinephrine to epinephrine, depends on intraadrenal cortisol.²⁶⁷

Cortisol deficiency increases sensitivity to insulin; for example, an increased frequency of hypoglycemia and diminishing insulin requirement are characteristic manifestations of Addison disease in type 1 diabetes.²⁶⁸

Fasting hypoglycemia can occur in patients with both primary and secondary glucocorticoid deficiency and is more common in neonates, infants, and young children than in adolescents and adults, and in those with more severe cortisol deficiency.²⁶⁹ Hypoglycemia is most likely to occur after prolonged fasting or during the stress of illness, especially if the usual daily glucocorticoid replacement has been interrupted by illness.^{270,271}

ACTH deficiency combined with GH deficiency, especially in congenital hypopituitarism, is more likely to cause hypoglycemia than primary adrenal insufficiency. Congenital ACTH deficiency, presenting with early hypoglycemia, has been reported with mutations of several genes involved in pituitary development (*POU1F1*, *PROPI*, *TPIT*).²⁶¹ ACTH insufficiency is not always clinically apparent in early infancy but may manifest later in childhood.

An increasing number of specific genetic defects resulting in isolated ACTH deficiency or resistance have been identified. Red hair and severe early-onset obesity are characteristic features of children with ACTH deficiency caused by proopiomelanocortin (POMC) mutations.²⁷² Hypoglycemia in an infant caused by ACTH deficiency has been attributed to a defect of prohormone cleavage.²⁷³ Idiopathic ACTH deficiency can be acquired in childhood, adolescence, or later adult life.²⁷⁴ Circumstantial evidence, such as an association with autoimmune thyroiditis, suggests autoimmune hypophysitis as a common cause and in some instances antipituitary antibodies have been demonstrated.²⁷⁵ In ACTH resistance, hyperpigmentation accompanies other manifestations of adrenal insufficiency.²⁷⁶

Iatrogenic suppression of adrenal function is a common cause of glucocorticoid insufficiency, but it may be overlooked because the typical electrolyte abnormalities of mineralocorticoid deficiency are absent. An adrenal crisis with prostration, vomiting, hypotension, and hypoglycemia can be triggered by a stressful event, in a child recently weaned from high-dose glucocorticoid therapy, and has also been reported on alternate day low-dose therapy. More often, hypoglycemia is caused by inhaled and nasal glucocorticoid preparations that are the mainstay of asthma and allergy treatment for millions of children.²⁷⁷ A serum cortisol level of less than 3 mcg/dL between 7 and 9 AM in a child receiving topical or inhaled glucocorticoids is diagnostic of adrenal insufficiency; if the level is 3

mcg/dL or higher, an ACTH stimulation test is required to evaluate adrenal function.²⁷⁷

Hypoglycemia may be a presenting feature of primary adrenal insufficiency,²⁷⁸ and often occurs during illness or starvation in patients being treated with replacement glucocorticoids.^{269,270} Primary adrenal insufficiency is associated with deficient epinephrine responses to hypoglycemia, and hypoglycemia manifested by neuroglycopenic rather than adrenergic symptoms may be less recognizable.²⁷⁹ Hypoglycemia is relatively frequent in treated patients with congenital adrenal hyperplasia.^{269,280}

As has been noted earlier for GH deficiency, the diagnosis of cortisol or ACTH deficiency, as the cause of a child's hypoglycemia, cannot be based on a single low cortisol value during hypoglycemia, and requires additional clinical evidence (such as a concomitant markedly elevated plasma ACTH concentration in the case of primary adrenal insufficiency) or a standard adrenal stimulation test.⁷²

Deficiency of Glucagon and Epinephrine

The importance of glucagon and epinephrine in the counterregulatory responses to hypoglycemia suggests that isolated deficiency of either hormone would be likely to cause hypoglycemia, and yet no instances of hypoglycemia caused by isolated, primary deficiency of either hormone have been well documented in children.

Although there have been case reports of reduced epinephrine excretion in patients with hypoglycemia, evidence from patients with type 1 diabetes and insulinomas suggests that reduced epinephrine excretion is likely to be the consequence rather than the cause of recurrent hypoglycemia. The defective epinephrine response that accompanies adrenal insufficiency can be at least partly restored by glucocorticoid replacement. Severe fasting hypoglycemia has been reported in a young child receiving the beta-blocker, propranolol.²⁸¹

Despite its crucial importance in acute glucose counterregulation, there have been no well-documented cases of childhood hypoglycemia caused by isolated glucagon deficiency. One case, often cited as an example of isolated glucagon deficiency, was subsequently proven to be an instance of familial SCHAD-HI.^{282,283}

Genetic Disorders of Gluconeogenesis

In the early postabsorptive period, the plasma glucose level is maintained by both glycogenolysis and gluconeogenesis. As fasting continues beyond 6 to 10 hours and liver glycogen is depleted, gluconeogenesis contributes an increasing proportion of circulating glucose (see Fig. 23.3). Alanine from muscle is a principal substrate for gluconeogenesis, but lactate derived from peripheral tissue glycolysis also contributes to glucose production via the Cori cycle, and in later stages of fasting glycerol, derived from lipolysis of adipose tissue triglycerides, becomes a major source of gluconeogenic substrate (FFA cannot be converted to glucose). Genetic defects of the four enzymes of gluconeogenesis required to bypass irreversible steps in glycolysis (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase [FDPase], and G6Pase) cause hypoglycemia, characterized by marked elevations of lactate (see Fig. 23.5; deficiency of G6Pase is described previously under the Glycogen Storage Disorders).

Pyruvate Carboxylase Deficiency

Pyruvate carboxylase is a biotin-containing protein consisting of four subunits that converts pyruvate to oxaloacetate, as the first step in the gluconeogenic pathway.²⁸⁴ Activation is dependent on acetyl CoA and occurs principally during mobilization

of FFA during fasting. Several forms of deficiency have been described, typically presenting as severe lactic acidosis and encephalopathy in early infancy, usually triggered by metabolic decompensation during illness.^{284,285} Hepatomegaly is common. Hypoglycemia is not invariably present in pyruvate carboxylase deficiency.^{286,287} Urine contains large amounts of alpha-ketoglutarate. The diagnosis can be confirmed by direct sequencing of the *PC* gene on 11q13. Management consists of frequent carbohydrate meals and intravenous dextrose support during illness.

Phosphoenolpyruvate Carboxykinase Deficiency

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate as the second key step in gluconeogenesis. Cytosolic and mitochondrial isoforms are encoded by *PEPCK1* and *PEPCK2*, respectively. Deficiencies of both forms have been reported in rare patients, but the clinical phenotype remains uncertain. It is only recently that patients with deficiency of the cytosolic form of PEPCK have been confirmed at the molecular level.²⁸⁸ These patients presented with intermittent episodes of hypoglycemia, without marked elevations of plasma lactate, but with increased urinary concentrations of tricarboxylic acid cycle intermediates. Several patients with deficiency of the mitochondrial form of PEPCK have been described based on enzymatic data, but have not been confirmed at the molecular level. These patients presented with hypotonia, hepatomegaly, failure to thrive, lactic acidosis, and hypoglycemia.

Fructose 1,6-Diphosphatase Deficiency

FDPase, or fructose 1,6-bisphosphatase, catalyzes the conversion of fructose 1,6-diphosphate to fructose-6-phosphate. FDPase is encoded by *FBP1* on chromosome 9q22.32. Deficiency of FDPase impairs the formation of glucose from lactate; glycerol; gluconeogenic amino acids, such as alanine; and fructose.

Hypoglycemia caused by FDPase deficiency was first described in 1970 by Baker and Winegrad.²⁸⁹ FDPase deficiency may present with hypoglycemia and lactic acidosis in the first days of life, but up to 50% of cases present later. Typical manifestations include hyperventilation because of lactic acidosis and ketoacidosis, seizures and coma from hypoglycemia, and hepatomegaly. Attacks are precipitated in infancy and childhood by prolonged fasting during intercurrent illness. Uric acid is elevated similar to type 1 GSD, secondary to sequestration of phosphate as fructose phosphates in the liver, and increased degradation of adenine nucleotides. The diagnosis should be suspected when fasting leads to hypoglycemia and lactic acidemia, with a poor glycemic response to glucagon. The diagnosis may be confirmed by genetic mutation analysis of *FBP1*.²⁹⁰ Treatment of acute attacks of hypoglycemia is with intravenous glucose and bicarbonate infusion. Chronic treatment is avoidance of prolonged fasting and a reduction, but not total elimination, of fructose from the diet.²⁹¹

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) was first described as an idiosyncrasy to ingestion of fructose,²⁹² and was later determined to be caused by recessive mutations of aldolase B (*ALDOB* at 9q31.1).^{293,294} Aldolase B plays an essential role in fructose metabolism by converting fructose-1-phosphate to dihydroxyacetone phosphate and glyceraldehyde, which are substrates for either gluconeogenesis via fructose 1,6-diphosphate or oxidation via conversion to pyruvate. Ingestion of fructose (e.g., as sucrose in fruit) in patients with aldolase B deficiency causes accumulation of fructose-1-phosphate in

liver, kidney, and intestinal cells, leading to intracellular phosphate depletion and both acute symptoms and potentially long-term organ damage. Phosphate depletion induces hypoglycemia by impairing glycogenolysis and, as noted earlier in G6Pase deficiency and FDPase deficiency, also causes hyperuricemia because of increased adenine nucleotide degradation.²⁹⁵ The hypoglycemia of HFI occurs immediately after fructose ingestion, rather than with fasting.

HFI presents in infancy, after fructose is introduced to the diet in fruits or as sucrose (table sugar). Fructose ingestion induces vomiting, abdominal pain, diarrhea, and hypoglycemia.²⁹⁶ With ingestion of substantial amounts of fructose, plasma lactic and uric acid levels rise and phosphorus, potassium, and bicarbonate levels fall. The severity of symptoms is proportional to the amount of fructose ingested; in early infancy, large amounts (e.g., in milk or formula made with sucrose) may produce shock, acute liver failure, and death. Older children are less likely to ingest toxic amounts because of abdominal pain induced by fructose. Acute treatment of hypoglycemia by intravenous glucose rapidly reverses symptoms and affected patients remain healthy if fructose exposure is discontinued. Chronic fructose ingestion causes failure to thrive and increasing liver dysfunction. The earliest renal effect is proximal tubular damage with glucosuria and phosphaturia; prolonged exposure to fructose can lead to kidney failure.

Fructose challenge is potentially dangerous and is not needed to make the diagnosis, because genetic mutation testing of *ALDOB* is available. Chronic treatment is the avoidance of all fructose-containing foods (fruits, sucrose, high-fructose corn syrup); because sorbitol is metabolized to fructose, it needs to be excluded as well.²⁹¹

Defects of Fatty Acid Oxidation

Fatty acid oxidation is the major source of energy for the heart and working skeletal muscle and, apart from the brain, becomes the body's dominant fuel during prolonged fasting. Lipolysis of adipose tissue triglyceride stores releases glycerol, as a substrate for hepatic gluconeogenesis, and FFA, which are used directly as fuel for peripheral tissues and, indirectly, to spare glucose consumption. FFA are converted by mitochondrial β -oxidation in liver to ketones (BOHB and acetoacetate), which are an alternative fuel for the brain. The key steps in transport of fatty acids into mitochondria, the β -oxidation cycle, and the ketone synthesis pathway are outlined in Chapter 7. Genetic disorders in all of these steps have been identified which, in general, present with life-threatening attacks of hypoketotic hypoglycemia, coma, and cardiovascular collapse induced by fasting stress. Defects interfering with early steps in long chain fatty acid oxidation tend to have more systemic features, including chronic cardiomyopathy and skeletal muscle weakness, whereas defects in later steps affecting shorter chain fatty acid oxidation, such as medium chain acyl-CoA dehydrogenase (MCAD) deficiency, may present chiefly with acute episodes of illness triggered by fasting. As a group, genetic disorders of fatty acid oxidation are relatively common (the incidence of MCAD deficiency is ~ 1 in 15,000 births in the United States, and 1 in 5000 in Northern Germany),²⁹⁷ and must be considered in the differential diagnosis of any child with hypoglycemia. Newborn screening programs exist in most countries, which can identify most of the genetic defects in fatty acid oxidation, based on analysis of acylcarnitine profiles (exceptions include HMG-CoA synthase deficiency). However, pediatric endocrinologists must also be able to recognize these fatty acid oxidation disorders, because many cases may present as new onset hypoglycemia at ages ranging from

the newborn period through infancy, and up to several years of age.

Hypoglycemia can manifest in the first days of life (see Chapter 7) in babies exposed to early fasting stress because of failed breastfeeding. However, typically, patients may not present until several months of age, when feeding intervals overnight lengthen to 8 to 12 hours of fasting. Genetic disorders of fatty acid oxidation are especially likely to become clinically apparent during the accelerated starvation of gastrointestinal illness. Because fatty acid oxidation disorders impair ketogenesis, hypoglycemia associated with these disorders is hypoketoneic; however, in contrast to HI disorders, plasma concentrations of FFA are increased. Acute attacks of illness may be accompanied by coma, fatty liver, and encephalopathy, which mimic Reye syndrome, and may not immediately respond to glucose.^{298,299} Episodes may be rapidly fatal and can resemble sudden infant death syndrome.³⁰⁰ Hypertrophic or dilated cardiomyopathy and skeletal muscle weakness are prominent features of certain defects, such as long chain acyl CoA dehydrogenase deficiency, carnitine transport deficiency, and carnitine-palmitoyl transferase 2 deficiencies.³⁰¹ In patients with primary carnitine deficiency caused by the plasma membrane carnitine transporter (*SLC22A5*), treatment with high-dose carnitine supplementation is required; the value of carnitine supplementation in other disorders is controversial.³⁰² Diagnosis can be based on an abnormal plasma acylcarnitine profile and confirmed by genetic mutation analysis.

The primary treatment of these disorders of fatty acid oxidation is avoidance of fasting. In younger children, fasting should be limited to less than 8 to 10 hours; older children may tolerate fasting as long as 10 to 12 hours. Episodes of acute illness with fasting should be treated by rapid institution of intravenous dextrose to raise plasma glucose to the high normal range.

Hypoglycemia Induced by Exogenous Agents

In children, hypoglycemia caused by exogenous agents usually represents accidental ingestions. Ethanol, salicylates, quinine, and beta-blockers, such as propranolol, are the agents most frequently implicated in childhood hypoglycemia.^{303,304}

Alcohol-Induced Hypoglycemia

Ethanol is a notorious cause of severe fasting hypoglycemia in young children and malnourished alcoholic adults, and occasionally in healthy adolescents who are intoxicated.³⁰⁵ Oxidation of ethanol in the liver causes an increase in the nicotinamide adenine dinucleotide (NAD):NAD⁺ ratio that impairs conversion of lactate to pyruvate and, thus, inhibits gluconeogenesis. This inhibition of gluconeogenesis can lead to hypoglycemia in the setting of fasting for long enough to deplete liver glycogen stores. Young children fasted overnight are particularly vulnerable, as are adolescents who have become intoxicated with alcohol without eating. The hypoglycemia is associated with lactic acidemia and hyperketonemia. Treatment with intravenous dextrose is required to reverse the hypoglycemia.

Salicylate Intoxication

In high doses, aspirin and other salicylates have been reported to cause hypoglycemia in both children and adults.³⁰⁶ Suggested mechanisms include stimulation of insulin release or inhibition of hepatic gluconeogenesis. Infants appear to be more susceptible than older children to salicylate-induced hypoglycemia. Because of concerns about a possible role of

salicylates in causing Reye syndrome, their routine use in young children has been largely abandoned.

Hypoglycin A and Jamaican Vomiting Sickness

Hypoglycemia caused by ingestion of unripe ackee fruit was first described as Jamaican vomiting sickness occurring in young children who were seeking potential foods because of undernutrition/starvation. The responsible toxin was identified as hypoglycin A, which is metabolized to a tricyclic compound that is a suicide-substrate for medium chain acyl-CoA dehydrogenase.⁷⁵ A similar illness has been recently linked to ingestion of unripe litchi fruit, which contains a related toxin (methylene cyclopropyl-glycine).⁷⁶ The toxins in both fruits produce a condition similar to MCAD deficiency (described earlier and in Chapter 7).

Hypoglycemia in Fasting, Starvation, Illness, and Stress

During prolonged fasting, FFA and ketones supply a progressively greater proportion of fuel and plasma glucose levels may fall below 50 mg/dL (2.8 mM), without autonomic or neuroglycopenic symptoms, or apparent ill effects because elevated levels of ketones provide adequate alternative fuel for the brain (see Fig. 23.2).^{16,25,307} This is especially likely to be seen in healthy young children and young women, because of their relatively smaller body stores of fuel and smaller body mass relative to brain size. Biochemical hypoglycemia, for example, is occasionally observed in younger children with excessive preoperative fasting.^{308,309}

The risk of biochemical, occasionally symptomatic, hypoglycemia is further increased in children during a wide variety of stressful illnesses, such as rewarming after profound hypothermia and near-drowning.^{310,311}

Hypoglycemia During Starvation and Malnutrition

Chronic malnutrition compromises the ability to maintain euglycemia, and hypoglycemia in malnourished infants and adults can be difficult to reverse.^{312,313} In African children with kwashiorkor, hypoglycemia has been reported to be a common terminal event.³¹⁴ In the developed world, anorexia nervosa is an important cause of severe malnutrition, and hypoglycemia is common and may occasionally be severe.^{315–318}

Hypoglycemia With Prolonged Exercise

Muscle uses glucose at a high rate during exercise; however hypoglycemia is normally prevented during prolonged exercise by a decrease in insulin secretion and increased secretion of glucagon and catecholamines.^{319–321} Nevertheless, hypoglycemia occasionally occurs during intense exercise, even in presumably healthy adults, and can be prevented by ingestion of complex carbohydrates, but not extra sugar.³²² Cases of hypoglycemia severe enough to cause seizures after a marathon or comparable endurance exercise have been reported.^{323,324} Exercise-induced HI because of promoter mutations of MCT1 (described previously) may explain at least some cases of hypoglycemia associated with exercise.³²⁵

Diarrheal Illness

Hypoglycemia is uncommon in previously healthy children with acute viral gastroenteritis, unless there has been a period of starvation (as occurs after prolonged consumption of water or sugar-free fluids) or of severe diarrhea.^{326,327} Hypoglycemia

in these settings is typically ketotic and has a good outcome. Because hypoglycemia is uncommon in otherwise healthy children, and previously unsuspected metabolic or hormonal disorders may present to an emergency department with hypoglycemia, it is important that a history of prolonged starvation be confirmed and that underlying conditions, especially the disorders of fatty acid oxidation, be ruled out.³²⁸

When diarrhea (e.g., cholera or shigella) develops in severely malnourished children, hypoglycemia is a grave prognostic sign and may be fatal because of depletion of gluconeogenic substrates and alternative fuels.³²⁷

Sepsis

Infectious diseases rarely cause hypoglycemia; indeed, hyperglycemia because of adrenergic stress responses is much more likely. However, septicemia, especially meningococemia (unrelated to adrenal insufficiency), may present with severe hypoglycemia.^{329,330} Research in animals suggests that cytokines, such as tumor necrosis factor may amplify glucose uptake.³³¹ Limited data in humans indicate appropriate suppression of insulin and elevation of counterregulatory hormones, as well as cytokines.³³²

Hypoglycemia in Specific Infections

Hypoglycemia has been reported to occur in approximately one-third of children with severe malaria and is a risk factor for increased mortality.³³³ Insulin levels are appropriately low, whereas lactate and alanine levels are high, suggesting that hepatic gluconeogenesis is impaired.³³⁴ Management should include provision of glucose and monitoring of plasma glucose concentrations. In addition, drugs for malaria treatment (in particular quinine) may aggravate hypoglycemia because of their ability to stimulate insulin release.³³⁵

Hypoglycemia has been repeatedly reported in infants with pertussis infection, which is caused by hyperinsulinemia and is not simply caused by fasting. Hyperinsulinemia, but without hypoglycemia, has also been shown to occur in mice after pertussis immunization.³³⁶ Evidence suggests that pertussis toxin amplifies the insulinogenic response to glucose rather than stimulating insulin secretion per se.^{337,338}

Hypoglycemia in Organ System Disease

Hypoglycemia is common in critically ill patients of all ages^{339,340} and can occur in failure or severe disease of nearly every major organ system. The liver is the principal source of glucose during the postabsorptive period, and under experimental conditions, hypoglycemia occurs after loss of 80% of the liver. Hypoglycemia in human liver disease is less predictable. In cirrhosis and progressive liver failure, glucose levels usually remain normal, even in patients with hepatic coma. However, fasting hypoglycemia can occur sporadically in many forms of liver disease, most likely caused by impaired glycogen reserves, with little dependence on the severity of liver impairment by other measures. Hypoglycemia has been reported in adults and children, with drug-induced hepatic injury, poisoning,³⁴¹ and in patients with infectious hepatitis³⁴² and other causes of hepatic injury.³⁴³ Hypoglycemia can also occur as a complication of various rare genetic diseases affecting the liver. For example, citrin deficiency has an intermediate, childhood-onset form characterized by growth retardation, episodic neurologic and behavioral abnormalities with hyperammonemia, and sometimes hypoglycemia.³⁴⁴

Renal gluconeogenesis also normally contributes to maintenance of blood glucose during fasting, and hypoglycemia may occur in patients with end-stage chronic renal failure.^{345–347} Because autonomic responses to hypoglycemia are often impaired in chronic renal failure, neuroglycopenic manifestations typically predominate. In many cases of hypoglycemia with renal disease, the hypoglycemia reflects a complication of dialysis or other procedures and the existence of a specific “uremic hypoglycemia” has been challenged.³⁴⁸

Many cases of fasting hypoglycemia associated with both acute and chronic pancreatitis have been reported in adults and children.^{349–351} Both hypoglycemia and hyperglycemia are recognized complications of mumps pancreatitis in children.³⁵²

The causes of hypoglycemia associated with severe heart disease, both cyanotic congenital heart disease in young children,³⁵³ and congestive heart failure in older children and adults^{354–356} are complex, with evidence of both increased glucose uptake and impaired gluconeogenesis. In infants, hypoglycemia can cause heart failure that improves with restoration of normal glucose levels.³⁵⁷

Skeletal muscle contributes substrates to gluconeogenesis during fasting, and fasting hypoglycemia occurs with several forms of muscular dystrophy and spinal muscle atrophy characterized by reduced muscle mass.^{358–361} A novel mechanism for hypoglycemia, resulting from intracranial disease, would be alteration of the afferent or efferent limbs of hypothalamic glucose sensing; this has been demonstrated in rodents and has been invoked in rare cases of hypoglycemia accompanying brain tumors and trauma.³⁶²

Hypoglycemia accompanied by lactic acidosis has been reported as both uncommon presentations and end-stage events in patients with acute and chronic leukemias, and lymphomas.³⁶³ Children on prolonged chemotherapy regimens have been found to have a high rate of fasting hypoglycemia caused by depleted glycogen and gluconeogenic precursors and to direct effects of oral purine analogs.^{364–366}

Hypoglycemia in the Intensive Care Unit

Hypoglycemia is common in critically ill children,³³⁹ is often asymptomatic and, especially when associated with increased glucose variability, is associated with poorer outcomes (increased morbidity, length of stay, and mortality rates).³⁶⁷ In addition to processes specific to the underlying diseases, other contributing factors may include substrate depletion, accelerated glucose consumption, undernutrition, impaired gluconeogenesis, cytokine effects, and adrenal insufficiency. Iatrogenic factors, such as misplaced infusion lines, changes in the rate of intravenous dextrose administration, or drug effects need to be considered.³⁶⁸ Abrupt discontinuation of parenteral nutrition may lead to hypoglycemia, particularly in children younger than 3 years of age; accordingly, a gradual taper of the infusion rate is recommended to reduce the risk of reactive hypoglycemia.³⁶⁸ Factors affecting reliability of glucose measurement (e.g., altered hematocrit, oxygenation, line draws, drugs) are more common in the intensive care unit (ICU).³⁶⁹ As noted earlier, it is important to not forget the possibility that an acute illness is unmasking a previously compensated disorder of glucose metabolism, especially in a young child.³²⁸

Insulin use aiming for tight glycemic control to improve outcomes in critically ill children has recently become the most common cause of hypoglycemia in the ICU.^{370,371} However, a recent multicenter randomized clinical trial showed that critically ill children with hyperglycemia did not benefit from tight control, 80 to 110 mg/dL, as compared with target plasma glucose levels of 150 to 180 mg/dL.³⁷²

Reactive Hypoglycemia and “Spells”

Reactive hypoglycemia refers to symptomatic hypoglycemia occurring 1 to 4 hours after a meal, and is rare in children, apart from those who have had gastric surgery (fundoplication for gastroesophageal reflux or gastric bypass surgery for obesity). However, children with symptoms attributed to hypoglycemia are not infrequently referred to endocrinology clinics. Whipple’s triad (symptoms typical of hypoglycemia, associated with a documented low plasma glucose level and relieved by treatment to raise glucose) is suggested by guidelines from the Pediatric Endocrinology Society and the Endocrine Society as necessary before entertaining a diagnosis hypoglycemia, because the symptoms of hypoglycemia are very nonspecific.^{63,66} Although home blood glucose meters may be helpful in testing the possibility of hypoglycemia, caution is needed in considering their use because of problems with accuracy of the results (see later in Artfactual Hypoglycemia). An OGTT should not be relied on to diagnose reactive hypoglycemia, because many normal individuals develop asymptomatic low glucose 2 to 4 hours after the glucose load. Minor dietary modifications to avoid high glycemic index foods and inclusion of foods that are more slowly digested, together with protein and fat, are usually sufficient to control symptoms in reactive hypoglycemia.

Artfactual Hypoglycemia

A low plasma glucose level discovered unexpectedly in an apparently “well” child, who had an outpatient chemistry profile for reasons unrelated to carbohydrate metabolism, is most often a spurious result. The most common cause of artfactual hypoglycemia is improper handling of samples before their arrival in the laboratory. If blood is drawn and allowed to stand, glucose is consumed by both erythrocytes and leukocytes.³⁷³ If a whole blood sample is allowed to clot at a room temperature, the serum glucose concentration may decrease by 7 to 20 mg/dL/h. The rate of glycolysis is temperature dependent and immediate refrigeration of samples reduces but does not completely eliminate glucose consumption.³⁷⁴ Use of collection tubes that separate cells from serum also prevents glycolysis. Fluoride inhibits but does not eliminate glycolysis, and glucose levels may decrease by 20% or more when processing is delayed for even a few hours.³⁷⁵ Postphlebotomy glycolysis consumes a relatively higher proportion of glucose when the specimen contains a low glucose level at the time it was obtained, and spurious hypoglycemia is more common when either the red cell or white cell counts are elevated, and has also been described in a hemolytic crisis with a high count of nucleated red blood cells.^{376,377}

In 2013 the International Organization for Standardization (ISO) stated that blood glucose meters are acceptably accurate if 95% or more of measurement results are within ± 15 mg/dL or $\pm 15\%$ (whichever is larger) of reference values. Thus according to ISO, if the true blood glucose is 60 mg/dL, measured glucose could be 45 to 75 mg/dL. The 2014 US Food and Drug Administration (FDA) guide for over-the-counter blood glucose meters requires 95% or more of results to be within $\pm 15\%$ of reference values and 99% or more of results within $\pm 20\%$ across the whole glycemic range. Based on the FDA criteria, if the true blood glucose is 60 mg/dL, measured glucose would be considered acceptably accurate in the range of 51 to 69 mg/dL.³⁷⁸ Recent evaluations of commercially available blood glucose meters show considerable differences in performance in the low glucose range.^{379–382} Moreover, accuracy in the low glucose range varies considerably among meters; for example, one study showed that when the reference plasma glucose was less than 70 mg/dL, mean absolute differences of

various meters ranged from 2 to 11 mg/dL and mean absolute relative differences (MARD) ranged from 4.3% to 24.2%.³⁷⁹ In another study, MARD ranged from 8.9% to 39.2%, when reference plasma glucose was less than 70 mg/dL.³⁸²

Continuous glucose monitoring (CGM) devices are increasingly being used to track patterns of glucose, but absolute nadirs may be inaccurate and their usefulness in evaluating hypoglycemia is still uncertain.^{383,384} There are concerns about accuracy of CGM at low glucose concentrations; nonetheless, in one study using a glucose monitoring system, a majority of parents of children with congenital HI found the glucose trend to be useful.³⁸⁵ CGM has also been used to signal impending hypoglycemia in studies of tight glycemic control, in critically ill children,^{372,386} and although potentially useful, their use has not been systematically evaluated in the diagnosis and management of diverse hypoglycemia disorders. Several reports have suggested that use of CGM may decrease the frequency and duration of hypoglycemia in high-risk newborn infants.^{384,387,388} Trials are needed in patients with congenital HI before CGM can be recommended as a routine alternative method for monitoring glucose levels during treatment. There are also no data on the use of CGM for diagnosis of hypoglycemia, such as using it in the outpatient setting as an alternative to an inpatient fasting test.

Despite the aforementioned considerations, it is important to stress that unexpected low glucose levels should not be ignored because of the possibility that a “fluke” laboratory test may detect an unrecognized chronic hypoglycemic condition.

EMERGENCY TREATMENT OF HYPOGLYCEMIA

Once the critical sample has been obtained, a minibolus of 0.2 g/kg of dextrose should be administered by intravenous infusion over 1 minute (2 mL/kg of 10% dextrose). This should be followed by a continuous intravenous infusion of 8 mg/kg/min using dextrose 10% solution. This rate of glucose administration may be rapidly and conveniently calculated by using the simple formula that 5 mL/kg/h of a 10% dextrose solution provides approximately 8 mg/kg/min of glucose. Glucose levels should be determined 15 minutes after the bolus has been given and while the maintenance glucose infusion is running. If hypoglycemia recurs, a bolus of 0.5 g/kg may be given (5 mL/kg of dextrose 10%) and the glucose infusion increased by 25% to 50%. For details of specific treatments of individual disorders, see the sections on each condition.

TABLE 23.5 Protocols for Fasting Studies

Type of Fast	Purpose	Fasting Duration Based on Age	Plasma Glucose and BOHB Monitoring	End of Fast
Diagnostic fast	Establish diagnosis underlying patient's hypoglycemia	<1 month: 18 h 1–12 months: 24 h >12 months: 36 h >13 years: 72 h	Bedside meter glucose and BOHB: Q3 until PG <70, then Q1 until <60, then Q30 min until <50 mg/dL	End fast for: duration, PG <50 mg/dL, BOHB \geq 2.0 mmol/L \times 2, or symptomatic ^a GST: when PG <50 mg/dL, give 1 mg glucagon IV/IM and then monitor PG at 10, 20, 30, 40 min (omit if BOHB >2 mmol/L)
Safety fast	Test efficacy of existing regimen	Diazoxide <1 month: 8–12 h; 1–12 month: 12 h; >1 year: 18 h Octreotide: 6–8 h (skip a feed) Lanreotide: 18 h if off dextrose; 12 h if on continuous intra-gastric dextrose	Bedside meter glucose and BOHB: Q3 until PG <70 mg/dL	End fast for: duration, PG confirmed <70 mg/dL, BOHB \geq 2.5–3 mmol/L \times 2
Cure fast	Prove resolution of disease in those who have received definitive therapy or are off of medication	<1 month: 18 h; 1–12 months: 24 h; >1 year: 36 h; >13 years: 48 h Insulinoma >13 years: 72 h	Bedside meter glucose and BOHB: Q3 until PG <70, then Q1 until <60, then Q30 min until <50 mg/dL	End fast for: duration, PG confirmed <50 mg/dL, BOHB \geq 3 mmol/L \times 2, or symptomatic Obtain HI draw: PG, FFA, BOHB, insulin, IGFBP-1, C-peptide GST: when PG <50 mg/dL (if BOHB <3 mmol/L), give 1 mg glucagon IV/IM and then monitor PG at 10, 20, 30, 40 min

^aAt the end of the diagnostic fast, obtain the critical sample. If PG <50 mg/dL and BOHB <1.5 mmol/L, this is most compatible with HI. Therefore **HI labs are the most important to draw first:** PG, FFA, BOHB, insulin (consider also C-peptide, cortisol, GH, ammonia, lactate, acylcarnitine profile, carnitine, IGFBP-1). A GST is indicated if HI is suspected.

If BOHB \geq 2 mmol/L at conclusion of fast, this suggests some form of ketotic hypoglycemia. Therefore ketotic hypoglycemia labs are important to draw first: PG, FFA, BOHB, cortisol, GH, lactate, acylcarnitine profile, carnitine, insulin, C-peptide, ammonia, IGFBP-1. GST is NOT indicated.

BOHB, Beta-hydroxybutyrate; FFA, free fatty acids; GST, glucagon stimulation test; HI, hyperinsulinemia; IGFBP-1, insulin-like growth factor binding protein 1; IM, intramuscular; IV, intravenous; PG, plasma glucose.

APPENDIX: USEFUL TESTS FOR DIAGNOSIS OF HYPOGLYCEMIA DISORDERS

Diagnostic Fasting Test: Maximum duration of fast is based on age: younger than 1 month 18 h, 1–12 months 24 h, 1–12 years 36 h, older than 13 years 72 h. Time intervals between the samples are based on the plasma glucose (PG) levels: monitor PG and plasma BOHB using respective bedside meters every 3 hours until PG is less than 70 mg/dL, then every hour until PG is less than 60 mg/dL, then every 30 minutes until PG is under 50 mg/dL. Ending criteria for fast include: maximum duration (based on patient age), plasma glucose confirmed less than 50 mg/dL, BOHB 2.0 (or more) mmol/L \times 2, or severely symptomatic. After confirming that PG is under 50 mg/dL (e.g., in STAT laboratory measurement), draw the critical blood samples for laboratory tests (monitoring at intermediate time points of insulin, lactate, BOHB, FFA is often useful, especially if acute changes might occur in response to patient developing adrenergic symptoms; Table 23.5).

The Critical Sample: refers to laboratory tests drawn at the time of a PG under 50 mg/dL to help determine the etiology of hypoglycemia. The critical sample may include a comprehensive metabolic panel, insulin, BOHB, FFA, lactate, GH, cortisol, C-peptide, ammonia, acylcarnitine profile, free and total carnitine, IGFBP-1, urine ketones, urine organic acids. Normal responses to fasting hypoglycemia (PG <50 mg/dL) in infants, children, and adults are similar: insulin less than 2 to 3 μ U/mL (lower limit of assay), lactate lower than 1.5 mmol/L, BOHB greater than 2.0 mmol/L, FFA greater than 1.0 mmol/L (see Ferrara et al.⁷¹)

Glucagon Stimulation Test: At the end of the fasting test, after the critical samples have been obtained (and if the fast was ended for a PG <50 mg/dL and BOHB <2.0 mmol/L), give 1 mg of glucagon intramuscularly or intravenously. PG on bedside meter should be 50 to 55 (or less) mg/dL before administering glucagon (time zero). Check PG at time zero, 10, 20, 30, and 40 minutes. If PG has not risen by more than 20 mg/dL after 20 minutes, consider terminating the study. A positive (abnormal) response is a rise in PG by at least 30 mg/dL within 40 minutes, indicating that excess insulin action restrained glycogen breakdown that was overcome by the action of the high dose of glucagon.^{71,389}

Oral Protein Tolerance Test for Protein-Sensitive Hyperinsulinism

- Patient must be fasting (nothing by mouth [NPO]) for more than 3 hours
- Verify patient allergies because protein powder contains cow milk protein (whey)
- Beneprotein protein powder 1.16 g/kg (mix protein powder in 4 ounces of Crystal Light)
- Administer protein drink by mouth or by nasogastric (NG) tube within less than 5 minutes
- Laboratory specimens: PG, insulin at times –15, 0, +15, +30, +45, +60, +90, +120, +150, and +180 minutes
- Terminate test if PG is less than 50 mg/dL during oral protein tolerance test
- Interpretation: Positive (abnormal) response is a fall in PG of more than 12 mg/dL and below 70 mg/dL.¹⁰⁵

Oral Glucose Tolerance Test for Postprandial Hypoglycemia

- Patient must be NPO 4 hours before the start of study

- Administer dextrose (e.g., Glucola) 1.75 g/kg PO within 15 to 20 minutes or via NG tube—maximum 75 g.
- Laboratory specimens at times –15, zero, +30, +60, +90, +120, +150, +180, +210, and +240 minutes for PG and insulin

Interpretation: Children with late dumping hypoglycemia caused by Nissen fundoplication have an exaggerated insulin increment ($220 \pm 310 \mu$ IU/mL [1610 ± 2250 pmol/L]), followed by a hypoglycemic nadir PG (47 ± 11 mg/dL [2.6 ± 0.6 mmol/L]) after oral glucose. They also usually, but not always, have an exaggerated rise in PG (180 ± 87 mg/dL [10 ± 4.9 mmol/L]) compared with controls: insulin increment $36 \pm 22 \mu$ IU/mL (255 ± 160 pmol/L); nadir PG 86 ± 21 mg/dL (4.8 ± 1.2 mmol/L); PG rise 58 ± 40 mg/dL (3.2 ± 2.2 mmol/L).¹⁷⁴

REFERENCES

1. Clarke DD, Sokoloff L. Circulation and energy metabolism of the brain. In: Siegel G, Agranoff B, Albers RW, Mielinoff P, eds. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. 5th ed. New York: Raven Press; 1994:645–680.
2. Cryer PE. Hypoglycemia, functional brain failure, and brain death. *J Clin Invest*. 2007;117(4):868–870.
3. Owen O, Morgan A, Kemp H, Sullivan J, Herrera M, Cahill Jr G. Brain metabolism during fasting. *J Clin Invest*. 1967;46:1589–1595.
4. Dalsgaard MK. Fuelling cerebral activity in exercising man. *J Cereb Blood Flow Metab*. 2006;26(6):731–750.
5. Fernandes J, Berger R, Smit G. Lactate as a cerebral metabolic fuel for glucose-6-phosphatase deficient children. *Pediatr Res*. 1984;18:335–339.
6. van de Ven KC, de Galan BE, van der Graaf M, Shestov AA, Henry PG, Tack CJ, et al. Effect of acute hypoglycemia on human cerebral glucose metabolism measured by ¹³C magnetic resonance spectroscopy. *Diabetes*. 2011;60(5):1467–1473.
7. van de Ven KC, van der Graaf M, Tack CJ, Heerschap A, de Galan BE. Steady-state brain glucose concentrations during hypoglycemia in healthy humans and patients with type 1 diabetes. *Diabetes*. 2012;61(8):1974–1977.
8. Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA, et al. Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideuteroglucose. *Diabetes*. 1977;26:1016–1023.
9. Haymond M, Karl I, Clarke W, Pagliara A, Santiago J. Differences in circulating gluconeogenic substrates during short-term fasting in men, women, and children. *Metabolism*. 1982;31:33–41.
10. Kaye R, Davidson MH, Williams ML, Kumagai M, Picou DM. The response of blood glucose, ketones, and plasma nonesterified fatty acids to fasting and epinephrine injection in infants and children. *J Pediatr*. 1961;59:836–847.
11. Stanley C, Baker L. Hyperinsulinism in infancy: diagnosis by demonstration of abnormal response to fasting hypoglycemia. *Pediatrics*. 1976;57:702–711.
12. van Veen MR, van Hasselt PM, de Sain-van der Velden MG, Verhoeven N, Hofstede FC, de Koning TJ, et al. Metabolic profiles in children during fasting. *Pediatrics*. 2011;127(4):e1021–e1027.
13. Chaussain J. Glycemic response to 24 hour fast in normal children and children with ketotic hypoglycemia. *J Pediatr*. 1973;82:438–443.
14. Chaussain J, Georges P, Olive G, Job J. Glycemic response to a 24-hour fast in normal children and children with ketotic hypoglycemia. II. Hormonal and metabolic changes. *J Pediatr*. 1974;85:776–781.
15. Chaussain J, Georges P, Calzada L, Job J. Glycemic response to 24-hour fast in normal children. III. Influence of age. *J Pediatr*. 1977;91:711–714.
16. Hojlund K, Wildner-Christensen M, Eshoj O, Skjaerbaek C, Holst JJ, Koldkjaer O, et al. Reference intervals for glucose, beta-cell polypeptides, and counterregulatory factors during prolonged fasting. *Am J Physiol Endocrinol Metab*. 2001;280(1):E50–E58.

17. Bonnefont J, Specola N, Vassault A, Lombes A, Ogier H, de Klerk J, et al. The fasting test in paediatrics: application to the diagnosis of pathological hypo- and hyperketotic states. *Eur J Paediatr*. 1990;150:80–85.
18. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. *J Clin Invest*. 1987;79(3):777–781.
19. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, et al. Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol*. 1991;260(1 Pt 1):E67–E74.
20. Fanelli C, Pampanelli S, Epifano L, Rambotti AM, Ciofetta M, Modarelli F, et al. Relative roles of insulin and hypoglycaemia on induction of neuroendocrine responses to, symptoms of, and deterioration of cognitive function in hypoglycaemia in male and female humans. *Diabetologia*. 1994;37(8):797–807.
21. Pagliara AS, Karl IE, De Vivo DC, Feigin RD, Kipnis DM. Hypoalaninemia: a concomitant of ketotic hypoglycemia. *J Clin Invest*. 1972;51(6):1440–1449.
22. Wolfsdorf J, Sadeghi-Nejad A, Senior B. Fat derived fuels during a 24 hour fast in children. *Eur J Paediatr*. 1982;138:141–144.
23. De Feo P, Perriello G, Torlone E, Ventura MM, Santeusano F, Brunetti P, et al. Demonstration of a role for growth hormone in glucose counterregulation. *Am J Physiol*. 1989;256(6 Pt 1):E835–E843.
24. Lamers KJ, Doesburg WH, Gabreels FJ, Lemmens WA, Romsom AC, Wevers RA, et al. The concentration of blood components related to fuel metabolism during prolonged fasting in children. *Clin Chim Acta*. 1985;152(1–2):155–163.
25. Lamers KJ, Doesburg WH, Gabreels FJ, Romsom AC, Renier WO, Wevers RA, et al. Reference values of blood components related to fuel metabolism in children after an overnight fast. *Clin Chim Acta*. 1985;145(1):17–26.
26. De Feo P, Perriello G, Torlone E, Ventura MM, Fanelli C, Santeusano F, et al. Contribution of cortisol to glucose counterregulation in humans. *Am J Physiol*. 1989;257(1 Pt 1):E35–E42.
27. Gosmanov NR, Szoke E, Israelian Z, Smith T, Cryer PE, Gerich JE, et al. Role of the decrement in intraislet insulin for the glucagon response to hypoglycemia in humans. *Diabetes Care*. 2005;28(5):1124–1131.
28. Cooperberg BA, Cryer PE. Insulin reciprocally regulates glucagon secretion in humans. *Diabetes*. 2010;59(11):2936–2940.
29. Hevener AL, Bergman RN, Donovan CM. Hypoglycemic detection does not occur in the hepatic artery or liver: findings consistent with a portal vein glucosensor locus. *Diabetes*. 2001;50(2):399–403.
30. McCrimmon RJ. Update in the CNS response to hypoglycemia. *J Clin Endocrinol Metab*. 2012;97(1):1–8.
31. Amiel S, Simonson D, Tamborlane W, DeFronzo R, Sherwin R. Rate of fall does not affect counterregulatory hormone responses to hypoglycemia in normal and diabetic humans. *Diabetes*. 1993;42(12):1791–1798.
32. Towler DA, Havlin CE, Craft S, Cryer P. Mechanism of awareness of hypoglycemia. Perception of neurogenic (predominantly cholinergic) rather than neuroglycopenic symptoms. *Diabetes*. 1993;42(12):1791–1798.
33. DeRosa MA, Cryer PE. Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab*. 2004;287(1):E32–E41.
34. Freinkel N, Metzger BE, Harris E, Robinson S, Mager M. The hypothermia of hypoglycemia. Studies with 2-deoxy-D-glucose in normal human subjects and mice. *N Engl J Med*. 1972;287(17):841–845.
35. Macdonald IA, Maggs DG. Cutaneous blood flow, sweating, tremor and temperature regulation in hypoglycaemia. In: Frier BM, Fisher BM, eds. *Hypoglycaemia and Diabetes: Clinical and Physiological Aspects*. London: Edward Arnold; 1993:132–143.
36. Gonder-Frederick L, Zrebiec J, Bauchowitz A, Lee J, Cox D, Ritterband L, et al. Detection of hypoglycemia by children with type 1 diabetes 6 to 11 years of age and their parents: a field study. *Pediatrics*. 2008;121(3):e489–e495.
37. Davis SN, Shavers C, Costa F. Differential gender responses to hypoglycemia are due to alterations in CNS drive and not glycaemic thresholds. *Am J Physiol Endocrinol Metab*. 2000;279(5):E1054–E1063.
38. Jones TW, Boulware SD, Kraemer DT, Caprio S, Sherwin RS, Tamborlane WV. Independent effects of youth and poor diabetes control on responses to hypoglycemia in children. *Diabetes*. 1991;40(3):358–363.
39. Galassetti P, Neill AR, Tate D, Ertl AC, Wasserman DH, Davis SN. Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *J Clin Endocrinol Metab*. 2001;86(8):3516–3524.
40. Jones TW, Porter P, Sherwin RS, Davis EA, O'Leary P, Frazer F, et al. Decreased epinephrine responses to hypoglycemia during sleep. *N Engl J Med*. 1998;338(23):1657–1662.
41. Fujii S, Tamai H, Kumai M, Takaichi Y, Nakagawa T, Aoki TT. Impaired glucagon secretion to insulin-induced hypoglycemia in anorexia nervosa. *Acta Endocrinol (Copenh)*. 1989;120(5):610–615.
42. Kerr D, Sherwin RS, Pavalkis F, Fayad PB, Sikorski L, Rife F, et al. Effect of caffeine on the recognition of and responses to hypoglycemia in humans. *Ann Intern Med*. 1993;119(8):799–804.
43. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes*. 1991;40(2):223–226.
44. Robinson AM, Parkin HM, Macdonald IA, Tattersall RB. Antecedent hypoglycaemia in non-diabetic subjects reduces the adrenaline response for 6 days but does not affect the catecholamine response to other stimuli. *Clin Sci (Lond)*. 1995;89(4):359–366.
45. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. *N Engl J Med*. 2013;369(4):362–372.
46. Mitrakou A, Fanelli C, Veneman T, Perriello G, Calderone S, Platanisiotis D, et al. Reversibility of unawareness of hypoglycemia in patients with insulinomas. *N Engl J Med*. 1993;329(12):834–839.
47. Hussain K, Bryan J, Christesen HT, Brusgaard K, Aguilar-Bryan L. Serum glucagon counterregulatory hormonal response to hypoglycemia is blunted in congenital hyperinsulinism. *Diabetes*. 2005;54(10):2946–2951.
48. Boyle PJ, Schwartz NS, Shah SD, Clutter WE, Cryer PE. Plasma glucose concentrations at the onset of hypoglycemic symptoms in patients with poorly controlled diabetes and in nondiabetics. *N Engl J Med*. 1988;318(23):1487–1492.
49. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes*. 1988;37:901–907.
50. Rankins D, Wellard RM, Cameron F, McDonnell C, Northam E. The impact of acute hypoglycemia on neuropsychological and neurometabolite profiles in children with type 1 diabetes. *Diabetes Care*. 2005;28(11):2771–2773.
51. Suh SW, Hamby AM, Swanson RA. Hypoglycemia, brain energetics, and hypoglycemic neuronal death. *Glia*. 2007;55(12):1280–1286.
52. Kinnala A, Rikalainen H, Lapinleimu H, Parkkola R, Kormanen M, Kero P. Cerebral magnetic resonance imaging and ultrasonography findings after neonatal hypoglycemia. *Pediatrics*. 1999;103(4 Pt 1):724–729.
53. Sharma P, Eesa M, Scott JN. Toxic and acquired metabolic encephalopathies: MRI appearance. *AJR Am J Roentgenol*. 2009;193(3):879–886.
54. Auer RN. Hypoglycemic brain damage. *Forensic Sci Int*. 2004;146(2–3):105–110.
55. Langan SJ, Deary IJ, Hepburn DA, Frier BM. Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. *Diabetologia*. 1991;34(5):337–344.
56. Menni F, de Lonlay P, Sevin C, Touati G, Peigne C, Barbier V, et al. Neurologic outcomes of 90 neonates and infants with persistent hyperinsulinemic hypoglycemia. *Pediatrics*. 2001;107(3):476–479.
57. Meissner T, Wendel U, Burgard P, Schaetzle S, Mayatepek E. Long-term follow-up of 114 patients with congenital hyperinsulinism. *Eur J Endocrinol*. 2003;149(1):43–51.
58. Steinkrauss L, Lipman TH, Hendell CD, Gerdes M, Thornton PS, Stanley CA. Effects of hypoglycemia on developmental outcome

- in children with congenital hyperinsulinism. *J Pediatr Nurs*. 2005;20(2):109–118.
59. Hershey T, Perantie DC, Warren SL, Zimmerman EC, Sadler M, White NH. Frequency and timing of severe hypoglycemia affects spatial memory in children with type 1 diabetes. *Diabetes Care*. 2005;28(10):2372–2377.
 60. Boluyt N, van Kempen A, Offringa M. Neurodevelopment after neonatal hypoglycemia: a systematic review and design of an optimal future study. *Pediatrics*. 2006;117(6):2231–2243.
 61. Avatapalle HB, Banerjee I, Shah S, Pryce M, Nicholson J, Rigby L, et al. Abnormal neurodevelopmental outcomes are common in children with transient congenital hyperinsulinism. *Front Endocrinol (Lausanne)*. 2013;4:60.
 62. Ludwig A, Enke S, Heindorf J, Empting S, Meissner T, Mohnike K. Formal neurocognitive testing in 60 patients with congenital hyperinsulinism. *Horm Res Paediatr*. 2018;89(1):1–6.
 63. Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2009;94(3):709–728.
 64. Beardsall K. Measurement of glucose levels in the newborn. *Early Hum Dev*. 2010;86(5):263–267.
 65. Whipple AO. The surgical therapy of hyperinsulinism. *J Int Chir*. 1938;3:237–276.
 66. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations from the pediatric endocrine society for evaluation and management of persistent hypoglycemia in neonates, infants, and children. *J Pediatr*. 2015;167(2):238–245.
 67. Davis MR, Shamooh H. Counterregulatory adaptation to recurrent hypoglycemia in normal humans. *J Clin Endocrinol Metab*. 1991;73(5):995–1001.
 68. Davis SN, Shavers C, Mosqueda-Garcia R, Costa F. Effects of differing antecedent hypoglycemia on subsequent counterregulation in normal humans. *Diabetes*. 1997;46(8):1328–1335.
 69. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C. Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci U S A*. 1994;91(20):9352–9356.
 70. Davis MR, Shamooh H. Deficient counterregulatory hormone responses during hypoglycemia in a patient with insulinoma. *J Clin Endocrinol Metab*. 1991;72(4):788–792.
 71. Ferrara C, Patel P, Becker S, Stanley CA, Kelly A. Biomarkers of insulin for the diagnosis of hyperinsulinemic hypoglycemia in infants and children. *J Pediatr*. 2016;168:212–219.
 72. Kelly A, Tang R, Becker S, Stanley CA. Poor specificity of low growth hormone and cortisol levels during fasting hypoglycemia for the diagnoses of growth hormone deficiency and adrenal insufficiency. *Pediatrics*. 2008;122(3):e522–e528.
 73. Stanley CA. Hyperinsulinism/hyperammonemia syndrome: insights into the regulatory role of glutamate dehydrogenase in ammonia metabolism. *Mol Genet Metab*. 2004;81(Suppl 1):S45–S51.
 74. Levitt Katz L, Satin-Smith M, Collett-Solberg P, Thornton P, Baker L, Stanley C, et al. Insulin-like growth factor binding protein-1 levels in the diagnosis of hypoglycemia due to hyperinsulinism. *J Pediatr*. 1997;131:193–199.
 75. Tanaka K, Kean EA, Johnson B. Jamaican vomiting sickness. Biochemical investigation of two cases. *N Engl J Med*. 1976;295(9):461–467.
 76. Shrivastava A, Kumar A, Thomas JD, Laserson KF, Bhushan G, Carter MD, et al. Association of acute toxic encephalopathy with litchi consumption in an outbreak in Muzaffarpur, India, 2014: a case-control study. *Lancet Glob Health*. 2017;5(4):e458–e466.
 77. Dekelbab BH, Sperling MA. Hypoglycemia in newborns and infants. *Adv Pediatr*. 2006;53:5–22.
 78. De Leon DD, Stanley CA. Mechanisms of disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab*. 2007;3(1):57–68.
 79. Stanley CA, De Leon DD, eds. *Monogenic Disorders of Insulin Secretion: Congenital Hyperinsulinism and Neonatal Diabetes March 15–16, 2012*. 2012. (synopses & abstracts). Pediatric Diabetes.
 80. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. *J Clin Endocrinol Metab*. 2016;101(3):815–826.
 81. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, et al. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. *J Clin Invest*. 2008;118(8):2877–2886.
 82. Palladino AA, Bennett MJ, Stanley CA. Hyperinsulinism in infancy and childhood: when an insulin level is not always enough. *Clin Chem*. 2008;54(2):256–263.
 83. Chevenne D, Trivin F, Porquet D. Insulin assays and reference values. *Diabetes Metab*. 1999;25(6):459–476.
 84. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab*. 2013;98(2):E355–E363.
 85. Thomas P, Cote G, Wohlik N, Haddad B, Mathew P, Rabi W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science*. 1995;268:426–429.
 86. Thomas P, Ye Y, Lightner E. Mutations of the pancreatic islet inward rectifier Kir6.2 also leads to familial persistent hyperinsulinemic hypoglycemia of infancy. *Hum Mol Genet*. 1996;5:1809–1812.
 87. Nestorowicz A, Wilson B, Schoor K, et al. Mutations in the sulfonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Hum Mol Genet*. 1996;5:1813–1822.
 88. Nestorowicz A, Inagaki N, Gono T, Schoor KP, Wilson BA, Glaser B, et al. A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes*. 1997;46(11):1743–1748.
 89. de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, et al. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *J Clin Invest*. 1997;100(4):802–807.
 90. Suchi M, MacMullen CM, Thornton PS, Adzick NS, Ganguly A, Ruchelli ED, et al. Molecular and immunohistochemical analyses of the focal form of congenital hyperinsulinism. *Mod Pathol*. 2006;19(1):122–129.
 91. Hardy OT, Hernandez-Pampaloni M, Saffer, JR, Suchi, M., Ruchelli, E., Zhuang, H., et al. Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodopa PET scan. *J Pediatr*. 150(2): 140–145.
 92. de Lonlay P, Giurgea I, Sempoux C, Touati G, Jaubert F, Rahier J, et al. Dominantly inherited hyperinsulinemic hypoglycaemia. *J Inherit Metab Dis*. 2005;28(3):267–276.
 93. Thornton P, Alter C, Levitt Katz L, Baler L, Stanley C. Short- and long-term use of octreotide in the treatment of congenital hyperinsulinism. *J Pediatr*. 1993;123:637–643.
 94. Le Quan Sang KH, Arnoux JB, Mamoune A, Saint-Martin C, Bellanne-Chantelot C, Valayannopoulos V, et al. Successful treatment of congenital hyperinsulinism with long-acting release octreotide. *Eur J Endocrinol*. 2012;166(2):333–339.
 95. Aynsley-Green A, Hussain K, Hall J, Saudubray JM, Nihoul-Fekete C, De Lonlay-Debeney P, et al. Practical management of hyperinsulinism in infancy. *Arch Dis Child Fetal Neonatal Ed*. 2000;82(2):F98–F107.
 96. de Lonlay-Debeney P, Poggi-Travert F, Fournet JC, Sempoux C, Vici CD, Brunelle F, et al. Clinical features of 52 neonates with hyperinsulinism. *N Engl J Med*. 1999;340(15):1169–1175.
 97. Bertrand J, Caquard M, Arnoux JB, Laborde K, Velho G, Verkarre V, et al. Glucose metabolism in 105 children and adolescents after pancreatectomy for congenital hyperinsulinism. *Diabetes Care*. 2012;35(2):198–203.
 98. Laje P, States LJ, Zhuang H, Becker SA, Palladino AA, Stanley CA, et al. Accuracy of PET/CT Scan in the diagnosis of the focal form of congenital hyperinsulinism. *J Pediatr Surg*. 2013;48(2):388–393.
 99. Adzick NS, De Leon DD, States LJ, Lord K, Bhatti TR, Becker SA, et al. Surgical treatment of congenital hyperinsulinism: Results from 500 pancreatectomies in neonates and children. *J Pediatr Surg*. 2019;54(1):27–32.
 100. Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, et al. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med*. 1998;338(19):1352–1357.

101. Treberg JR, Clow KA, Greene KA, Brosnan ME, Brosnan JT. Systemic activation of glutamate dehydrogenase increases renal ammoniogenesis: implications for the hyperinsulinism/hyperammonemia syndrome. *Am J Physiol Endocrinol Metab.* 2010;298(6):E1219–E1225.
102. Weinzimer S, Stanley C, Berry G, Yudkoff M, Tuchman M, Thornton P. A syndrome of congenital hyperinsulinism and hyperammonemia. *J Pediatr.* 1997;130:661–664.
103. Zammarchi E, Filippi L, Novembre E, Donati M. Biochemical evaluation of a patient with a familial form of leucine-sensitive hypoglycemia and concomitant hyperammonemia. *Metabolism.* 1996;45:957–960.
104. Raizen DM, Brooks-Kayal A, Steinkrauss L, Tennekoon GI, Stanley CA, Kelly A. Central nervous system hyperexcitability associated with glutamate dehydrogenase gain of function mutations. *J Pediatr.* 2005;146(3):388–394.
105. Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr.* 2001;138(3):383–389.
106. Kelly A, Ng D, Ferry Jr RJ, Grimberg A, Koo-McCoy S, Thornton PS, et al. Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab.* 2001;86(8):3724–3728.
107. Treberg JR, Brosnan ME, Watford M, Brosnan JT. On the reversibility of glutamate dehydrogenase and the source of hyperammonemia in the hyperinsulinism/hyperammonemia syndrome. *Adv Enzyme Regul.* 2010;50(1):34–43.
108. Huijman JG, Duran M, de Klerk JB, Rovers MJ, Scholte HR. Functional hyperactivity of hepatic glutamate dehydrogenase as a cause of the hyperinsulinism/hyperammonemia syndrome: effect of treatment. *Pediatrics.* 2000;106(3):596–600.
109. Matschinsky FM, Glaser B, Magnuson MA. Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities. *Diabetes.* 1998;47(3):307–315.
110. Gloyn AL. Glucokinase (GCK) mutations in hyper- and hypoglycemia: maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemia of infancy. *Hum Mutat.* 2003;22(5):353–362.
111. Sayed S, Matschinsky F, Stanley C. Hyperinsulinism due to activating mutations of glucokinase. In: Stanley C, De Leon D, eds. *Monogenic Hyperinsulinemic Hypoglycemia Disorders.* Frontiers in Diabetes. Karger; Basel; 2012:146–157.
112. Sayed S, Langdon DR, Odili S, Chen P, Buettger C, Schiffman AB, et al. Extremes of clinical and enzymatic phenotypes in children with hyperinsulinism caused by glucokinase activating mutations. *Diabetes.* 2009;58(6):1419–1427.
113. Cuesta-Munoz AL, Huopio H, Otonkoski T, Gomez-Zumaquero JM, Nanto-Salonen K, Rahier J, et al. Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. *Diabetes.* 2004;53(8):2164–2168.
114. Maiorana A, Manganozzi L, Barbetti F, Bernabei S, Gallo G, Cusmai R, et al. Ketogenic diet in a patient with congenital hyperinsulinism: a novel approach to prevent brain damage. *Orphanet J Rare Dis.* 2015;10: 120.
115. Quintens R, Hendrickx N, Lemaire K, Schuit F. Why expression of some genes is disallowed in beta-cells. *Biochem Soc Trans.* 2008;36 (Pt 3):300–305.
116. Pinney SE, Ganapathy K, Bradfield J, Stokes D, Sasson A, Mackiewicz K, et al. Dominant form of congenital hyperinsulinism maps to HK1 region on 10q. *Horm Res Paediatr.* 2013;80(1):18–27.
117. McQuarrie I. Idiopathic spontaneously occurring hypoglycemia in infants; clinical significance of problem and treatment. *AMA Am J Dis Child.* 1954;87(4):399–428.
118. Henquin JC, Sempoux C, Marchandise J, Godecharles S, Guiot Y, Nenquin M, et al. Congenital hyperinsulinism caused by hexokinase I expression or glucokinase-activating mutation in a subset of beta-cells. *Diabetes.* 2013;62(5):1689–1696.
119. Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE, et al. Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. *Am J Hum Genet.* 2007;81(3):467–474.
120. Otonkoski T, Meissner T. Exercise-induced hyperinsulinism: a failure of monocarboxylate transporter 1 expression silencing. In: DeLeon DD, Stanley CA, eds. *Monogenic Hyperinsulinemic Hypoglycemia Disorders.* Frontiers in Diabetes. Karger; 2012:172–181.
121. Narayan SB, Master SR, Sireci AN, Bierl C, Stanley PE, Li C, et al. Short-chain 3-hydroxyacyl-coenzyme A dehydrogenase associates with a protein super-complex integrating multiple metabolic pathways. *PLoS One.* 2012;7(4):e35048.
122. Li C, Chen P, Palladino A, Narayan S, Russell LK, Sayed S, et al. Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem.* 2010;285(41):31806–31818.
123. Kapoor RR, Locke J, Colclough K, Wales J, Conn JJ, Hattersley AT, et al. Persistent hyperinsulinemic hypoglycemia and maturity-onset diabetes of the young due to heterozygous HNF4A mutations. *Diabetes.* 2008;57(6):1659–1663.
124. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, et al. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med.* 2007;4(4):e118.
125. Pingul MM, Hughes N, Wu A, Stanley CA, Gruppuso PA. Hepatocyte nuclear factor 4alpha gene mutation associated with familial neonatal hyperinsulinism and maturity-onset diabetes of the young. *J Pediatr.* 2011;158(5):852–854.
126. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab.* 2012;97(10):E2026–E2030.
127. Rozenkova K, Malikova J, Nessa A, Dusatkova L, Bjorkhaug L, Obermannova B, et al. High incidence of heterozygous ABC8 and HNF1A mutations in Czech patients with congenital hyperinsulinism. *J Clin Endocrinol Metab.* 2015;100(12):E1540–E1549.
128. Tung JY, Boodhansingh K, Stanley CA, De Leon DD. Clinical heterogeneity of hyperinsulinism due to HNF1A and HNF4A mutations. *Pediatr Diabetes.* 2018;19(5):910–916.
129. Ferrara CT, Boodhansingh KE, Paradies E, Giuseppe F, Steinkrauss LJ, Topor LS, et al. Novel hypoglycemia phenotype in congenital hyperinsulinism due to dominant mutations of uncoupling protein 2. *J Clin Endocrinol Metab.* 2017;102(3):942–949.
130. Meissner T, Friedmann B, Okun JG, Schwab MA, Otonkoski T, Bauer T, et al. Massive insulin secretion in response to anaerobic exercise in exercise-induced hyperinsulinism. *Horm Metab Res.* 2005;37(11):690–694.
131. Kalish J, Boodhansingh K, Bhatti T, Ganguly A, Conlin L, Becker S, et al. Congenital hyperinsulinism in children with paternal 11p uniparental isodisomy and Beckwith-Wiedemann syndrome. *J Med Genet.* 2016;53(1):53–61.
132. Bogershausen N, Gatinos V, Riehmer V, Kayserli H, Becker J, Thoenes M, et al. Mutation update for Kabuki syndrome genes KMT2D and KDM6A and further delineation of X-linked Kabuki syndrome subtype 2. *Hum Mutat.* 2016;37(9):847–864.
133. Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, et al. Congenital hyperinsulinism in infants with Turner Syndrome: possible association with monosomy X and KDM6A haploinsufficiency. *Horm Res Paediatr.* 2018;1–10.
134. Freeze HH. Genetic defects in the human glycome. *Nat Rev Genet.* 2006;7:537.
135. Ng B, Freeze H. Perspectives on glycosylation and its congenital disorders. *Trend Genet.* 2018;34(6):466–476.
136. Böhles H, Sewell AC, Gebhardt B, Reinecke-Lüthge A, Klöppel G, Marquardt T. Hyperinsulinaemic hypoglycaemia—Leading symptom in a patient with congenital disorder of glycosylation Ia (phosphomannomutase deficiency). *J Inherit Metab Dis.* 2001;24(8):858–862.
137. de Lonlay P, Cueur M, Vuillaumier-Barrot S, Beaune G, Castelnau P, Kretz M, et al. Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: A new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. *J Pediatr.* 1999;135(3):379–383.
138. Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. Congenital disorder of glycosylation Id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. *J Clin Endocrinol Metab.* 2005;90(7):4371–4375.

139. Enns GM, Steiner RD, Buist N, Cowan C, Leppig KA, McCracken MF, et al. Clinical and molecular features of congenital disorder of glycosylation in patients with type 1 sialotransferrin pattern and diverse ethnic origins. *J Pediatr*. 2002;141(5):695–700.
140. Babovic-Vuksanovic D, Patterson MC, Schwenk WF, O'Brien JF, Vockley J, Freeze HH, et al. Severe hypoglycemia as a presenting symptom of carbohydrate-deficient glycoprotein syndrome. *J Pediatr*. 1999;135(6):775–781.
141. de Lonlay P, Seta N. The clinical spectrum of phosphomannose isomerase deficiency, with an evaluation of mannose treatment for CDG-Ib. *Biochim Biophys Acta*. 2009;1792(9):841–843.
142. Harms H, Zimmer K-P, Kurnik K, Bertele-Harms R, Weidinger S, Reiter K. Oral mannose therapy persistently corrects the severe clinical symptoms and biochemical abnormalities of phosphomannose isomerase deficiency. *Acta Paediatr*. 2002;91(10):1065–1072.
143. Tegtmeier LC, Rust S, van Scherpenzeel M, Ng BG, Losfeld ME, Timal S, et al. Multiple phenotypes in phosphoglucomutase 1 deficiency. *N Engl J Med*. 2014;370(6):533–542.
144. Wong SY, Gadomski T, van Scherpenzeel M, Honzik T, Hansikova H, Holmefford KSB, et al. Oral D-galactose supplementation in PGM1-CDG. *Genet Med*. 2017;19(11):1226–1235.
145. Hussain K, Challis B, Rocha N, Payne F, Minic M, Thompson A, et al. An activating mutation of AKT2 and human hypoglycemia. *Science*. 2011;334(6055):474.
146. Leiter SM, Parker VER, Welters A, Knox R, Rocha N, Clark G, et al. Hypoinsulinaemic, hypoketotic hypoglycaemia due to mosaic genetic activation of PI3-kinase. *Eur J Endocrinol*. 2017;177(2):175–186.
147. Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S, et al. Clinical course of genetic diseases of the insulin receptor (Type A and Rabson-Mendenhall Syndromes): a 30-year prospective. *Medicine*. 2004;83(4):209–222.
148. Højlund K, Hansen T, Lajer M, Henriksen JE, Levin K, Lindholm J, et al. A novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the human insulin receptor gene. *Diabetes*. 2004;53:1592–1598.
149. Kawashima Y, Nishimura R, Utsunomiya A, Kagawa R, Funata H, Fujimoto M, et al. Leprechaunism (Donohue syndrome): a case bearing novel compound heterozygous mutations in the insulin receptor gene. *Endocr J*. 2013;60(1):107–112.
150. Roth SI, Schedewie HK, Bier DM, Conaway HH, Olefsky J, Rubenstein A, et al. Hepatic ultrastructure in leprechaunism. Hepatic ultrastructural evidence suggesting a syndrome with defective hepatic glucose release. *Virchows Arch A Pathol Anat Histol*. 1982;397(2):121–130.
151. Bier DM, Schedewie H, Larner J, Olefsky J, Rubenstein A, Fiser RH, et al. Glucose kinetics in leprechaunism: accelerated fasting due to insulin resistance. *J Clin Endocrinol Metab*. 1980;51(5):988–994.
152. al-Gazali LI, Khalil M, Devadas K. A syndrome of insulin resistance resembling leprechaunism in five sibs of consanguineous parents. *J Med Genet*. 1993;30:470–475.
153. Rosenberg AM, Haworth JC, Degroot G, Trevenen CL, Rechler MM. A case of leprechaunism with severe hyperinsulinemia. *Am J Dis Child*. 1980;134(2):170–175.
154. Brown RJ, Cochran E, Gorden P. Metreleptin improves blood glucose in patients with insulin receptor mutations. *J Clin Endocrinol Metab*. 2013;98(11):E1749–E1756.
155. Semple RK, Savage DB, Cochran EK, Gorden P, O'Rahilly S. Genetic syndromes of severe insulin resistance. *Endocr Rev*. 2011;32(4):498–514.
156. Agostini M, Schoenmakers E, Mitchell C, Szatmari I, Savage D, Smith A, et al. Non-DNA binding, dominant-negative, human PPAR γ mutations cause lipodystrophic insulin resistance. *Cell Metab*. 2006;4(4):303–311.
157. Marks V, Teale JD. Hypoglycemia: factitious and felonious. *Endocrinol Metab Clin North Am*. 1999;28(3):579–601.
158. (1983). Meadow and Munchausen. *Lancet*, 1(8322), 456.
159. Marks V. Murder by insulin. *Med Leg J*. 1999;67(Pt 4):147–163.
160. Moriyama M, Hayashi N, Ohyabu C, Mukai M, Kawano S, Kumagai S. Performance evaluation and cross-reactivity from insulin analogs with the ARCHITECT insulin assay. *Clin Chem*. 2006;52:1423–1426.
161. Owen WE, Roberts WL. Cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. *Clin Chem*. 2004;50:257–259.
162. Sperling MA. Insulin biosynthesis and C-peptide. Practical applications from basic research. *Am J Dis Child*. 1980;134(12):1119–1121.
163. Uchigata Y, Eguchi Y, Takayama-Hasumi S, Omori Y. Insulin autoimmune syndrome (Hirata disease): clinical features and epidemiology in Japan. *Diabetes Res Clin Pract*. 1994;22(2–3):89–94.
164. Redmon JB, Nuttall FQ. Autoimmune hypoglycemia. *Endocrinol Metab Clin North Am*. 1999;28(3):603–618. vii.
165. Goldman J, Baldwin D, Rubenstein AH, Klink DD, Blackard WG, Fisher LK, et al. Characterization of circulating insulin and proinsulin-binding antibodies in autoimmune hypoglycemia. *J Clin Invest*. 1979;63(5):1050–1059.
166. Meschi F, Dozio N, Bognetti E, Carrà M, Cofano D, Chiumello G. An unusual case of recurrent hypoglycaemia: 10-year follow up of a child with insulin auto-immunity. *Eur J Pediatr*. 1992;151(1):32–34.
167. Rovira A, Valverde I, Escorihuela R, Lopez-Linares M. Autoimmunity to insulin in a child with hypoglycemia. *Acta Paediatr*. 1982;71(2):343–345.
168. Elias D, Cohen IR, Shechter Y, Spierer Z, Golander A. Antibodies to insulin receptor followed by anti-idiotypic: antibodies to insulin in child with hypoglycemia. *Diabetes*. 1987;36:348–354.
169. Burch HB, Clement S, Sokol MS, Landry F. Reactive hypoglycemic coma due to insulin autoimmune syndrome: case report and literature review. *Am J Med*. 1992;92(6):681–685.
170. Bortolotti D, Mothe-Satney I, Ferrari P, Gautier N, Sonke J, Pallé S, et al. Spontaneous hypoglycaemia in the presence of both anti-insulin antibody and anti-insulin receptor antibody. *Diabetes Metab*. 2006;32(6):598–603.
171. Bhatti TR, Ganapathy K, Huppmann AR, Conlin L, Boodhasingh KE, MacMullen C, et al. Histologic and molecular profile of pediatric insulinomas: evidence of a paternal parent-of-origin effect. *J Clin Endocrinol Metab*. 2016;101(3):914–922.
172. Peranteau WH, Palladino AA, Bhatti TR, Becker SA, States LJ, Stanley CA, et al. The surgical management of insulinomas in children. *J Pediatr Surg*. 2013;48(12):2517–2524.
173. Tack J, Arts J, Caenepeel P, De Wulf D, Bisschops R. Pathophysiology, diagnosis and management of postoperative dumping syndrome. *Nat Rev Gastroenterol Hepatol*. 2009;6(10):583–590.
174. Palladino AA, Sayed S, Levitt Katz LE, Gallagher PR, De Leon DD. Increased glucagon-like peptide-1 secretion and postprandial hypoglycemia in children after Nissen fundoplication. *J Clin Endocrinol Metab*. 2009;94(1):39–44.
175. De Leon D. Role of incretin hormones in hyperinsulinemic hypoglycemia. In: Stanley C, De Leon D, eds. *Monogenic Hyperinsulinemic Hypoglycemia Disorders*. *Frontiers in Diabetes*. Basel: S. Karger AG; 2012:79–86. 21.
176. Calabria AC, Charles L, Givler S, De Leon DD. Postprandial hypoglycemia in children after gastric surgery: clinical characterization and pathophysiology. *Horm Res Paediatr*. 2016;85(2):140–146.
177. Salehi M, Vella A, McLaughlin T, Patti ME. Hypoglycemia after gastric bypass surgery: current concepts and controversies. *J Clin Endocrinol Metab*. 2018;103(8):2815–2826.
178. Samuk I, Afriat R, Horne T, Bistrizter T, Barr J, Vinograd I. Dumping syndrome following Nissen fundoplication, diagnosis, and treatment. *J Pediatr Gastroenterol Nutr*. 1996;23(3):235–240.
179. Zung A, Zadik Z. Acarbose treatment of infant dumping syndrome: extensive study of glucose dynamics and long-term follow-up. *J Pediatr Endocrinol Metab*. 2003;16(6):907–915.
180. Ng DD, Ferry Jr RJ, Kelly A, Weinzimer SA, Stanley CA, Katz LE. Acarbose treatment of postprandial hypoglycemia in children after Nissen fundoplication. *J Pediatr*. 2001;139(6):877–979.
181. De Cunto A, Barbi E, Minen F, Ventura A. Safety and efficacy of high-dose acarbose treatment for dumping syndrome. *J Pediatr Gastroenterol Nutr*. 2011;53(1):113–114.
182. Sperling M, Drash A. Evolution of diabetes mellitus from hypoglycemia. *Am J Dis Child*. 1971;121(1):5–9.
183. Poon M, Hussain K. Postprandial hyperinsulinaemic hypoglycaemia and type 1 diabetes mellitus. *Arch Dis Child*. 2007;92(8):714–715.

184. Diabetes Prevention Trial - Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med.* 2002;346(22):1685–1691.
185. Harris S. Hyperinsulinism and dysinsulinism (Insulinogenic Hypoglycemia). *Endocrinology.* 1932;16(1):29–42.
186. Conn JW, Fajans SS, Seltzer HS. Spontaneous hypoglycemia as an early manifestation of diabetes mellitus. *Diabetes.* 1956;5(6):437–442.
187. Sussman KE, Stimmler L, Birenboim H. Plasma insulin levels during reactive hypoglycemia. *Diabetes.* 1966;15(1):1–4.
188. Horton ES, Sheikholislam BM, Bressler R. Combined sulfonylurea-phenformin therapy of the dysinsulinism of early diabetes mellitus. *Ann N Y Acad Sci.* 1968;148(3):778–786.
189. Freinkel N, Metzger BE. Oral glucose tolerance curve and hypoglycemia in the fed state. *N Engl J Med.* 1969;280(15):820–828.
190. Kelly A, Moran A. Update on cystic fibrosis-related diabetes. *J Cyst Fibros.* 2013;12(4):318–331.
191. Battezzati A, Battezzati PM, Costantini D, Seia M, Zazzeron L, Russo MC, et al. Spontaneous hypoglycemia in patients with cystic fibrosis. *Eur J Endocrinol.* 2007;156(3):369–376.
192. Radike K, Molz K, Holl RW, Poeter B, Hebestreit H, Ballmann M. Prognostic relevance of hypoglycemia following an oral glucose challenge for cystic fibrosis-related diabetes. *Diabetes Care.* 2011;34(4):e43.
193. Mannik LA, Chang KA, Annoh POK, Sykes J, Gilmour J, Robert R, et al. Prevalence of hypoglycemia during oral glucose tolerance testing in adults with cystic fibrosis and risk of developing cystic fibrosis-related diabetes. *J Cyst Fibros.* 2018;17(4):536–541.
194. Daughaday WH. Hypoglycemia in patients with non-islet cell tumors. *Endocrinol Metab Clin North Am.* 1989;18(1):91–101.
195. Agus MS, Katz LE, Satin-Smith M, Meadows AT, Hintz RL, Cohen P. Non-islet-cell tumor associated with hypoglycemia in a child: successful long-term therapy with growth hormone. *J Pediatr.* 1995;127(3):403–407.
196. Chung J, Henry RR. Mechanisms of tumor-induced hypoglycemia with intraabdominal hemangiopericytoma. *J Clin Endocrinol Metab.* 1996;81(3):919–925.
197. Le Roith D. Tumor-induced hypoglycemia. *N Engl J Med.* 1999;341(10):757–758.
198. Dynkevich Y, Rother KI, Whitford I, Qureshi S, Galiveeti S, Szulc AL, et al. Tumors, IGF-2, and hypoglycemia: insights from the clinic, the laboratory, and the historical archive. *Endocr Rev.* 2013;34(6):798–826.
199. Glasheen J, Sorensen M. Burkitt's lymphoma presenting with lactic acidosis and hypoglycemia—a case presentation. *Leukemia Lymphoma.* 2005;46(2):281–283.
200. Elhomsey GC, Eranki V, Albert SG, Fesler MJ, Parker SM, Michael AG, et al. "Hyper-Warburgism," a cause of asymptomatic hypoglycemia with lactic acidosis in a patient with Non-Hodgkin's lymphoma. *J Clin Endocrinol Metab.* 2012;97(12):4311–4316.
201. Weinstein DA, Correia CE, Saunders AC, Wolfsdorf JI. Hepatic glycogen synthase deficiency: an infrequently recognized cause of ketotic hypoglycemia. *Mol Genet Metab.* 2006;87(4):284–288.
202. Aynsley-Green A, Williamson DH, Gitzelmann R. Hepatic glycogen synthetase deficiency: definition of syndrome from metabolic and enzyme studies on a 9-year-old girl. *Arch Dis Child.* 1977;52:573–579.
203. Nessa A, Kumaran A, Kirk R, Dalton A, Ismail D, Hussain K. Mutational analysis of the GYS2 gene in patients diagnosed with ketotic hypoglycaemia. *J Pediatr Endocrinol Metab.* 2012;25(9–10):963–967.
204. Brown LM, Corrado MM, van der Ende RM, Derks TG, Chen MA, Siegel S, et al. Evaluation of glycogen storage disease as a cause of ketotic hypoglycemia in children. *J Inherit Metab Dis.* 2015;38(3):489–493.
205. Chou JY, Jun HS, Mansfield BC. Glycogen storage disease type I and G6Pase-beta deficiency: etiology and therapy. *Nat Rev Endocrinol.* 2010;6(12):676–688.
206. Bandsma RH, Smit GP, Kuipers F. Disturbed lipid metabolism in glycogen storage disease type I. *Eur J Pediatr.* 2002;161(Suppl 1):S65–S69.
207. Wolfsdorf JI, Crigler Jr JF. Effect of continuous glucose therapy begun in infancy on the long-term clinical course of patients with type I glycogen storage disease. *J Pediatr Gastroenterol Nutr.* 1999;29(2):136–143.
208. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood.* 2002;100(10):3776–3781.
209. Lee PJ, Dalton RN, Shah V, Hindmarsh PC, Leonard JV. Glomerular and tubular function in glycogen storage disease. *Pediatr Nephrol.* 1995;9(6):705–710.
210. Chen Y-T, Coleman RA, Scheinman JI, Kolbeck PC, Sidbury JB. Renal disease in type glycogen storage disease. *N Engl J Med.* 1988;318:7–11.
211. Chen Y-T, Scheinman JI, Park HK, Coleman RA, Roe CR. Amelioration of proximal renal tubular dysfunction in type 1 glycogen storage disease with dietary therapy. *N Engl J Med.* 1990;323:590–593.
212. Wolfsdorf JI, Laffel LMB, Crigler Jr JF. Metabolic control and renal dysfunction in type I glycogen storage disease. *J Inherit Metab Dis.* 1997;20(4):559–568.
213. Weinstein DA, Wolfsdorf JI. Effect of continuous glucose therapy with uncooked cornstarch on the long-term clinical course of type 1a glycogen storage disease. *Eur J Pediatr.* 2002;161(Suppl 1):S35–S39.
214. Franco LM, Krishnamurthy V, Bali D, Weinstein DA, Arn P, Clary B, et al. Hepatocellular carcinoma in glycogen storage disease type Ia: a case series. *J Inherit Metab Dis.* 2005;28(2):153–162.
215. Visser G, Rake JP, Fernandes J, Labrune P, Leonard JV, Moses S, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type Ib: results of the European Study on Glycogen Storage Disease type I. *J Pediatr.* 2000;137(2):187–191.
216. Melis D, Minopoli G, Balivo F, Marcolongo P, Parini R, Paci S, et al. Vitamin E improves clinical outcome of patients affected by glycogen storage disease type Ib. *JIMD Rep.* 2016;25:39–45.
217. Rake JP, ten Berge AM, Visser G, Verlind E, Niezen-Koning KE, Buys CH, et al. Glycogen storage disease type Ia: recent experience with mutation analysis, a summary of mutations reported in the literature and a newly developed diagnostic flow chart. *Eur J Pediatr.* 2000;159(5):322–330.
218. Kishnani PS, Austin SL, Abdenur JE, Arn P, Bali DS, Boney A, et al. Diagnosis and management of glycogen storage disease type I: a practice guideline of the American College of Medical Genetics and Genomics. *Genet Med.* 2014;16(11):e1.
219. Wolfsdorf JI, Keller RJ, Landy H, Crigler Jr JF. Glucose therapy for glycogenosis type 1 in infants: comparison of intermittent uncooked cornstarch and continuous overnight glucose feedings. *J Pediatr.* 1990;117(3):384–391.
220. Wolfsdorf JI, Ehrlich S, Landy HS, Crigler Jr JF. Optimal daytime feeding regimen to prevent postprandial hypoglycemia in type 1 glycogen storage disease. *Am J Clin Nutr.* 1992;56:587–592.
221. Correia CE, Bhattacharya K, Lee PJ, Shuster JJ, Theriaque DW, Shankar MN, et al. Use of modified cornstarch therapy to extend fasting in glycogen storage disease types Ia and Ib. *Am J Clin Nutr.* 2008;88(5):1272–1276.
222. Ross KM, Brown LM, Corrado MM, Chengsupanimit T, Curry LM, Ferrecchia IA, et al. Safety and efficacy of chronic extended release cornstarch therapy for glycogen storage disease type I. *JIMD Rep.* 2016;26:85–90.
223. Weinstein DA, Somers MJ, Wolfsdorf JI. Decreased urinary citrate excretion in type 1a glycogen storage disease. *J Pediatr.* 2001;138(3):378–382.
224. Visser G, Rake JP, Labrune P, Leonard JV, Moses S, Ullrich K, et al. Granulocyte colony-stimulating factor in glycogen storage disease type Ib. Results of the European Study on Glycogen Storage Disease Type 1. *Eur J Pediatr.* 2002;161(Suppl 1):S83–S87.
225. Visser G, Rake JP, Labrune P, Leonard JV, Moses S, Ullrich K, et al. Consensus guidelines for management of glycogen storage disease type 1b - European Study on Glycogen Storage Disease Type 1. *Eur J Pediatr.* 2002;161(Suppl 1):S120–S123.
226. Kasaparka CS, Cinasal Demir G, Hasanoglu A, Tumer L. Continuous glucose monitoring in children with glycogen storage disease type I. *Eur J Clin Nutr.* 2014;68(1):101–105.
227. Vertilus SM, Austin SL, Foster KS, Boyette KE, Bali DS, Li JS, et al. Echocardiographic manifestations of Glycogen Storage Disease

- III: increase in wall thickness and left ventricular mass over time. *Genet Med*. 2010;12(7):413–423.
228. Kishnani PS, Austin SL, Arn P, Bali DS, Boney A, Case LE, et al. Glycogen storage disease type III diagnosis and management guidelines. *Genet Med*. 2010;12(7):446–463.
 229. Dagli AI, Zori RT, McCune H, Ivsic T, Maisenbacher MK, Weinstein DA. Reversal of glycogen storage disease type III-related cardiomyopathy with modification of diet. *J Inherit Metab Dis*. 2009;32(Suppl 1):S103–S106.
 230. Dagli AI, Weinstein DA. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LH, et al. eds. *Glycogen storage disease type VI*. Seattle (WA): GeneReviews(R); 1993.
 231. Willems PJ, Gerver WJ, Berger R, Fernandes J. The natural history of liver glycogenosis due to phosphorylase kinase deficiency: a longitudinal study of 41 patients. *Eur J Pediatr*. 1990;149(4):268–271.
 232. Weinstein DA, Steuerwald U, De Souza CFM, Derks TGJ. Inborn errors of metabolism with hypoglycemia: glycogen storage diseases and inherited disorders of gluconeogenesis. *Clin North Am*. 2018;65(2):247–265.
 233. Roscher A, Patel J, Hewson S, Nagy L, Feigenbaum A, Kronick J, et al. The natural history of glycogen storage disease types VI and IX: Long-term outcome from the largest metabolic center in Canada. *Mol Genet Metab*. 2014;113(3):171–176.
 234. Greene HL, Ghishan FK, Brown B, McClenathan DT, Freese D. Hypoglycemia in type IV glycogenosis: hepatic improvement in two patients with nutritional management. *J Pediatr*. 1988;112(1):55–58.
 235. Santer R, Schneppenheim R, Dombrowski A, Gotze H, Steinmann B, Schaub J. Mutations in GLUT2, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. *Nat Genet*. 1997;17(3):324–326.
 236. Santer R, Steinmann B, Schaub J. Fanconi-Bickel syndrome—a congenital defect of facilitative glucose transport. *Curr Mol Med*. 2002;2(2):213–227.
 237. Al-Haggag M. Fanconi-Bickel syndrome as an example of marked allelic heterogeneity. *World J Nephrol*. 2012;1(3):63–68.
 238. Fridman E, Zeharia A, Markus-Eidlitz T, Haimi Cohen Y. Phenotypic variability in patients with fanconi-bickel syndrome with identical mutations. *JIMD Rep*. 2015;15:95–104.
 239. Ross S, Josephs H. Observations on the metabolism of recurrent vomiting. *Am J Dis Child*. 1924;28(4):447–457.
 240. Colle E, Ulstrom R. Ketotic hypoglycemia. *J Pediatr*. 1964;64:632–651.
 241. Haymond M. Hypoglycemia in infants and children. *Endocrinol Metab Clin North Am*. 1989;18:211–252.
 242. Pagliara AS, Karl IE, Haymond M, Kipnis DM. Hypoglycemia in infancy and childhood. I. *J Pediatr*. 1973;82(3):365–379.
 243. Pagliara AS, Karl IE, Haymond M, Kipnis DM. Hypoglycemia in infancy and childhood. II. *J Pediatr*. 1973;82(4):558–577.
 244. Senior B. Ketotic hypoglycemia. A tale (tail) of Gauss? *J Pediatr*. 1973;82:555–556.
 245. Stanley CA. Parsing ketotic hypoglycaemia. *Arch Dis Child*. 2006;91(6):460–461.
 246. van Hasselt PM, Ferdinandusse S, Monroe GR, Ruiten JP, Turkenburg M, Geerlings MJ, et al. Monocarboxylate transporter 1 deficiency and ketone utilization. *N Engl J Med*. 2014;371(20):1900–1907.
 247. Huidekoper HH, Duran M, Turkenburg M, Ackermans MT, Sauerwein HP, Wijburg FA. Fasting adaptation in idiopathic ketotic hypoglycemia: a mismatch between glucose production and demand. *Eur J Pediatr*. 2008;167(8):859–865.
 248. Bodamer OA, Hussein K, Morris AA, Langhans CD, Rating D, Mayatepek E, et al. Glucose and leucine kinetics in idiopathic ketotic hypoglycaemia. *Arch Dis Child*. 2006;91(6):483–486.
 249. Haymond M, Karl I, Pagliara A. Ketotic hypoglycemia: an amino acid substrate limited disorder. *J Clin Endocrinol Metab*. 1974;38:521–530.
 250. Senior B, Loridan L. Gluconeogenesis and insulin in the ketotic variety of childhood hypoglycemia and in control children. *J Pediatr*. 1969;74:529–539.
 251. Wolfsdorf J, Sadeghi-Nejad A, Senior B. Hypoalaninemia and ketotic hypoglycemia: cause or consequence? *Eur J Paediatr*. 1982;138:28–31.
 252. Brasel JA, Wright JC, Wilkins L, Blizzard RM. An evaluation of seventy-five patients with hypopituitarism beginning in childhood. *Am J Med*. 1965;38:484–498.
 253. Goodman HG, Grumbach MM, Kaplan SL. Growth and growth hormone. II. A comparison of isolated growth-hormone deficiency and multiple pituitary-hormone deficiencies in 35 patients with idiopathic hypopituitary dwarfism. *N Engl J Med*. 1968;278(2):57–68.
 254. Hopwood NJ, Forsman PJ, Kenny FM, Drash AL. Hypoglycemia in hypopituitary children. *Am J Dis Child*. 1975;129(8):918–926.
 255. Johnson JD, Hansen RC, Albritton WL, Werthemann U, Christiansen RO. Hypoplasia of the anterior pituitary and neonatal hypoglycemia. *J Pediatr*. 1973;82(4):634–641.
 256. Thornton PS. Hypoglycemia. In: Moshang Jr T, ed. *Pediatric Endocrinology: The Requisites in Pediatrics*. St. Louis: Elsevier Mosby; 2005.
 257. Lovinger R, Kaplan S, Grumbach M. Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus. *J Pediatr*. 1975;87:1171–1181.
 258. Spray CH, McKiernan P, Waldron KE, Shaw N, Kirk J, Kelly DA. Investigation and outcome of neonatal hepatitis in infants with hypopituitarism. *Acta Paediatr*. 2000;89(8):951–954.
 259. Kaufman FR, Costin G, Thomas DW, Sinatra FR, Roe TF, Neustein HB. Neonatal cholestasis and hypopituitarism. *Arch Dis Child*. 1984;59(8):787–789.
 260. Hussain K, Hindmarsh P, Aynsley-Green A. Spontaneous hypoglycemia in childhood is accompanied by paradoxically low serum growth hormone and appropriate cortisol counterregulatory hormonal responses. *J Clin Endocrinol Metab*. 2003;88(8):3715–3723.
 261. Mehta A, Dattani MT. Developmental disorders of the hypothalamus and pituitary gland associated with congenital hypopituitarism. *Best Pract Res Clin Endocrinol Metab*. 2008;22(1):191–206.
 262. Roe TF, Kogut MD. Hypopituitarism and ketotic hypoglycemia. *Am J Dis Child*. 1971;121(4):296–299.
 263. Lorentz Jr WB. Ketotic hypoglycemia and hypopituitarism. *Pediatrics*. 1979;63(3):414–415.
 264. Wolfsdorf J, Sadeghi-Nejad A, Senior B. Hypoketonemia and age-related fasting hypoglycemia in growth hormone deficiency. *Metabolism*. 1983;32:457–462.
 265. Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone (GH) insensitivity due to primary GH receptor deficiency. *Endocr Rev*. 1994;15(3):369–390.
 266. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958–2003. *J Clin Endocrinol Metab*. 2004;89(3):1031–1044.
 267. Zuckerman-Levin N, Tiosano D, Eisenhofer G, Bornstein S, Hochberg Z. The importance of adrenocortical glucocorticoids for adrenomedullary and physiological response to stress: a study in isolated glucocorticoid deficiency. *J Clin Endocrinol Metab*. 2001;86(12):5920–5924.
 268. Cryer PE, Binder C, Bolli GB, Cherrington AD, Gale EA, Gerich JE, et al. Hypoglycemia in IDDM. *Diabetes*. 1989;38(9):1193–1199.
 269. Artavia-Loria E, Chaussain JL, Bougneres PF, Job JC. Frequency of hypoglycemia in children with adrenal insufficiency. *Acta Endocrinol*, 279 Supplementum. 1986;275–278.
 270. Christiansen JJ, Djurhuus CB, Gravholt CH, Iversen P, Christiansen JS, Schmitz O, et al. Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. *J Clin Endocrinol Metab*. 2007;92(9):3553–3559.
 271. Hinde FR, Johnston DI. Hypoglycaemia during illness in children with congenital adrenal hyperplasia. *Br Med J (Clin Res Ed)*. 1984;289(6458):1603–1604.
 272. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet*. 1998;19(2):155–157.
 273. Nussey SS, Soo SC, Gibson S, Gout I, White A, Bain M, et al. Isolated congenital ACTH deficiency: a cleavage enzyme defect? *Clin Endocrinol (Oxf)*. 1993;39(3):381–385.
 274. Andrioli M, Pecori Giralaldi F, Cavagnini F. Isolated corticotrophin deficiency. *Pituitary*. 2006;9(4):289–295.

275. Jensen MD, Handwerger BS, Scheithauer BW, Carpenter PC, Mirakian R, Banks PM. Lymphocytic hypophysitis with isolated corticotropin deficiency. *Ann Intern Med.* 1986;105(2):200–203.
276. Clark AJ, Weber A. Molecular insights into inherited ACTH resistance syndromes. *Trends Endocrinol Metab.* 1994;5(5):209–214.
277. Kapadia CR, Nebesio TD, Myers SE, Willi S, Miller BS, Allen DB, et al. Endocrine effects of inhaled corticosteroids in children. *JAMA Pediatr.* 2016;170(2):163–170.
278. Hsieh S, White PC. Presentation of primary adrenal insufficiency in childhood. *J Clin Endocrinol Metab.* 2011;96(6):E925–E928.
279. Verma S, Green-Golan L, VanRyzin C, Drinkard B, Mehta SP, Weise M, et al. Adrenomedullary function in patients with non-classic congenital adrenal hyperplasia. *Horm Metab Res.* 2010;42(8):607–612.
280. Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, et al. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *N Engl J Med.* 2000;343(19):1362–1368.
281. McBride JT, McBride MC, Viles PH. Hypoglycemia associated with propranolol. *Pediatrics.* 1973;51(6):1085–1087.
282. Vidnes J, Oyasaeter S. Glucagon deficiency causing severe neonatal hypoglycemia in a patient with normal insulin secretion. *Pediatr Res.* 1977;11:943–949.
283. Molven A, Rishaug U, Matre GE, Njolstad PR, Sovik O. Hunting for a hypoglycemia gene: severe neonatal hypoglycemia in a consanguineous family. *Am J Med Genet.* 2002;113(1):40–46.
284. Marin-Valencia I, Roe CR, Pascual JM. Pyruvate carboxylase deficiency: mechanisms, mimics and anaplerosis. *Mol Genet Metab.* 2010;101(1):9–17.
285. Robinson B. Lactic acidemia: disorders of pyruvate carboxylase and pyruvate dehydrogenase. In: *The Online Metabolic and Molecular Bases of Inherited Disease [Internet]*. New York: McGraw-Hill; 2009.
286. Robinson BH, Oei J, Saudubray JM, Marsac C, Bartlett K, Quan F, et al. The French and North American phenotypes of pyruvate carboxylase deficiency, correlation with biotin containing protein by 3H-biotin incorporation, 35S-streptavidin labeling, and Northern blotting with a cloned cDNA probe. *Am J Hum Genet.* 1987;40(1):50–59.
287. García-Cazorla A, Rabier D, Touati G, Chadeaux-Vekemans B, Marsac C, de Lonlay P, et al. Pyruvate carboxylase deficiency: Metabolic characteristics and new neurological aspects. *Ann Neurol.* 2006;59(1):121–127.
288. Vieira P, Cameron J, Rahikkala E, Keski-Filppula R, Zhang LH, Santra S, et al. Novel homozygous PCK1 mutation causing cytosolic phosphoenolpyruvate carboxykinase deficiency presenting as childhood hypoglycemia, an abnormal pattern of urine metabolites and liver dysfunction. *Mol Genet Metab.* 2017;120(4):337–341.
289. Baker L, Winegrad AI. Fasting hypoglycaemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity. *Lancet.* 1970;2(7662):13–16.
290. Santer R, du Moulin M, Shahinyan T, Vater I, Maier E, Muntau AC, et al. A summary of molecular genetic findings in fructose-1,6-bisphosphatase deficiency with a focus on a common long-range deletion and the role of MLPA analysis. *Orphanet J Rare Dis.* 2016;11(1):44.
291. Steinmann B, Gitzelmann R, Van den Bergh G. Disorders of fructose metabolism. In: Valle D, Beaudet A, Vogelstein B, et al., eds. *The Online Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill; 2009.
292. Chambers RA, Pratt RT. Idiosyncrasy to fructose. *Lancet.* 1956;271(6938):340.
293. Hers HG, Joassin G. Anomalie de l'aldolase hépatique dans l'intolérance au fructose. *Enzymol Biol Clin.* 1961;1:4–14.
294. Bouteldja N, Timson DJ. The biochemical basis of hereditary fructose intolerance. *J Inheret Metab Dis.* 2010;33(2):105–112.
295. Kaufmann U, Froesch ER. Inhibition of phosphorylase-a by fructose-1-phosphate, alpha-glycerophosphate and fructose-1,6-diphosphate: explanation for fructose-induced hypoglycaemia in hereditary fructose intolerance and fructose-1,6-diphosphatase deficiency. *Eur J Clin Invest.* 1973;3(5):407–413.
296. Ali M, Rellos P, Cox TM. Hereditary fructose intolerance. *J Med Genet.* 1998;35(5):353–365.
297. Matern D, Rinaldo P. *Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency*. GeneReviews® [Internet]; 2000.
298. Hale D, Bennett M. Fatty acid oxidation disorders: a new class of metabolic diseases. *J Pediatr.* 1992;121:1–11.
299. Roe C, Ding J. Mitochondrial fatty acid oxidation disorders. In: Valle D, Beaudet A, Vogelstein B, et al., eds. *The Online Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill; 2009.
300. Treacy EP, Lambert DM, Barnes R, Boriack RL, Vockley J, O'Brien LK, et al. Short-chain hydroxyacyl-coenzyme A dehydrogenase deficiency presenting as unexpected infant death: a family study. *J Pediatr.* 2000;137(2):257–259.
301. El-Gharbawy A, Vockley J. Inborn errors of metabolism with myopathy. *defects of fatty acid oxidation and the carnitine shuttle system Pediatr Clin North Am.* 2018;65(2):317–335.
302. Treem W, Stanley C, et al. Primary carnitine deficiency due to failure of carnitine transport in kidney, muscle, and fibroblasts. *N Engl J Med.* 319:1331.
303. Murad MH, Coto-Yglesias F, Wang AT, Sheidaee N, Mullan RJ, Elamin MB, et al. Clinical review: Drug-induced hypoglycemia: a systematic review. *J Clin Endocrinol Metab.* 2009;94(3):741–745.
304. Ben Salem C, Fathallah N, Hmouda H, Bouraoui K. Drug-induced hypoglycaemia: an update. *Drug Saf.* 2011;34(1):21–45.
305. Ernst AA, Jones K, Nick TG, Sanchez J. Ethanol ingestion and related hypoglycemia in a pediatric and adolescent emergency department population. *Acad Emerg Med.* 1996;3(1):46–49.
306. Limbeck GA, Ruvalcaba RA, Samols E, Kelley VC. Salicylates and hypoglycemia. *Am J Dis Child.* 1965;109(2):165–167.
307. Merimee TJ, Tyson JE. Stabilization of plasma glucose during fasting: Normal variations in two separate studies. *N Engl J Med.* 1974;291(24):1275–1278.
308. Morrice JJ, Taylor KM, Blair JL, Young DG. Preoperative plasma glucose level. *Arch Dis Child.* 1974;49(11):898–900.
309. O'Flynn PE, Milford CA. Fasting in children for day case surgery. *Ann R Coll Surg Engl.* 1989;71(4):218–219.
310. Strapazzon G, Nardin M, Zanon P, Kaufmann M, Kritzinger M, Brugger H. Respiratory failure and spontaneous hypoglycemia during noninvasive rewarming from 24.7 degrees C (76.5 degrees F) core body temperature after prolonged avalanche burial. *Ann Emerg Med.* 2012;60(2):193–196.
311. Boles JM, Mabelle S, Scheydecker JL, Garo B, Garre M. Hypoglycaemia in salt water near-drowning victims. *Intens Care Med.* 1988;14(1):80.
312. Elias AN, Gwinup G. Glucose-resistant hypoglycemia in inanition. *Arch Intern Med.* 1982;142(4):743–746.
313. Kerpel-Fronius E, Kaiser E. Hypoglycaemia in infantile malnutrition. *Acta Paediatr Scand.* 1967;Suppl 172: 119+.
314. Wharton B. Hypoglycaemia in children with kwashiorkor. *Lancet.* 1970;1:171–173.
315. Ratcliffe PJ, Bevan JS. Severe hypoglycaemia and sudden death in anorexia nervosa. *Psychol Med.* 1985;15(3):679–681.
316. Yamada Y, Fushimi H, Inoue T, Nishinaka K, Kameyama M. Anorexia nervosa with recurrent hypoglycemic coma and cerebral hemorrhage. *Intern Med.* 1996;35(7):560–563.
317. Rich LM, Caine MR, Findling JW, Shaker JL. Hypoglycemic coma in anorexia nervosa. Case report and review of the literature. *Arch Intern Med.* 1990;150(4):894–895.
318. Sakurai-Chin C, Ito N, Taguchi M, Miyakawa M, Takeshita A, Takeuchi Y. Hypoglycemic coma in a patient with anorexia nervosa coincident with acute exacerbation of liver injury induced by oral intake of nutrients. *Intern Med.* 2010;49(15):1553–1556.
319. Hoelzer DR, Dalsky GP, Clutter WE, Shah SD, Holloszy JO, Cryer PE. Glucoregulation during exercise: hypoglycemia is prevented by redundant glucoregulatory systems, sympathochromaffin activation, and changes in islet hormone secretion. *J Clin Invest.* 1986;77(1):212–221.
320. Hirsch IB, Marker JC, Smith LJ, Spina RJ, Parvin CA, Holloszy JO, et al. Insulin and glucagon in prevention of hypoglycemia during exercise in humans. *Am J Physiol.* 1991;260(5 Pt 1):E695–E704.
321. Marker JC, Hirsch IB, Smith LJ, Parvin CA, Holloszy JO, Cryer PE. Catecholamines in prevention of hypoglycemia during exercise in humans. *Am J Physiol.* 1991;260(5 Pt 1):E705–E712.

322. Brun JF, Dumortier M, Fedou C, Mercier J. Exercise hypoglycemia in nondiabetic subjects. *Diabetes Metab.* 2001;27(2 Pt 1):92–106.
323. French JK, Frengley PA. Hypoglycemia induced seizures following a marathon. *N Z Med J.* 1983;96(732):407.
324. Field JB. Exercise and deficient carbohydrate storage and intake as causes of hypoglycemia. *Endocrinol Metab Clin North Am.* 1989;18(1):155–161.
325. Marquard J, Welters A, Buschmann T, Barthlen W, Vogelgesang S, Klee D, et al. Association of exercise-induced hyperinsulinaemic hypoglycaemia with MCT1-expressing insulinoma. *Diabetologia.* 2013;56(1):31–35.
326. Reid SR, Losek JD. Hypoglycemia complicating dehydration in children with acute gastroenteritis. *J Emerg Med.* 2005;29(2):141–145.
327. Bennish ML, Azad AK, Rahman O, Phillips RE. Hypoglycemia during diarrhea in childhood. Prevalence, pathophysiology, and outcome. *N Engl J Med.* 1990;322(19):1357–1363.
328. Weinstein D, Raymond K, Korson M, Weiner D, Wolfsdorf J. High incidence of unrecognized metabolic and endocrinologic disorders in acutely ill children with previously unrecognized hypoglycemia. *Pediatr Res.* 2001;49:103.
329. Miller SI, Wallace Jr RJ, Musher DM, Septimus EJ, Kohl S, Baughn RE. Hypoglycemia as a manifestation of sepsis. *Am J Med.* 1980;68(5):649–654.
330. Preyra I, Worster A. Hypoglycemia in bacterial septicemia. *Can J Emerg Med.* 2003;5(4):268–270.
331. Maitra S, Wojnar M, Lang C. Alterations in tissue glucose uptake during the hyperglycemic and hypoglycemic phases of sepsis. *Shock.* 2000;13(5):379–385.
332. Romijn JA, Godfried MH, Wortel C, Sauerwein HP. Hypoglycemia, hormones and cytokines in fatal meningococcal septicemia. *J Endocrinol Invest.* 1990;13(9):743–747.
333. White NJ, Miller KD, Marsh K, Berry CD, Turner RC, Williamson DH, et al. Hypoglycaemia in African children with severe malaria. *Lancet.* 1987;1(8535):708–711.
334. Krishna S, Waller DW, Kuile FT, Kwiatkowski D, Crawley J, Craddock CFC, et al. Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Trans R Soc Trop Med Hyg.* 1994;88(1):67–73.
335. Phillips RE, Looareesuwan S, White NJ, Chanthavanich P, Karbwang J, Supanaranond W, et al. Hypoglycaemia and antimalarial drugs: quinidine and release of insulin. *Br Med J (Clinical research ed).* 1986;292:1319–1321.
336. Furman BL, Walker E, Sidey FM, Wardlaw AC. Slight hyperinsulinaemia but no hypoglycaemia in pertussis patients. *J Med Biol.* 1988;25(3):183–186.
337. Furman BL, Wardlaw AC, Stevenson LQ. Bordetella pertussis-induced hyperinsulinaemia without marked hypoglycaemia: a paradox explained. *Br J Exp Pathol.* 1981;62(5):504–511.
338. Wang Y, Park S, Bajpayee NS, Nagaoka Y, Boulay G, Birnbaumer L, et al. Augmented glucose-induced insulin release in mice lacking G_{α2}, but not G_{α1} or G_i proteins. *Proc Natl Acad Sci.* 2011;108:1693–1698.
339. Faustino EVS, Hirshberg EL, Bogue CW. Hypoglycemia in critically ill children. *J Diabetes Sci Technol.* 2012;6(1):48–57.
340. Fischer KF, Lees JA, Newman JH. Hypoglycemia in Hospitalized Patients. *N Engl J Med.* 1986;315(20):1245–1250.
341. deCastro FJ, Jaeger R, Gleason WA. Liver damage and hypoglycemia in acute iron poisoning. *Clin Toxicol.* 1977;10(3):287–289.
342. Felig P, Brown WV, Levine RA, Klatskin G. Glucose homeostasis in viral hepatitis. *N Engl J Med.* 1970;283(26):1436–1440.
343. Arky RA. Hypoglycemia associated with liver disease and ethanol. *Endocrinol Metab Clin North Am.* 1989;18(1):75–90.
344. Kobayashi K, Saheki T, Song Y. *Citrin deficiency*; 2012. In: GeneReviews® [Internet]. [Internet]. Seattle University of Washington.
345. Wells TG, Ulstrom RA, Nevins TE. Hypoglycemia in pediatric renal allograft recipients. *J Pediatr.* 1988;113(6):1002–1007.
346. Peitzman SJ, Agarwal BN. Spontaneous Hypoglycemia in end-stage renal failure. *Nephron.* 1977;19(3):131–139.
347. Arem R. Hypoglycemia associated with renal failure. *Endocrinol Metab Clin North Am.* 1989;18(1):103–121.
348. Toth EL, Lee DW. 'Spontaneous'/uremic hypoglycemia is not a distinct entity: substantiation from a literature review. *Nephron.* 1991;58(3):325–329.
349. Nigro G. Pancreatitis with hypoglycemia-associated convulsions following rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr.* 1991;12(2):280–282.
350. Friedlander EO. Hyperinsulinism secondary to disease of the pancreas and organs adjacent to the pancreas: a review of the world literature. *Ann Intern Med.* 1960;52(4):838–848.
351. Laraya-Cuasay LR, Wolfe R, Hughes WT. Acute pancreatitis and hypoglycemia. *Clin Pediatr.* 1968;7(9):525–528.
352. Teng R-J, Wu T-J, Ho M-M. Mumps infection complicated by transient hyperinsulinemic hypoglycemia. *Pediatr Infect Dis J.* 1997;16(4):416–417.
353. Haymond MW, Strauss AW, Arnold KJ, Bier DM. Glucose homeostasis in children with severe cyanotic congenital heart disease. *J Pediatr.* 1979;95(2):210–213.
354. Benzing 3rd G, Schubert W, Sug G, Kaplan S. Simultaneous hypoglycemia and acute congestive heart failure. *Circulation.* 1969;40(2):209–216.
355. Hedayati HA, Beheshti M. Profound spontaneous hypoglycemia in congestive heart failure. *Curr Med Res Opin.* 1977;4(7):501–504.
356. Mellinkoff SM, Tumulty PA. Hepatic hypoglycemia. *N Engl J Med.* 1952;247(20):745–750.
357. Amatayakul O, Cumming GR, Haworth JC. Association of hypoglycaemia with cardiac enlargement and heart failure in newborn infants. *Arch Dis Child.* 1970;45(243):717–720.
358. Shu S, Cruse RP, Redmond GP. Hypoglycemia in a child with congenital muscular dystrophy. *Brain Dev.* 1989;11(1):62–65.
359. Bruce AK, Jacobsen E, Dossing H, Kondrup J. Hypoglycaemia in spinal muscular atrophy. *Lancet.* 1995;346(8975):609–610.
360. Aboumoussa A, Hoogendijk J, Charlton R, Barresi R, Herrmann R, Voit T, et al. Caveolinopathy – New mutations and additional symptoms. *Neuromuscul Disord.* 2008;18(7):572–578.
361. Orngreen MC, Zacho M, Hebert A, Laub M, Vissing J. Patients with severe muscle wasting are prone to develop hypoglycemia during fasting. *Neurology.* 2003;61(7):997–1000.
362. Hadfield MG, Vennart GP, Rosenblum WI. Hypoglycemia: invasion of the hypothalamus by lymphosarcoma. Metastasis to blood glucose regulating centers. *Arch Pathol.* 1972;94(4):317–321.
363. Eadington DW. Hypoglycaemia and metabolic acidosis in a patient with an acute leukaemia. *Scott Med J.* 1988;33(4):309–310.
364. Halonen P, Salo MK, Mäkiperna A. Fasting hypoglycemia is common during maintenance therapy for childhood acute lymphoblastic leukemia. *J Pediatr.* 2001;138(3):428–431.
365. Halonen P, Salo M, Schmiegelow K, Mäkiperna A. Investigation of the mechanisms of therapy-related hypoglycaemia in children with acute lymphoblastic leukaemia. *Acta Paediatr.* 2003;92(1):37–42.
366. Ziino O, Russo D, Orlando MA, Benigno V, Locatelli F, Aricò M. Symptomatic hypoglycemia in children receiving oral purine analogues for treatment of childhood acute lymphoblastic leukemia. *Med Pediatr Oncol.* 2002;39(1):32–34.
367. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, Kache S, Wilson DM. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. *Pediatrics.* 2006;118:173–179.
368. Stout SM, Cober MP. Metabolic effects of cyclic parenteral nutrition infusion in adults and children. *Nutr Clin Pract.* 2010;25(3):277–281.
369. Dungan K, Chapman J, Braithwaite SS, Buse J. Glucose measurement: confounding issues in setting targets for inpatient management. *Diabetes Care.* 2007;30:403–409.
370. Vlasselaers D, Milants I, Desmet L, Wouters PJ, Vanhorebeek I, van den Heuvel I, et al. Intensive insulin therapy for patients in paediatric intensive care: a prospective, randomised controlled study. *Lancet.* 2009;373(9663):547–556.
371. Macrae D, Grieve R, Allen E, Sadique Z, Morris K, Pappachan J, et al. A randomized trial of hyperglycemic control in pediatric intensive care. *N Engl J Med.* 2014;370(2):107–118.
372. Agus MS, Wypij D, Hirshberg EL, Srinivasan V, Faustino EV, Luckett PM, et al. Tight glycemic control in critically ill children. *N Engl J Med.* 2017;376(8):729–741.
373. Horwitz DL. Factitious and artifactual hypoglycemia. *Endocrinol Metab Clin North Am.* 1989;18(1):203–210.

374. Giampietro O, Navalesi R, Buzzigoli G, Boni C, Benzi L. Decrease in plasma glucose concentration during storage at -20 degrees C. *Clin Chem*. 1980;26(12):1710–1712.
375. de Pasqua A, Mattock MB, Phillips R, Keen H. Errors in blood glucose determination. *Lancet*. 1984;2(8412):1165.
376. Billington CJ, Casciato DA, Choquette DL, Morley JE. Artifactual hypoglycemia associated with polycythemia vera. *JAMA*. 1983;249(6):774–775.
377. Macaron CI, Kadri A, Macaron Z. Nucleated red blood cells and artifactual hypoglycemia. *Diabetes Care*. 1981;4(1):113–115.
378. Heinemann L, Zijlstra E, Pleus S, Freckmann G. Performance of blood glucose meters in the low-glucose range: current evaluations indicate that it is not sufficient from a clinical point of view. *Diabetes Care*. 2015;38(9):e139–e140.
379. Klaff LJ, Brazg R, Hughes K, Tideman AM, Schachner HC, Stenger P, et al. Accuracy evaluation of contour next compared with five blood glucose monitoring systems across a wide range of blood glucose concentrations occurring in a clinical research setting. *Diabetes Technol Ther*. 2015;17(1):8–15.
380. Freckmann G, Link M, Schmid C, Pleus S, Baumstark A, Haug C. System accuracy evaluation of different blood glucose monitoring systems following ISO 15197:2013 by using two different comparison methods. *Diabetes Technol Ther*. 2015;17(9):635–648.
381. Freckmann G, Pleus S, Link M, Baumstark A, Schmid C, Hogel J, et al. Accuracy evaluation of four blood glucose monitoring systems in unaltered blood samples in the low glycemic range and blood samples in the concentration range defined by ISO 15197. *Diabetes Technol Ther*. 2015;17(9):625–634.
382. Ekhlaspour L, Mondesir D, Lautsch N, Balliro C, Hillard M, Magyar K, et al. Comparative Accuracy of 17 Point-of-Care Glucose Meters. *J Diabetes Sci Technol*. 2017;11(3):558–566.
383. Hay Jr WW, Rozance PJ. Continuous glucose monitoring for diagnosis and treatment of neonatal hypoglycemia. *J Pediatr*. 2010;157(2):180–182.
384. Harris DL, Battin MR, Weston PJ, Harding JE. Continuous glucose monitoring in newborn babies at risk of hypoglycemia. *J Pediatr*. 2010;157(2):198–202. e1.
385. Alsaif H, Turner L, Yung Z, Didi M, Senniappan S. Continuous flash glucose monitoring in children with congenital hyperinsulinism; first report on accuracy and patient experience. *Int J Pediatr Endocrinol*. 2018;2018:3.
386. Steil GM, Langer M, Jaeger K, Alexander J, Gaies M, Agus MSD. Value of continuous glucose monitoring for minimizing severe hypoglycemia during tight glycemic control. *Pediatr Critical Care Med*. 2011;12(6):643–648.
387. Uettwiller F, Chemin A, Bonnemaïson E, Favrais G, Saliba E, Labarthe F. Real-time continuous glucose monitoring reduces the duration of hypoglycemia episodes: a randomized trial in very low birth weight neonates. *PLOS One*. 2015;10(1), e0116255.
388. Galderisi A, Facchinetti A, Steil GM, Ortiz-Rubio P, Cavallin F, Tamborlane WV, et al. Continuous glucose monitoring in very preterm infants: a randomized controlled trial. *Pediatrics*. 2017;140.
389. Finegold DN, Stanley CA, Baker L. Glycemic response to glucagon during fasting hypoglycemia: an aid in the diagnosis of hyperinsulinism. *J Pediatr*. 1980;96(2):257–259.
390. Cryer PE. Banting Lecture. Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes*. 1994;43(11):1378–1389.

Joan C. Han, Ram Weiss

CHAPTER OUTLINE

INTRODUCTION	939		
ENERGY BALANCE	939		
Regulation of Food Intake	940		
HOMEOSTATIC SYSTEM FOR ENERGY BALANCE	941		
The Afferent System	941		
Central Processing	943		
The Efferent System	945		
NONHOMEOSTATIC REGULATION OF ENERGY BALANCE	947		
The Nucleus Accumbens and the Hedonic Pathway of Food Reward	947		
The Amygdala and the Stress Response	947		
DYSREGULATION OF ENERGY BALANCE	947		
Leptin Resistance	947		
Counterregulatory Mechanisms That Oppose Weight Loss	948		
Feed-Forward Mechanism That Promote Weight Gain	949		
ENERGY EXCESS—OBESITY	949		
Definition	949		
Prevalence and Epidemiology	949		
Global Prevalence	951		
Racial and Ethnic Considerations	951		
Predictive Factors	952		
METABOLIC IMPACT OF CHILDHOOD OBESITY	952		
Insulin Resistance	952		
Lipid Partitioning	953		
Vascular Changes	954		
Adipocytokines	955		
		Comorbidities Related to Insulin Resistance	956
		Other Endocrine Comorbidities	958
		Other Nonendocrine Comorbidities	958
		FACTORS ASSOCIATED WITH THE CURRENT EPIDEMIC OF OBESITY	958
		Genetics	958
		Epigenetics and Developmental Programming	958
		Environmental Factors	959
		Dietary Factors	960
		DISORDERS OF OBESITY	964
		“Classic” Endocrine Disorders With an Obesity Phenotype	964
		Genetic Obesity Disorders	966
		Syndromic Obesity Disorders	969
		Hypothalamic Obesity	970
		EVALUATION AND TREATMENT OF PEDIATRIC OBESITY	971
		Diagnostic Approach	971
		Lifestyle Modification	972
		Pharmacotherapy	973
		Bariatric Surgery	975
		ENERGY INADEQUACY	977
		Starvation Versus Cachexia	977
		Failure to Thrive	977
		Cancer Cachexia	979
		The Diencephalic Syndrome	980
		Anorexia Nervosa	980
		CONCLUSIONS	982
		References	984

INTRODUCTION

The global prevalence of pediatric obesity has increased by a staggering eightfold over the past 4 decades with a current estimate of 124 million children, ages 5 to 19, having obesity worldwide.¹ The concept of the adipocyte functioning as an endocrine organ rather than simply serving as a repository for lipid storage emerged a quarter of a century ago when leptin was identified as the first of many adipokines produced by fat tissue.² Thus given that the pathophysiology of obesity is a form of endocrine derangement at its core, and because many of the complications associated with obesity have an endocrine component in nature, pediatric endocrinologists are increasingly receiving referrals for the treatment of obesity and its comorbidities. Type 2 diabetes, which was once rare in children and is typically associated with obesity, now comprises approximately one-third of all new diagnoses of pediatric diabetes and continues to rise by nearly 5% in annual incidence rate among youth.³

This chapter provides a framework for understanding the regulation of energy balance and summarizes approaches for

assessing and managing pediatric patients with obesity. On the flip side of the energy-balance coin, disorders of energy inadequacy are also discussed in this chapter, and they provide insight on the converse problem of nutritional insufficiency. Although the relative dearth of current knowledge on how best to ameliorate the complex biologic, behavioral, and environmental contributors to energy imbalance poses a therapeutic challenge, recent advances in novel “precision medicine” approaches that target specific defects in energy homeostasis provide insights into potentially effective treatments for both obesity and underweight disorders.

ENERGY BALANCE

The major components of energy balance are energy intake, energy expenditure, and energy storage. Sources of energy intake include carbohydrate (4 kCal/g), protein (~4 kCal/g), fat (9 kCal/g), and alcohol (7 kCal/g), typically negligible in children, but possibly significant in some adolescents. Energy expenditure is comprised of basal metabolic rate ([BMR],

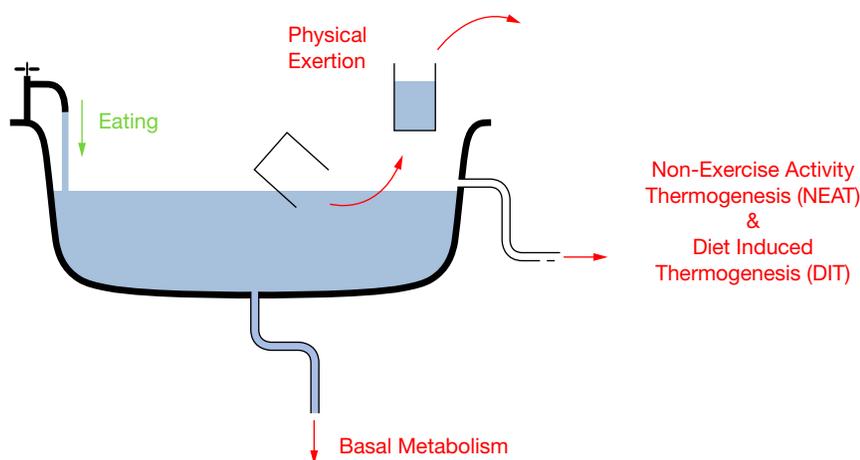


Fig. 24.1 Determinants of total body energy stores as represented by water in a bathtub. (Figure by Clint Kinney, PhD, modified from Rogers, P.J., Brunstrom, J.M. (2016). Appetite and energy balancing. *Physiol Behav*, 164, 465–471).

energy needed to maintain bodily functions, highly correlated with lean muscle and organ mass, and affected by various disease states), thermic effect of food (energy used to digest food, diet-induced thermogenesis [DIT]), and physical activity (exercise and nonexercise activity thermogenesis [NEAT], which includes activities of daily living, fidgeting, and posture maintenance). Energy storage, primarily in the form of fat, occurs when intake exceeds expenditure.⁴ Using a bathtub (Fig. 24.1) as an analogy for energy balance with the water level representing total energy stores, the water volume (total body fat) is determined by intermittent addition of water from the faucet (meals), episodic removal of water buckets (physical exertion), continual drainage of water from a bottom drain (BMR), and overflow drainage of water from an upper drain (NEAT and DIT) that is positioned at the set point for maximal water level (individual homeostatic set point).⁵ Changes in faucet flow rate, size of buckets, aperture diameter of the drains, and height of overflow set point together determine the water level in the bathtub.

Human energy balance is regulated by intricately connected homeostatic and non-homeostatic mechanisms. The homeostatic system maintains body fat stores within a fairly tight range for each individual, remarkably within around 10 kCal/d on average,^{6,7} such that even small perturbations in balance can lead to cumulative weight gain over time.⁸ The non-homeostatic system mediates environmental and cognitive factors that motivate eating, including the reward aspects of food intake and learned behaviors that lead to food consumption for reasons other than fulfillment of nutritive needs. The neural circuitry and neuroendocrine hormones of the homeostatic and non-homeostatic processes interact and overlap to form a complex system that integrates temporal, spatial, and contextual signals of metabolic status and higher-order brain function.⁹ Because survival depends on an adequate supply of energy, the evolutionary calibration of energy balance favors food consumption and promotes excess energy storage to protect against starvation when food is scarce.⁵ Consequently, in our modern era, in places where food is readily available in abundance, especially highly palatable, energy-dense foods, the inhibitory mechanisms for appetite regulation may be inadequate for preventing overconsumption.

Regulation of Food Intake

Human meal patterning is typically characterized by discrete bouts of food consumption interspersed with periods of fasting. The time-course of each meal follows a cycle, comprised

of cephalic, gastric, and intestinal phases followed by a postabsorptive state.¹⁰ The cephalic phase consists of the physiologic responses to the thought, sight, smell, and taste of food in anticipation of ingestion. In the gastric phase, ingested food enters the stomach and digestion formally begins. As food leaves the stomach, the intestinal phase begins, where secreted pancreatic enzymes enhance digestion and nutrient absorption. Finally, in the postabsorptive state, nutrient absorption from the meal is complete and circulating glucose concentrations are maintained by glycogenolysis and gluconeogenesis until initiation of the next meal.

Short-term regulation of appetite determines satiety (time period between meals before hunger prompts meal initiation) and satiation (sensation of having eaten enough, leading to meal termination). Food intake is driven by a combination of hunger, social context, and sensory inputs.⁷ Hunger is mediated by a drop in anorexic (appetite-suppressing) signals in the postabsorptive state following the prior meal, and by a rise in ghrelin, an orexigenic (appetite-stimulating) peptide secreted by the enteroendocrine cells of the stomach during fasting. Nutrient ingestion leads to a rise in anorexic signals, including gastric wall stretch and mechanical contact with food, secretion of intestinal peptides (e.g., cholecystokinin [CCK], peptide YY, glucagon-like peptide 1 [GLP-1], etc.), and entry of digested nutrients into circulation.¹⁰ The vagal nerve is the primary neural connection between the gastrointestinal (GI) tract and the central nervous system (CNS).¹¹ It is the longest cranial nerve and contains both sensory and motor fibers involved in the regulation of parasympathetic (“rest and digest”) homeostasis.¹² Vagal sensory afferents from the gut terminate in the hindbrain where connections to cortical, forebrain, and midbrain regions are involved in vagal motor efferent regulation of GI motility and secretory functions for digestion.¹³ Importantly, the GI tract is also innervated by the spinal nerves, which convey sensory inputs from the intestinal tract to the homeostatic centers. Satiation is reached when ghrelin drops; gastric distension triggers anorexic vagal afferents to the hindbrain; and increases in glucose, insulin, and anorexic peptides lead to slowing of gastric motility and signaling of fullness to the CNS. Satiety, which determines the interval until the next meal, is influenced by the quantity and composition of the prior meal and additional physiologic contributors, such as gut microbiota, fermentation products, and bile acids.¹⁴

Nonnutritive aspects of meal regulation include food palatability and neuropsychologic factors, such as mindfulness, stress, cognitive demands, and alertness.¹⁵ Food craving is linked to the hedonic aspects of eating because of activation

of the mesocorticolimbic reward pathway. Opioid receptor activation mediates the reward sensation of food and the pleasure of eating, whereas dopaminergic activation mediates the reward value of food and the motivation to obtain food.¹⁶ Social context (gatherings and events where food consumption is expected) and sensory inputs (sight and smell of palatable food) can drive food intake in the absence of hunger, making environmental contingencies and stimulus control just as critical to address as physiologic cues for obesity management.⁵

HOMEOSTATIC SYSTEM FOR ENERGY BALANCE

The CNS is the control center for energy homeostasis. Afferent signals from the periphery deliver energy status information to the brain, which processes these cues and then delivers efferent signals to modulate energy intake and expenditure. The system is directed by several hypothalamic nuclei, which receive an array of hormonal, neural, environmental, and cortical inputs. These signals activate, based on their directionality, anorexigenic or orexigenic signal transduction pathways. The output of the system is conveyed via the autonomic nervous system and is translated by peripheral organs into food-seeking behavior, energy conservation/wasting, and physiologic adaptations (a simplified vision of these pathways is shown in Fig. 24.2). The physiologic target of energy homeostasis is to maintain stable levels of total body fat rather than overall weight per se. Therefore leptin, which is secreted in proportion to total fat mass, functions suitably as a key circulating signal to convey adiposity status to the CNS.^{17–19} Binding of these hormones to their respective receptors within the hypothalamic arcuate nucleus (ARC) upregulates anorexic/catabolic signals from proopiomelanocortin (POMC)/cocaine-amphetamine-related transcript (CART) expressing neurons and downregulates orexigenic/anabolic signals from Agouti-related peptide (AgRP)/neuropeptide Y (NPY) expressing neurons. A schematic of these energy homeostasis pathways is shown in Fig. 24.3, and further details about individual hormones are described later.²⁰

The Afferent System

Monitoring of metabolic status occurs in multiple regions of the body and assesses circulating forms of energy that are available for immediate use and fluctuate over the course of a meal cycle, as well as more constant stored forms of energy, namely fat. Direct detection of circulating forms of energy uses specific receptors for micronutrients²¹ or cellular detection systems, such as glycolytic flux generating varying amounts of adenosine triphosphate (ATP) depending on glucose availability.²² Indirect surveillance involves paracrine and endocrine hormones that reflect energy status and also stretch receptors in the GI tract that detect volume of material within the gut, nutritive or otherwise, rather than micronutrients specifically. The bloodstream and the autonomic nervous system, mainly the afferent vagus nerve, are the primary routes for transmission of metabolic information from the periphery to the CNS. The mediobasal hypothalamus (MBH), contains the ARC, ventromedial hypothalamic nucleus [VMH], and median eminence [ME] and dorsal vagal complex (DVC, contains the nucleus of the solitary tract [NTS], the area postrema [AP], and dorsal motor nucleus of the vagus nerve [DMV]) of the brainstem medulla have a special fenestrated blood-brain barrier (BBB),²³ allowing circulating hormones and micronutrients to diffuse into these brain regions for detection by specific receptors and sensing mechanisms.^{22,24–26} The DVC also receives sensory afferents from the vagal nerve, which innervates the GI tract from the esophagus to the colon and delivers information from chemical and mechanical sensors in the gut to the CNS.²⁷

Positive Regulation

In addition to intrinsic neurocircuitry that favors positive energy balance by default, currently, one additional positive regulatory signal from the periphery is known to enhance this drive.

Ghrelin. Ghrelin is an octanoylated 28-amino-acid peptide that is a ligand of the growth hormone secretagogue receptor

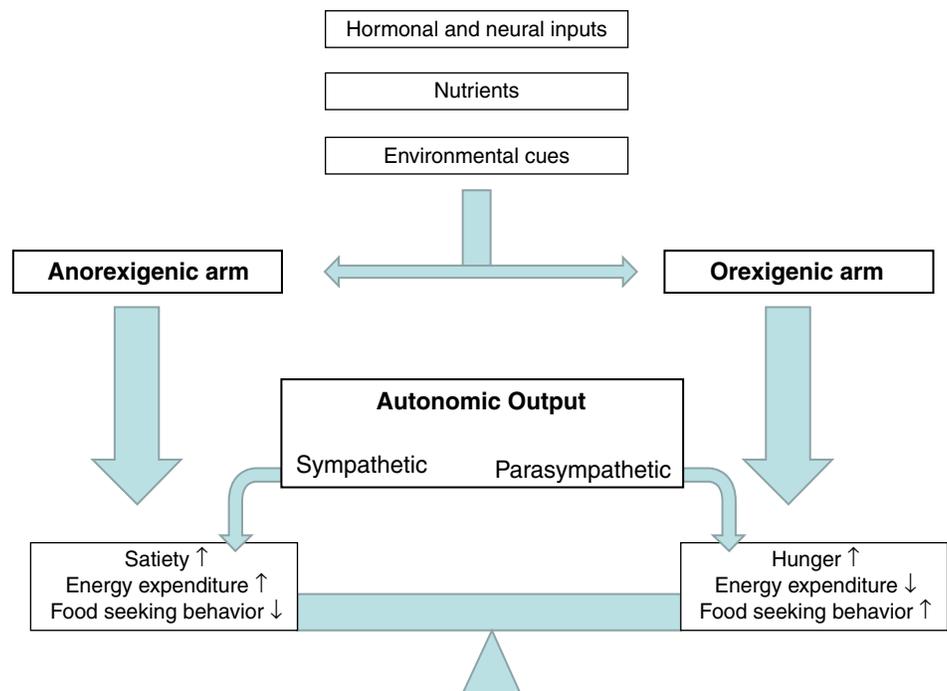


Fig. 24.2 Simplified view of the homeostatic energy system and its inputs/outputs. Hypothalamic nuclei govern homeostasis. Their inputs include hormones (such as leptin or ghrelin), neural inputs (directly from the gastrointestinal tract), nutrients, and environmental cues. The integration of these signals leads to an autonomic output that determines the dominance of the orexigenic or anorexigenic arms.

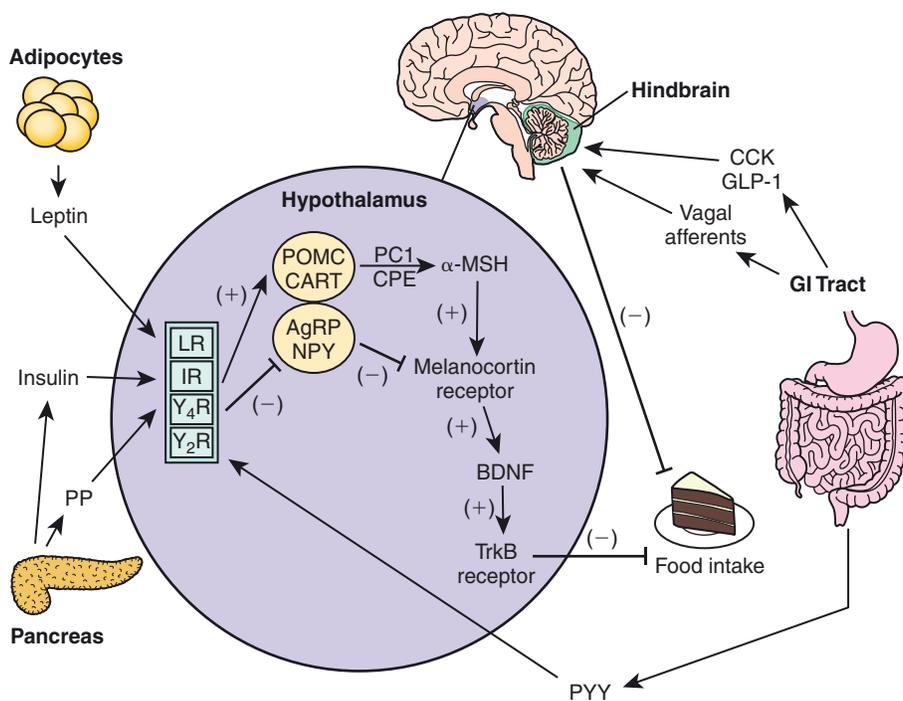


Fig. 24.3 Schematic of energy homeostasis pathways. Lines with arrowheads show stimulatory action. Lines with perpendicular end-blocks show inhibitory action. *AgRP*, Agouti-related peptide; *BDNF*, brain-derived neurotrophic factor; *CART*, cocaine-amphetamine-related transcript; *CCK*, cholecystokinin; *CPE*, carboxypeptidase E; *GI*, gastrointestinal; *GLP-1*, glucagon-like peptide 1; *IR*, insulin receptor; *LR*, leptin receptor; *MSH*, melanocyte-stimulating hormone; *NPY*, neuropeptide Y; *PC1*, prohormone convertase 1; *POMC*, proopiomelanocortin; *PP*, pancreatic polypeptide; *PYY*, peptide YY; *TrkB*, tropomyosin-related kinase B; *Y₂R*, type-2 neuropeptide Y receptor; *Y₄R*, type-4 neuropeptide Y receptor. (Figure modified from Han, J.C., Lawlor, D. A., Kimm, S.Y. (2010). Childhood obesity. *Lancet*, 375(9727), 1737–1748.)

(GHSR) and endogenously secreted by the stomach and duodenum in the fasting state.²⁸ Ghrelin circulates within the bloodstream to the CNS, where it binds GHSRs in the hypothalamus and brainstem, accessed through fenestrations and selective transport across the BBB.^{29–31} Pituitary GHSR stimulation induces growth hormone (GH) release whereas VMH GHSR stimulation promotes positive energy balance through increased hunger, food intake, fat deposition, and reduced energy expenditure.^{32–35} To date, ghrelin is the only known peripherally produced orexigenic peptide hormone. Ghrelin secretion by the stomach terminates upon entry of nutrients into the stomach and intestines after meal consumption.¹² Ghrelin rises in conjunction with increasing hunger, and ghrelin peaks at the conclusion of satiety when voluntary food consumption begins.³⁶ Exogenous ghrelin infusion induces food intake, supporting the role of ghrelin in triggering meal initiation.³⁷ Individuals with obesity compared with normal weight controls have a globally lower plasma ghrelin concentration but retain a similar circadian pattern of rise in ghrelin with fasting and decrease with meal intake,³⁸ suggesting that ghrelin is responsive to, rather than a cause of, obesity. Weight loss because of dietary restriction leads to a rise in ghrelin whereas gastric bypass surgery results in decreased ghrelin,³⁸ which may contribute to the differences in weight regain after weight loss by these different approaches. Importantly, ghrelin is activated from its prohormone form by binding of octanoic acid to a serine residue by ghrelin O-acetyltransferase (GOAT). The unacylated form is active as a homeostatic signal yet does not bind to the GH receptor.³⁹

Negative Regulation

Satiation, satiety, and expenditure of excess energy are achieved by negative regulatory elements that respond acutely to intake and to long-term energy stores.

Stretch Receptors. Ingested material entering the stomach applies pressure to the gastric wall although mechanosensitive receptors formed by vagal afferents whose cell bodies reside in the nodose ganglion and terminals lie within the NTS of the

brainstem DVC. The stretch signal slows gastric emptying, which promotes retention of food in the stomach allowing satiation to be reached.¹²

Glucose. Digestion of carbohydrates leads to liberation of glucose and other sugars, which are readily absorbed into the hepatic portal vein and taken up by liver hepatocytes. A significant portion of the incoming glucose also enters into systemic circulation,⁴⁰ and blood glucose levels are detected by glucose-sensing neurons in the brain that are involved in regulation of glucose homeostasis, as well as energy balance.^{41–43} A subset of glucose-excited neurons responds to postprandial increases in glucose by stimulating energy expenditure through thermogenesis,⁴² whereas a subset of glucose-inhibited neurons respond to increased glucose by reducing the activity of hunger circuitry with the CNS.⁴⁴

Insulin and Amylin. Glucose entry into circulation from meal absorption triggers a proportional release of the peptide hormones insulin and amylin from pancreatic β -cells.⁴⁵ Circulating insulin induces cellular uptake and utilization of glucose and glycogen synthesis,⁴⁶ and enters the CNS through active transport across the BBB⁴⁷ or through fenestrations in the BBB at the MBH and DVC to promote satiety and increasing energy expenditure.⁴⁸ Amylin enhances satiation by slowing gastric emptying⁴⁹ and mediates satiety through its actions in AP of the DVC.⁵⁰

Cholecystokinin. As food enters the proximal small intestine, enteroendocrine I-cells in the duodenum and proximal jejunum respond to nutrients, most strongly to long-chain free fatty acids (FFAs), by secreting CCK, an 8-amino-acid peptide with paracrine and endocrine hormone actions. Locally, CCK binds receptors in the pylorus to promote delayed gastric emptying, and stimulates vagal afferent receptors within the proximal intestine that transmit signals to the NTS and AP of the DVC to induce satiation.⁵¹ CCK also circulates within the bloodstream to the BBB-fenestrated MBH and DVC to suppress hunger and promote meal termination.⁵²

Peptide YY, Glucagon-Like Peptide-1, and Oxyntomodulin. As food enters the lower GI tract, enteroendocrine L-cells residing primarily in the ileum and colon become activated

and secrete the peptide hormones peptide YY (PYY), GLP-1, and oxyntomodulin into circulation.⁵³ The segment of intestine that secretes PYY, GLP-1, and oxyntomodulin is both downstream and considerably longer than that of the region where CCK is released, and thus the passage of food through this later segment of the gut leads to prolonged satiety between meals.⁵⁴

PYY circulates within the bloodstream to the brain as peptide fragments, predominantly as the 34-amino-acid PYY₃₋₃₆,⁵⁵ and binds type-2 neuropeptide Y receptors (Y2Rs), predominantly in the ARC,⁵⁶ where it suppresses orexigenic NPY signaling and activates anorexigenic POMC signaling, together inducing satiation and satiety. PYY also acts in a paracrine fashion by locally activating intestinal afferent vagal neurons that transmit anorexigenic signals to the DVC.⁵⁷

GLP-1 is produced by prohormone convertase 1 (PC1)-mediated proteolytic cleavage of proglucagon within L-cells, and acts on the stomach to slow gastric emptying, contributing to satiation through food retention. GLP-1 also circulates to the MBH and DVC where it activates its receptor to induce satiation and satiety. In addition to these effects on energy homeostasis, GLP-1 also acts as an incretin (enhancer of glucose-dependent insulin secretion) upon binding of GLP-1 receptors expressed in pancreatic β -cells, thereby enhancing insulin-mediated negative energy balance.⁵⁸

Oxyntomodulin is also produced through PC1-mediated proteolytic cleavage of proglucagon as a separate product from GLP-1.⁵⁹ Oxyntomodulin activates the GLP-1 receptor, but appears to act as a biased agonist that preferentially engages different intracellular signaling pathways from that of GLP-1, resulting in extended actions on satiation and satiety.⁶⁰ Oxyntomodulin also activates the glucagon receptor, which stimulates energy expenditure through increased thermogenesis.⁶¹

Pancreatic Polypeptide. Food ingestion stimulates, via CCK and vagal efferent signals delivered to the pancreas, the secretion of pancreatic polypeptide (PP) from pancreatic PP-cells into circulation.^{62,63} Circulating PP slows gastric emptying and mediates satiety by activating type-4 NPY receptors (Y4Rs) in the hypothalamus that increase anorexigenic POMC neuronal activity and decrease orexigenic NPY expression.^{64–66} Postprandial PP levels remain elevated for several hours, likely persisting into the postabsorptive phase and mediating satiety.⁶⁷

Bile Acids and Fibroblast Growth Factor 19. Bile acids are synthesized from cholesterol in the liver and are secreted into the duodenum after a meal, serving as lipid emulsifiers and signaling molecules of nutritional status via the G-protein-coupled receptor 19 (GPCR19) and farnesoid X-activated receptor (FXR). GPCR19 and FXR are expressed in the small intestine, liver, and adipose tissue. Activation of GPCR19 stimulates GLP-1 secretion and increases colonic peristalsis. Activation of FXR induces fibroblast growth factor 19 (FGF19) secretion into the bloodstream. FGF19 regulates lipid and glucose metabolism within the liver and also acts within the CNS to reduce food intake and increase energy expenditure.¹²

Leptin. Adipocytes secrete the 167-amino-acid peptide hormone leptin in proportion to the amount of stored body fat.^{68,69} Typically, this secretion follows a circadian pattern with higher levels at night during sleep.⁷⁰ However, when the post-absorptive phase progresses into extended fasting and subsequent starvation, leptin levels rapidly decrease, leading to potent food-seeking behavior.^{71–76} This striking effect indicates that an acute drop in leptin serves as a starvation signal and confirms leptin's primary role as a protector of energy stores rather than as a preventer of obesity.⁷⁷ Importantly, this acute effect is achieved regardless of basal leptin levels, thus starvation or extended fasting induces the same metabolic adaptations and behaviors in individuals with and without obesity.

Leptin secretion is enhanced by markers of nutrient availability, including glucose, insulin, and cortisol, which all rise with meal intake,^{78–80} and is suppressed by catecholamines released with activation of the sympathetic nervous system (SNS, consistent with the “fight or flight” response requiring diversion of attention away from “rest and digest” behaviors).⁸¹ Leptin is a pre-requisite signal of sufficient energy stores to permit initiation of high-energy processes, such as puberty and pregnancy.^{82,83} Programming of relative leptin concentrations by early caloric intake may be one mechanism that links early over nutrition with later obesity.⁸⁴

Leptin accesses the CNS through the BBB fenestrations of the MBH and DVC and is also actively transported across the BBB.^{85,86} Leptin's primary site of action is the MBH but it also acts in other regions in the CNS and periphery. Leptin receptors are expressed by white adipocytes suggesting paracrine autoregulation,⁸⁷ liver hepatocytes,⁸⁸ pancreatic islet cells including insulin-secreting β -cells,⁸⁹ neurons throughout the brain, and a portion of brain vascular endothelial cells (cerebrovascular cells) that form the BBB.^{90–92} The leptin receptor (a member of the cytokine receptor superfamily) has four isoforms, formed by differential messenger ribonucleic acid (mRNA) splicing: ObRa, an isoform with a shortened intracellular domain, which may function as a transporter; ObRb, the intact full-length receptor; ObRc, also with a short intracellular domain; and ObRe, without an intracellular domain, but which may function as a soluble receptor.⁹³ Leptin receptor (LepR) activation leads to three primary neuronal signals. The first is the opening of an ATP-sensitive potassium channel, which hyperpolarizes the neuron and decreases its firing rate.⁹⁴ The second is the activation of a cytoplasmic Janus kinase 2 (JAK2), which phosphorylates a tyrosine moiety on proteins of a family called *signal transducers and activators of transcription* (STAT-3).⁹⁵ The phosphorylated STAT-3 translocates to the nucleus, where it promotes leptin-dependent gene transcription.⁹⁶ However, leptin also activates the insulin receptor substrate 2/phosphatidylinositol-3-kinase (IRS-2/PI3K) second messenger system, which increases neurotransmission of the central anorexigenic signaling pathway.⁹⁷

LepR activation in hypothalamic and brainstem regions suppresses the activity of orexigenic neurons and activates anorexigenic activity that increases energy expenditure.^{98–102} LepR activation increases SNS efferent tracts connected to adipocytes and acts to reduce leptin secretion, suggesting that leptin, like classic hormones, may be autoregulated by an endocrine feedback loop.¹⁰³ Leptin also appears to have a role in regulating energy thermogenesis through reduction in the thermoregulatory tolerance of colder temperatures, in essence increasing the body's thermostat to a higher temperature set-point.^{104,105} Thus when leptin levels are reduced during fasting, body temperature is not defended as strongly and thermogenic energy expenditure is therefore decreased to conserve energy stores.

Central Processing

The peripheral afferent signals outlined earlier reach the CNS and act primarily within the hypothalamus and brainstem, where they are integrated by a gated neural circuit, designed to promote net catabolic or anabolic effects (see Fig. 24.2).

Central Catabolic Signals

POMC/CART Neurons. The ARC houses neurons that coexpress POMC and CART. POMC is a peptide that is proteolytically cleaved by PC1 and carboxypeptidase E (CPE) to form different peptides depending on neuron type and location. Cleaved products include β -endorphin, adrenocorticotrophic hormone (ACTH), and, in ARC POMC-expressing neurons

(ARC^{POMC}), α -melanocyte-stimulating hormone (α -MSH). Both overfeeding and peripheral leptin infusion induce the synthesis of POMC and α -MSH within the ARC.¹⁰⁶ ARC^{POMC} neurons are also directly activated by insulin, glucose, and serotonin.¹⁰⁷ ARC^{POMC} neurons are inhibited by ghrelin and AgRP neurons.¹⁰⁸ α -MSH induces anorexia by binding to receptors within the paraventricular hypothalamic nucleus (PVN) and lateral hypothalamus (LHA). CART is a hypothalamic neuropeptide induced by leptin and reduced by fasting. Intrahypothalamic infusion of CART blocks appetite, whereas antagonism of endogenous CART increases caloric intake.¹⁰⁹

Melanocortin Receptors. The cleaved peptide products of POMC bind to and activate various 7-transmembrane G-protein-coupled melanocortin receptors (MCRs): MC1R in skin and hair stimulates production of the dark pigment, melanin; MC2R in the adrenal glands stimulates production of glucocorticoids; and melanocortin-4 receptor (MC4R) and melanocortin-3 receptor (MC3R) in the CNS induce negative energy balance. Activation of MC4R in the PVN and LHA results in a state of satiety, whereas intracerebroventricular (ICV) administration of MC4R antagonists in rodents stimulates feeding.¹¹⁰ MC4R-null mice display severe hyperphagia and obesity. MC3R-null mice display milder obesity and are not hyperphagic but appear to have higher feeding efficiency and greater fat partitioning.^{111–113}

Brain-Derived Neurotrophic Factor. Brain-derived neurotrophic factor (BDNF) is an activity-dependent neurotrophin that regulates synaptic plasticity.¹¹⁴ BDNF has additionally been shown to play an important role in energy homeostasis as a downstream mediator of the leptin-melanocortin pathway.¹¹⁵ BDNF expression in the VMH is regulated by nutritional state and MC4R signaling.^{116,117} Selective deletion of BDNF from VMH and dorsomedial hypothalamic nucleus (DMH) of adult mice causes hyperphagia and obesity,¹¹⁶ whereas infusion of BDNF into VMH of wild-type (WT) rats reduces food intake and increases energy expenditure.^{118,119} In the anterior PVN, BDNF suppresses food intake and increases locomotor activity, whereas in medial and posterior PVN, BDNF stimulates thermogenesis through increased SNS outflow.¹²⁰ BDNF haploinsufficiency in both humans and rodents is associated with obesity, which in mice, can be prevented with pair-feeding, suggesting hyperphagia as the primary driver of weight gain.^{121–125} BDNF dysfunction may also contribute to overeating behaviors found in Prader-Willi syndrome (PWS) and Smith-Magenis syndrome (SMS), as well as in common eating disorders.^{126–131} The BDNF Val66Met polymorphism, which impairs activity-dependent BDNF secretion, is linked to binge episodes in bulimia nervosa and binge eating disorder,¹³² and BDNF hypermethylation is associated with bulimia nervosa.¹³³ The intronic BDNF rs12291063 variant (homozygous in ~10% of individuals with African ancestry) is associated with reduced VMH BDNF expression and increased adiposity.¹³⁴ Together these observations indicate that BDNF insufficiency may underlie common, as well as rare causes of overeating behaviors.

Tropomyosin-Related Kinase B Receptor. Tropomyosin-related kinase B (TrkB) is encoded by the *NTRK2* gene and is the cognate receptor for BDNF. Chemogenetic activation of TrkB-expression neurons in the DMH suppresses feeding during the dark cycle when mice are physiologically hungry, whereas chemogenetic inhibition of these neurons promoted feeding during the light cycle when mice are physiologically satiated. Selective *Ntrk2* deletion in the DMH of adult mice induces hyperphagia, decreased energy expenditure, and obesity.¹³⁵

Norepinephrine. Norepinephrine (NE) neurons in the locus coeruleus synapse on VMH neurons to regulate food intake.¹³⁶ In rodents, the actions of NE on food intake seem paradoxical, as intrahypothalamic NE infusion stimulates food intake through

effects on central α_2 - and β -adrenergic receptors,¹³⁷ whereas central infusion of α_1 -agonists markedly reduces food intake.¹³⁸

In human studies using molecular neuroimaging, NE transporter availability was negatively associated with perceptions of hunger and the strength of this association was stronger among individuals with obesity compared with normal-weight controls, suggesting that NE has a role in modulating hunger.¹³⁹

Serotonin (5-hydroxytryptamine). 5-Hydroxytryptamine (5-HT) has been implicated in the perception of satiety based on many lines of evidence: (1) injection of 5-HT into the hypothalamus increases satiety, particularly with respect to carbohydrate¹⁴⁰; (2) central administration of 5-HT_{2c} receptor agonists increase satiety, whereas antagonists induce feeding¹⁴¹; (3) administration of selective 5-HT reuptake inhibitors induce early satiety¹⁴²; (4) leptin increases 5-HT turnover¹⁴³; and (5) the 5-HT_{2c}R-KO mouse exhibits increased food intake and body weight.¹⁴⁴ The role of 5-HT in the transduction of the satiety signal may have both central and peripheral components, as intestinal 5-HT is secreted into the bloodstream during a meal, where it may have an impact on GI neuronal function and muscle tone, and may bind to 5-HT receptors in the NTS (discussed previously) to promote satiety.¹⁴⁵ Molecular neuroimaging in humans suggests that obesity is driven by decreased serotonin-mediated homeostatic feedback in response to food intake.¹⁴⁶

Central Anabolic Signals

AgRP/NPY Neurons. NPY and AgRP colocalize to a different set of neurons within the ARC, immediately adjacent to those expressing POMC/CART.¹⁴⁷ The ARC houses the only population of neurons that express AgRP, and the majority of these neurons coexpress NPY.¹⁴⁷ These two orexigenic peptides are secreted from nerve terminals as peptide neurotransmitters, in addition to the inhibitory small-molecule neurotransmitter gamma-aminobutyric acid (GABA). AgRP is the human homolog of the protein agouti, which is present in abundance in the Agouti yellow mouse (A^y-a).¹⁴⁸ AgRP is an endogenous competitive antagonist of all MCRs, accounting for the yellow color in these mice because of its inhibitory actions at the MCR1 receptors of the fur. AgRP also antagonizes MC4R and MC3R, thereby attenuating the ability of α -MSH to suppress appetite and fat deposition.¹⁴⁹ AgRP neurons also suppress SNS-mediated “browning” of white adipose tissue which would otherwise increase energy expenditure,^{150,151} thus reducing thermogenesis and promoting positive energy balance. AgRP neurons are directly activated by the orexigenic hormone ghrelin¹⁵², dopamine, which may originate from dopamine neurons residing within the ARC¹⁵³; and several glutamatergic projections from other hypothalamic nuclei including the DMH (which exhibits leptin BBB transport and activity) and the PVH.¹⁵⁴ AgRP neurons are inhibited by leptin,¹⁵⁵ insulin,¹⁵⁶ PYY,⁵⁵ and by GABAergic projections from the DMH and other arcuate neurons.¹⁵⁷

NPY has numerous functions within the hypothalamus, including initiation of feeding, regulation of gonadotropin secretion, and modulation of adrenal responsiveness.^{158,159} NPY acts as an orexigen and it also stimulates adipogenesis.¹⁶⁰ ICV infusion of NPY in rats rapidly leads to hyperphagia, energy storage, and obesity,^{161,162} mediated through Y₁ and Y₅ receptors. Fasting and weight loss increase NPY expression in the ARC, accounting for increased hunger, whereas PYY_{3–36} (through Y₂ receptors) and leptin decrease NPY mRNA.^{55,163}

Melanin-Concentrating Hormone. Melanin-concentrating hormone (MCH) is a 17-amino-acid orexigenic peptide expressed in the zona incerta and LHA. MCH-knockout mice are hypophagic and lean,¹⁶⁴ whereas transgenic MCH-overexpressing mice develop obesity and insulin resistance.¹⁶⁵ ICV

administration of MCH stimulates food intake, similar to that seen with NPY administration.¹⁶⁶ Interestingly, endogenous MCH circulation within the cerebrospinal fluid (CSF) may in fact represent an alternative neural communication mechanism in the regulation of food intake with rise in CSF concentrations of MCH driving the initiation of food intake.¹⁶⁷

Orexins A and B. Orexin A and B are orexigenic peptides (33 and 28 amino acids in length, respectively) produced within the hypothalamus and modulate both energy balance and autonomic function in mice.^{168–170} Orexins stimulate release of NPY, corticotropin-releasing hormone (CRH), and SNS outflow leading to increased food intake, wakefulness, blood pressure, and energy expenditure. The orexins also appear to bridge the homeostatic and non-homeostatic mechanisms that regulate food intake and may play a role in reward-based learning and memory.¹⁷¹ Orexin neurons in the LHA process the hedonic aspects of food and drugs of abuse, whereas orexin neurons in the perifornical and DMH regulate arousal and stress response.¹⁷²

Endocannabinoids. Tetrahydrocannabinol, the main psychotropic component of marijuana, has long been known to stimulate food intake. The endogenous endocannabinoid (EC) receptor, CB₁, is expressed in corticotropin-releasing hormone (CRH) neurons in the PVN, in CART neurons in the VMN, and in MCH- and orexin-positive neurons in the LHA and perifornical region.¹⁷³ Fasting and feeding are associated with high and low levels of ECs in the hypothalamus, respectively. For example, CB₁ receptor-knockout mice have increased CRH and reduced CART expression. In the leptin-deficient *ob/ob* mice, hypothalamic EC levels are increased, whereas leptin infused intravenously reduces these levels, indicating that a direct negative control is exerted by leptin on the EC system. Glucocorticoids increase food intake by stimulating EC synthesis and secretion, whereas leptin blocks this effect.¹⁷⁴ Also the presence of CB₁ receptors on afferent vagal neurons suggests that EC may be involved in mediating satiety signals originating in the gut.¹⁷⁵

The Efferent System

The MCRs in the PVN and LHA transduce the anorexigenic and orexigenic information coming from the VMH, to modulate activity of the SNS, and the efferent vagus, which promotes energy storage (Fig. 24.4). In this way, peripheral energy balance can be modulated acutely to provide requisite energy for metabolic needs, and store the rest.

The Sympathetic Nervous System and Energy Expenditure

Anorexigenic pressure increases energy expenditure through activation of the SNS.¹⁷⁶ For instance, leptin administration to leptin-deficient *ob/ob* mice promotes increased brown adipose tissue lipolysis, thermogenesis, renovascular activity, and increased movement, all associated with increased energy expenditure and enhanced weight loss.¹⁷⁷ Similarly, insulin administration acutely increases SNS activity in normal rats and in humans.^{178,179} The SNS increases energy expenditure in four ways: (1) by innervating the hypothalamus and appetite centers in the medulla to reduce appetite, (2) by increasing thyroid-stimulating hormone (TSH) secretion to increase thyroid hormone release and energy expenditure, (3) by innervating skeletal muscles to increase energy expenditure, and (4) by innervating β_3 -adrenergic receptors in white adipose tissue to promote lipolysis.

Activation of the SNS increases energy expenditure by the skeletal muscle, by activating β_2 -adrenergic receptors,¹⁸⁰ which in turn increase the expression of numerous genes in skeletal muscle,¹⁸¹ especially those involved in carbohydrate metabolism. SNS activation stimulates glycogenolysis, incites

myocardial energy expenditure, increases in glucose and fatty acid oxidation, and increases protein synthesis.¹⁸²

Activation of the SNS in rodents stimulates the β_3 -adrenergic receptor of brown adipose tissue to promote lipolysis.¹⁸³ In humans, activation of the β_3 -adrenergic receptor increases cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA). PKA acts in two separate molecular pathways to increase energy expenditure. First, PKA phosphorylates the cAMP response element binding protein (CREB), which induces expression of peroxisome proliferator-activated receptor (PPAR) γ -coactivator-1 α (PGC-1 α). PGC-1 α then binds to enhancer elements on the uncoupling protein-1 (UCP1) gene, which increases the expression and activity of uncoupling proteins (UCPs) 1 and 2.^{184,185} UCPs reduce the proton gradient across the inner membranes of mitochondria, thereby diverting protons from storage in the form of ATP to heat production. Originally, UCPs were discovered in brown adipose tissue and were found to be responsible for thermogenesis. UCP1 is an inner membrane mitochondrial protein that uncouples proton entry from ATP synthesis;¹⁸⁶ therefore UCP1 expression dissipates energy as heat, thus reducing the energy efficiency of the adipose tissue. However, UCP2 has been found in most tissues and UCP3 in skeletal muscle. Second, PKA activation activates the enzyme hormone-sensitive lipase (HSL), which is responsible for lipolysis of intracellular triglyceride to its component FFAs. The FFAs also induce UCP1, further increasing energy expenditure. The FFAs released from the adipocyte also travel to the liver where they are used for energy by being metabolized into two-carbon fragments. Lipolysis reduces leptin expression; thus a negative feedback loop is achieved between leptin and the SNS (see Fig. 24.4).

The Efferent Vagus and Energy Storage

In response to declining levels of leptin or persistent orexigenic pressure, the LHA and PVN send efferent projections residing in the medial longitudinal fasciculus to the DMV, activating the efferent vagus.¹⁸⁷ The efferent vagus opposes the SNS by promoting energy storage in four ways: (1) by slowing the heart rate, myocardial oxygen consumption is reduced; (2) the vagus nerve promotes alimentary peristalsis, pyloric opening, and energy substrate absorption; (3) through direct effects on the adipocyte, the vagus nerve promotes insulin sensitivity to increase the clearance of energy substrate into adipose tissue; and (4) through effects on the β -cells, the vagus increases postprandial insulin secretion,^{12,188–191} which promotes energy deposition into adipose tissue.

Retrograde tracing of white adipose tissue reveals an abundance of efferents originating at the DMV.¹⁹¹ These efferents synapse on the M₁ muscarinic receptor on the adipocyte, which increases insulin sensitivity of the adipocyte. Denervation of white adipose tissue results in a reduction of glucose and FFA uptake, and an induction of HSL, which promotes lipolysis—both of which reduce the efficiency of insulin-induced energy storage. Thus vagal modulation of the adipocyte augments storage of both glucose and FFAs by improving adipose insulin sensitivity¹⁹² (see Fig. 24.4).

The DMV also sends efferent projections to the β -cells of the pancreas.¹⁹³ This pathway is responsible for the “cephalic” or preabsorptive phase of insulin secretion, which is glucose independent and can be blocked by atropine.¹⁹⁴ Overactive vagal neurotransmission increases insulin secretion from β -cells in response to an oral glucose load through three distinct but overlapping mechanisms¹⁹⁵ (see Fig. 24.4):

1. Vagal firing increases acetylcholine availability and binding to the M₃ muscarinic receptor on the β -cell, which is coupled to a sodium channel within the pancreatic β -cell membrane.¹⁹⁶ As glucose enters the β -cell after ingestion of a

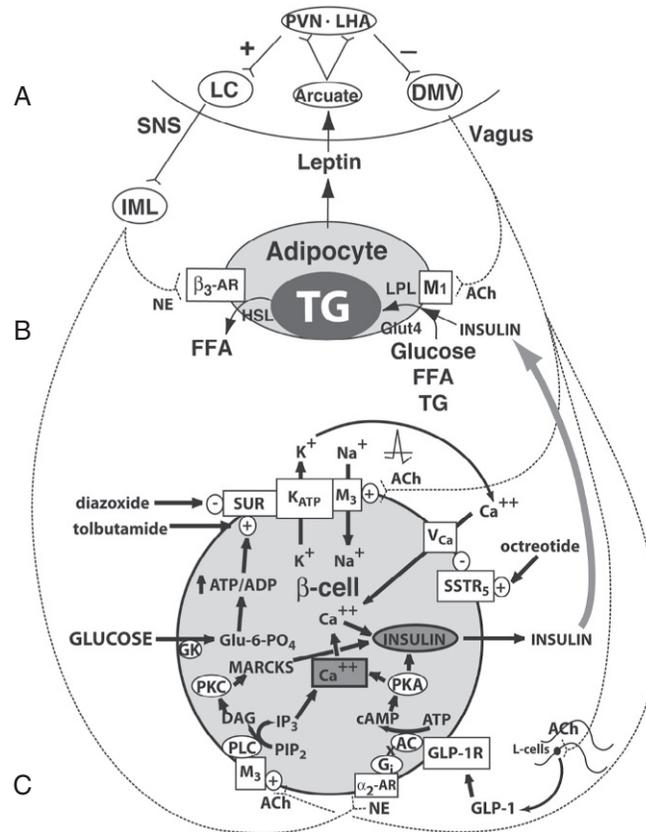


Fig. 24.4 Central regulation of leptin signaling, autonomic innervation of the adipocyte and β -cell, and the starvation response. (A) The arcuate nucleus transduces the peripheral leptin signal as one of sufficiency or deficiency. In leptin sufficiency, efferents from the hypothalamus synapse in the locus coeruleus, which stimulates the sympathetic nervous system. In leptin deficiency or resistance, efferents from the hypothalamus stimulate the dorsal motor nucleus of the vagus. (B) Autonomic innervation and hormonal stimulation of white adipose tissue. In leptin sufficiency, norepinephrine binds to the β_3 -adrenergic receptor, which stimulates hormone-sensitive lipase, promoting lipolysis of stored triglyceride into free fatty acids. In leptin deficiency or resistance, vagal acetylcholine increases adipose tissue insulin sensitivity (documented only in rats to date), promotes uptake of glucose and free fatty acids for lipogenesis, and promotes triglyceride uptake through activation of lipoprotein lipase. (C) Autonomic innervation and hormonal stimulation of the β -cell. Glucose entering the cell is converted to glucose-6-phosphate by the enzyme glucokinase, generating adenosine triphosphate (ATP), which closes an ATP-dependent potassium channel, resulting in cell depolarization. A voltage-gated calcium channel opens, allowing for intracellular calcium influx, which activates neurosecretory mechanisms leading to insulin vesicular exocytosis. In leptin sufficiency, norepinephrine binds to α_2 -adrenergic receptors on the β -cell membrane to stimulate inhibitory G-proteins, decrease adenylyl cyclase and its product cyclic adenosine monophosphate (cAMP), and thereby reduce protein kinase A levels and insulin release. In leptin deficiency or resistance, the vagus stimulates insulin secretion through three mechanisms.⁸⁰⁵ First, acetylcholine binds to an M_3 muscarinic receptor, opening a sodium channel, which augments the ATP-dependent cell depolarization, increasing the calcium influx, and insulin exocytosis. Second, acetylcholine activates a pathway that increases protein kinase C, which also promotes insulin secretion. Third, the vagus innervates L-cells of the small intestine, which secrete glucagon-like peptide-1, which activates protein kinase A, contributing to insulin exocytosis. Octreotide binds to a somatostatin receptor on the β -cell, which is coupled to the voltage-gated calcium channel, limiting calcium influx and the amount of insulin released in response to glucose (reprinted with kind permission of Springer Science and Business media). α_2 -AR, α_2 -Adrenergic receptor; β_3 -AR, β_3 -adrenergic receptor; AC, adenylyl cyclase; ACh, acetylcholine; DAG, diacylglycerol; DMV, dorsal motor nucleus of the vagus; FFA, free fatty acids; G_i , inhibitory G-protein; GK, glucokinase; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; Glu-6-PO₄, glucose-6-phosphate; GLUT4, glucose transporter-4; HSL, hormone-sensitive lipase; IML, intermediolateral cell column; IP₃, inositol triphosphate; LC, locus coeruleus; LHA, lateral hypothalamic area; LPL, lipoprotein lipase; MARCKS, myristoylated alanine-rich protein kinase C substrate; NE, norepinephrine; PIP₂, phosphatidylinositol pyrophosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PVN, paraventricular nucleus; SUR, sulfonylurea receptor; SSTR₅, somatostatin-5 receptor; TG, triglyceride; V_{Ca}, voltage-gated calcium channel. (From Lustig, R.H. (2006). Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the first law of thermodynamics. *Nat Clin Pract Endo Metab*, 2, 447–458. With permission from Nature Publishing Group.)⁹⁰⁴

meal, the enzyme glucokinase phosphorylates glucose to form glucose-6-phosphate, increasing intracellular ATP, which induces closure of the ATP-dependent potassium channel. Upon channel closure, the β -cell experiences an ATP concentration-dependent β -cell depolarization^{197,198} and the opening of a separate voltage-gated calcium channel within the membrane. Intracellular calcium influx increases acutely, which results in rapid insulin vesicular exocytosis. Concomitant opening of the sodium channel by vagally mediated acetylcholine augments β -cell depolarization,

which in turn augments the intracellular calcium influx and results in insulin hypersecretion.^{199–201}

2. Vagally mediated acetylcholine increases phospholipases A₂, C, and D within the β -cell, which hydrolyze intracellular phosphatidylinositol to diacylglycerol (DAG) and inositol triphosphate (IP₃).¹⁹⁵ DAG is a potent stimulator of protein kinase C (PKC),²⁰² which phosphorylates myristoylated alanine-rich protein kinase C substrate (MARCKS), which then binds actin and calcium-calmodulin and induces insulin vesicular exocytosis.²⁰³ IP₃ potentiates the release of calcium

within β -cells from intracellular stores, which also promotes insulin secretion.²⁰⁴

3. The vagus also stimulates the release of GLP-1 from intestinal L-cells, which circulates and binds to a GLP-1 receptor within the β -cell membrane. Activation of this receptor induces a calcium-calmodulin-sensitive adenylyl cyclase, with conversion of intracellular ATP to cAMP, which then activates PKA. PKA causes both the release of intracellular calcium stores and the phosphorylation of vesicular proteins, each contributing to an increase in insulin exocytosis.^{205,206}

In the efferent pathway, insulin is responsible for shunting blood-borne nutrients into adipose for storage. Indeed, the primary hormonal signal for adipogenesis is insulin.²⁰⁷ Within the adipocyte, insulin increases: (1) glucose transporter 4 (GLUT 4) expression, (2) acetyl-CoA carboxylase, (3) fatty acid synthase, and (4) lipoprotein lipase.²⁰⁸ Thus the net effect of insulin on the adipocyte is the rapid clearance and storage of circulating glucose and lipid thereby promoting energy storage.

NON-HOMEOSTATIC REGULATION OF ENERGY BALANCE

The non-homeostatic determinants of energy balance are higher order functions that integrate cognitive functioning and environmental cues that prompt nonnutritive reasons for eating. Homeostatic feeding is needed for survival, whereas hedonic feeding is driven by the reward aspects of palatable food.²⁰⁹ In contrast to rodents or other mammals, hedonistic drives in humans may override homeostatic drives and dictate eating behavior.

The Nucleus Accumbens and the Hedonic Pathway of Food Reward

The hedonic pathway comprises the ventral tegmental area (VTA) and the nucleus accumbens (NA), with inputs from various components of the limbic system, including the striatum, amygdala, hypothalamus, and hippocampus. These pathways also mediate the hedonic response to drugs of abuse, such as nicotine and morphine. In fact, administration of morphine to the NA increases food intake in a dose-dependent fashion.²¹⁰ When functional, the hedonic pathway helps curtail food intake in situations where energy stores are replete; however, when dysfunctional, this pathway can increase food intake leading to obesity.

The VTA appears to mediate feeding on the basis of palatability rather than energy need. The dopaminergic projection from the VTA to the NA mediates the motivating, rewarding, and reinforcing properties of various stimuli, such as food and addictive drugs. Leptin and insulin receptors are expressed in the VTA, and both hormones have been implicated in modulating rewarding responses to food and other pleasurable stimuli.²¹¹ For instance, fasting and food restriction (when insulin and leptin levels are low) increase the addictive properties of drugs of abuse, whereas ICV leptin can reverse these effects.²¹² In rodent models of addiction, increased addictive behavior (and pleasurable response from a food reward), as measured by dopamine release and dopamine receptor signaling, is greater after food deprivation.²¹³ In humans with leptin deficiency, alterations in activity in the NA can be seen using functional magnetic resonance imaging (MRI) scanning, and these changes subside with administration of exogenous leptin.²¹⁴ Acutely, insulin increases expression and activity of the dopamine transporter, which clears and removes dopamine from the synapse; thus acute insulin exposure blunts the reward of food.²¹⁵ Furthermore, insulin appears to inhibit the ability

of VTA agonists (e.g., opioids) to increase intake of sucrose.²¹⁶ Finally, insulin blocks the ability of rats to form a conditioned place preference association to a palatable food.²¹⁷ However, insulin resistance of this pathway may lead to increased reward perception of food by way of reduced dopamine clearance from the synapse and prolongation of the postsynaptic hedonistic response.

One question that has garnered increasing interest is whether any macronutrient has addictive properties. In animal studies, sugar has been shown to induce the four criteria for addiction: (1) bingeing, (2) withdrawal, (3) craving, and (4) cross-sensitization with other drugs of abuse.²¹⁸ Within fast food, sugar and caffeine satisfy the criteria presented in the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) for dependence in humans.²¹⁹ However, the question of whether food addiction exists, and whether it can explain patients with obesity, remains contested,²²⁰ although a recent systematic review of the literature supports this notion of “highly-palatable food addiction” based on the criteria of impaired control, social impairment, risky use, and tolerance/withdrawal, at least as a paradigm for consideration in treatment strategies.²²¹

The Amygdala and the Stress Response

The VMH and VTA-NA mediate satiety when energy stores are replete, but they appear to be easily overridden by amygdala activation and resultant stress, a state of physiologic insulin resistance (Fig. 24.5). Numerous lines of evidence suggest that the stress glucocorticoids corticosterone (in the rodent) or cortisol (in the human) are essential for the full expression of obesity, which helps to explain the disruptive role of stress in weight regulation.²²²

Stress and glucocorticoids are integral in promoting adiposity and the metabolic syndrome. Adrenalectomized rats maintained pharmacologically with high levels of corticosterone demonstrate that exogenous fat intake is directly proportional to circulating corticosterone concentrations,²²³ whereas amygdala activation by stress is dampened by the ingestion of energy-dense food.²²⁴ In intact rats, corticosterone stimulates eating, particularly of high-fat food, and in humans, cortisol administration increases food intake.²²⁵ Human research shows increased caloric intake of “comfort foods” (i.e., those with high energy density) after acute stress,²²⁶ and that the stress response contributes to leptin resistance (discussed later).²²⁷ Several studies in children have observed relationships between stress and unhealthy dietary practices, including increased snacking, and an elevated risk for problems with weight during adolescence and adulthood.²²⁸ In a controlled study of 9-year-old children who scored high on dietary restraint and who felt more stressed by laboratory challenges tended to eat more comfort food.²²⁹ Adverse childhood experiences are also associated with later development of obesity and cardiometabolic risk factors suggesting a role of stress in longitudinal weight gain and metabolic health.²³⁰

DYSREGULATION OF ENERGY BALANCE

Leptin Resistance

Most children with obesity have high leptin levels but do not have receptor mutations, manifesting what is commonly referred to as *functional leptin resistance*. Leptin resistance prevents exogenous leptin administration from promoting weight loss.²³¹ The response to most weight-loss regimens plateaus rapidly because of the rapid fall of peripheral leptin levels which induces a “starvation response” immediately, regardless of baseline values, and potentially because of reaching a

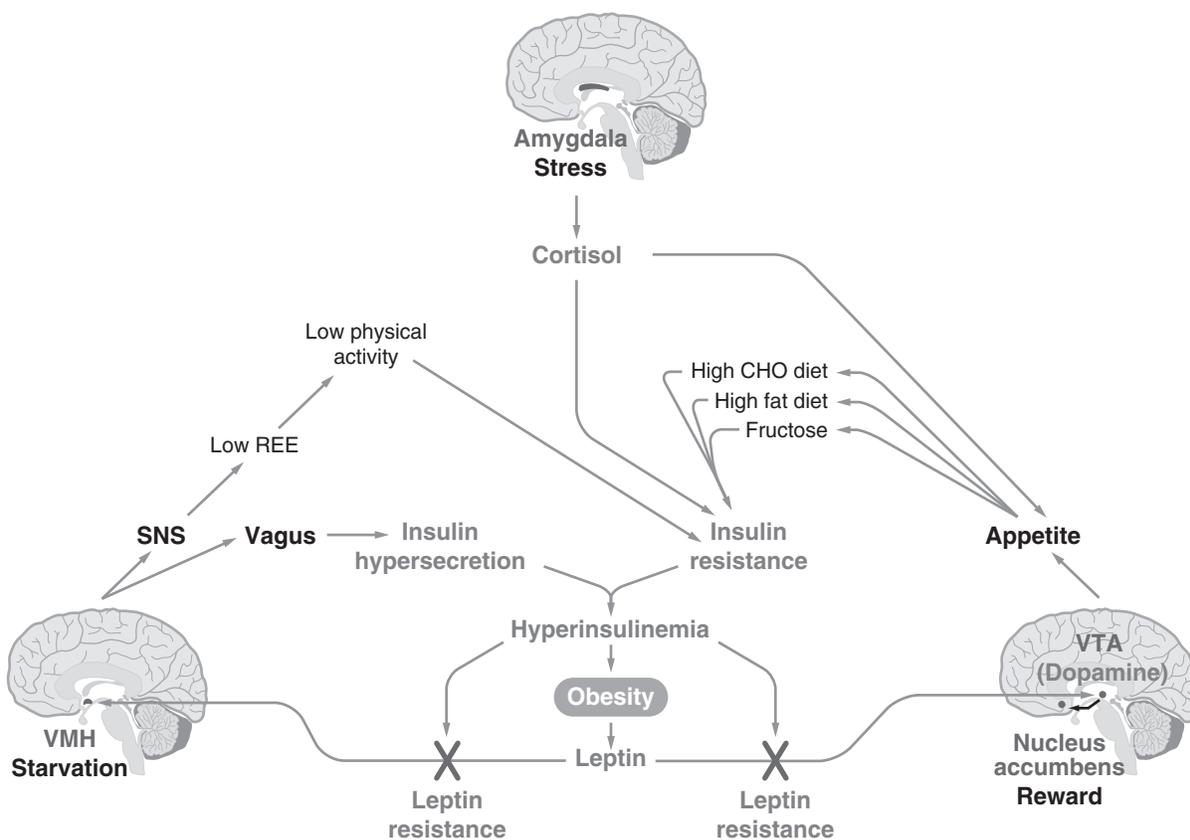


Fig. 24.5 The “limbic triangle.” Three areas of the CNS conspire to drive food intake and reduce physical activity, resulting in persistent weight gain. The ventromedial hypothalamus (VMH) transduces the leptin signal from adipocytes to reduce energy intake and increase energy expenditure; however, hyperinsulinemia prevents leptin signaling, promoting the “starvation response.” The ventral tegmental area (VTA) transduces the leptin signal to reduce dopamine neurotransmission to the nucleus accumbens, reducing food intake; however, hyperinsulinemia prevents leptin signaling here as well, increasing dopamine and promoting the “reward” of food. The amygdala transduces fear and stress, which results in increased cortisol release from the adrenal cortex. The elevated cortisol also drives energy-rich food intake and promotes insulin resistance, further interfering with leptin signaling at the other two central nervous system sites. Thus activation of any aspect of the limbic triangle turns on a positive feedback loop, promoting continued weight gain and obesity. (From Mietus-Snyder, M.L., Lustig, R.H. (2008). Childhood obesity: adrift in the “limbic triangle.” *Ann Rev Med*, 59, 147–162.)⁹⁰⁵

personal “leptin threshold,”²³² which is likely genetically determined. Leptin decline causes the VMH to sense a reduction in peripheral energy stores, which modulates a decrease in resting energy expenditure (REE) to conserve energy, analogous to a starvation response,²³³ but occurring at elevated leptin levels.

The cause of leptin resistance is unknown, but it may have several etiologies.²³⁴ Leptin crosses the BBB via a saturable transporter, which limits the amount of leptin reaching its receptor in the VMH^{235,236}; this transporter operates more efficiently at lower levels of leptin, while preventing increased signaling at higher levels.²³⁷ Activation of the leptin receptor induces the intraneuronal expression of suppressor of cytokine signaling-3 (SOCS-3), which limits leptin signal transduction in an autoregulatory fashion.²³⁸ Because the presence of hyperleptinemia has been shown to be a prerequisite for development of leptin resistance, it has been postulated that leptin-induced expression of leptin signaling inhibitors may be an initial step in the process. Other studies suggest that obesity itself induces hypothalamic inflammation, gliosis, and endoplasmic reticulum (ER) stress that impair responsiveness to leptin.²³⁴

The standard method for producing insulin resistance and obesity in rodents is a high-fat diet. Dietary fat promotes leptin resistance through its effects on hypertriglyceridemia,⁸⁵ which limits access of peripheral leptin to the VMH, and also by interfering with leptin signal transduction upstream of STAT-3, its primary second messenger.²³⁹ One likely modulator of this pathway is the enzyme PI3K, which is the downstream effector

of insulin action in POMC neurons²⁴⁰ and which appears to account for the effects of dietary fat on leptin resistance and obesity.²⁴¹

Two clinical paradigms have been shown to improve leptin sensitivity. After weight loss through caloric restriction, exogenous administration of leptin can then increase REE back to baseline and permit further weight loss,^{242,243} suggesting that the weight loss itself improves leptin sensitivity. Second, suppression of insulin correlates with improvement in leptin sensitivity and promotes weight loss,²⁴⁴ suggesting that hyperinsulinemia promotes leptin resistance by interfering with leptin signal transduction in the VMH and VTA.²⁴⁵ Indeed, insulin reduction strategies can effectively promote weight loss in children with hyperinsulinemia by improving leptin sensitivity.²⁴⁶

Counterregulatory Mechanisms That Oppose Weight Loss

Because the homeostatic system for energy balance was developed to protect against starvation, body fat stores are avidly protected. Therefore dietary restriction, even before the onset of weight loss, triggers counterregulatory mechanisms to oppose the perceived threat of starvation, regardless of baseline adipose tissue stores.⁷¹ Gastric secretion of ghrelin acutely rises, which increases pituitary GH release, to stimulate lipolysis to

provide energy substrate for catabolism. Ghrelin stimulates NPY/AgRP to antagonize α -MSH/CART. Decline of leptin reduces α -MSH/CART as well. This leads to decreased MC4R and MC3R occupancy leading to reduced anorexigenic and catabolic signaling with a net effect of increased feeding behavior and higher energy efficiency (with reduced fat oxidation). Appetite proportionally increases leading to food consumption above baseline by approximately 100 kCal/d per kilogram of lost weight.²⁴⁷ Meanwhile, total and resting energy expenditures decline in an attempt to conserve energy.²³³ Specifically, UCP1 levels within adipose tissue decline²⁴⁸ as a result of decreased SNS activity.²⁴⁹ In spite of decreased SNS tone at the adipocyte, there is clearly an obligate lipolysis (because of insulin suppression and upregulation of HSL), which is necessary to maintain energy delivery to the musculature and brain in the form of liver-derived ketone bodies. In addition, in the weight-reduced state, vagal tone is increased to slow the heart rate and myocardial oxygen consumption, increase β -cell insulin secretion in response to glucose, and increase adipose insulin sensitivity—all directed to increase energy storage.²⁴⁹ These counterregulatory mechanisms together serve to drive weight regain and can even persist for years after the initial onset of weight loss,²⁵⁰ therefore rendering maintenance of reduced body weight exceedingly challenging especially in light of the feed-forward mechanisms described later. In other words, the metabolic adaptations aimed at conserving energy and returning to the body weight before weight loss are maintained for years following the weight loss and achievement of weight plateau rendering the individual prone to weight gain. Upon comparison of two individuals with similar weight and body composition, a weight-stable patient and a patient who lost weight to achieve this measurement, to maintain the current body weight—the patient who lost weight will have to consume less energy and spend more energy in comparison with the weight-stable counterpart.

Feed-Forward Mechanism That Promote Weight Gain

Feed-forward signaling bridges homeostatic and hedonic mechanisms of appetite regulation, a concept developed based on the observation that a hungry mouse will have appropriately elevated AgRP neuronal firing in the fasted state but these AgRP neurons are then acutely suppressed when the mouse is presented with food, even before the onset of eating.⁷ In addition, gut signals are also released that anticipate the imminent arrival of ingested food, setting in motion, digestive processes before actual food intake. Because hunger is an aversive experience, rapid reduction in AgRP firing and the sudden removal of hunger sensations induce an acute reward experience, perhaps serving as a positive reinforcer of the environmental cue of food availability. For example, readily visible and appealing packaging of processed foods encourages hedonistic consumption and establishes a learned behavior that prompts subsequent return to the environment where such foods were available. Along the same lines, fast food restaurants and advertising targeting youth rely heavily on connecting images of food with other pleasurable stimuli (toys, games, fun, etc.), further compounding the reward effect of already highly palatable food.²⁵¹ This feed-forward mechanism has potential implications as we consider the role of the built environment and its role in promoting food consumption.

ENERGY EXCESS—OBESITY

The rise in the prevalence of obesity in children and adolescents is one of the most alarming public health issues facing the

world today. Although the rise in the prevalence of obesity in children and adolescents seems to have leveled in some parts of the world,²⁵² many others, especially developing countries and migrant populations,²⁵³ are still experiencing a steady increase. Obesity is associated with significant health problems in children and is an early risk factor for much of adult non-communicable disease morbidity and mortality,²⁵⁴ and an important factor in increasing healthcare expenditures.²⁵⁵ Childhood obesity tends to track into adulthood,²⁵⁶ and those who continue to have obesity as adults have a significant risk for the development of obesity-driven morbidity with an excess risk associated with the length of exposure to obesity.²⁵⁷ In contrast, children with obesity who lost weight and became non-obese adults do not have an increased risk for such morbidity.²⁵⁸ These observations identify obesity in early childhood as a major window of opportunity for obesity prevention efforts with a potential lifelong impact.

Definition

The theoretical definition of obesity is a degree of somatic overweight that affords detrimental health consequences.²⁵⁹ Based on morbidity and mortality statistics, and with a desire to prevent future risk of morbidity, we practically define obesity as a statistical magnitude of overweight for a population, keeping in mind that morbidity and mortality vary with degree of overweight in different racial, ethnic, and socioeconomic groups.²⁶⁰

The majority of obesity in adulthood has its origins in childhood,^{261,262} making obesity a pediatric concern and the prevention and treatment of obesity a pediatric goal. Body mass index (BMI) is also the accepted marker in children.²⁶³ In childhood, comparison of BMI with normal curves for age²⁶⁴ allows for categorization of BMI above the 85th percentile as overweight, and above the 95th percentile as obesity (Fig. 24.6A and B). The World Health Organization (WHO)²⁶⁵ categorizes adult overweight into four subgroups based on BMI (weight [kg] \div height [m]²): BMI 25 to 30 (overweight); BMI 30 to 35, Grade 1 (moderate obesity); BMI 35 to 40, Grade 2 (severe obesity); and BMI over 40, Grade 3 (morbid obesity). A similar degree of obesity categorization can also be used in the pediatric population using BMI centiles where the 95th centile for age and sex is used as a reference point and 100% to 120% of the 95th percentile for age and sex is grade 1, 120% to 140% is grade 2, and over 140% is grade 3.²⁶⁶ Such categorization demonstrates that cardiometabolic risk increases with rising degrees of obesity.²⁶⁶

Although BMI is the standard indicator of obesity for statistical purposes and within populations, it should be noted that BMI does not take into account body composition parameters such as total body fat, (as well as both subcutaneous and visceral fat), muscle, and bone. Furthermore, BMI in children is age, sex, and puberty dependent, thus BMI z-score is a more accurate assessment of childhood adiposity. Lastly, waist circumference (an indirect measure of intraabdominal visceral fat) has emerged as a more accurate indicator of metabolic disturbance in children.²⁶⁷ These limitations of BMI indicate that it is a useful index for population and epidemiologic studies yet should be used with caution when assessing an individual child in the clinical setting.

Prevalence and Epidemiology

The prevalence of childhood obesity in the United States has increased dramatically during the past 30 years,^{268,269} and continues to do so, although the comparison of longitudinal and cross-sectional data is difficult because of different definitions and measurement parameters between epidemiologic studies. The most recent estimates of obesity prevalence and trends in

the United States are based on data from the 2011 to 2014 National Health and Nutrition Examination Survey (NHANES V).^{252,270} NHANES demonstrates that the epidemic of childhood obesity in the United States seems to have stabilized in some age groups but not in others. Overall, in 2013 to 2014, 9.4% (95% confidence interval [CI], 6.8–12.6) of infants and toddlers and 17% (95% CI, 15.5–18.6) of children and adolescents from 2 through 19 years of age had obesity. Of note, the prevalence of extreme obesity (>120% of 95th percentile for age and sex) was 5.8% (95% CI, 4.9–6.8). Trend analyses over a 25-year period indicated a significant increase in obesity prevalence among children aged 2 to 5 years between 1988 and 1994 and 2003 and 2004 that slightly declined in 2013 to 2014 (7.2%, 13.9%, and 9.4%, respectively). Among children aged 6 to 11 years old, the prevalence of obesity increased from 1988 to 1994 to 2007 to 2008 and remained stable in 2013 to 2014 (11.3%, 19.6%, and 17.4%, respectively). Among adolescents 12 to 19 years of age, the prevalence of obesity significantly increased from 1988 to 1994 to 2013 to 2014 (10.5% and 20.6%, respectively, $P < .001$). Of note, the prevalence of extreme obesity increased among children 6 to 11 years old between 1988 and 1994 to 2013 and 2014 (3.6% and 4.3%, respectively, $P = .02$) and among adolescents aged 12 to 19 years (2.6% and 9.1%, respectively, $P < .001$). Importantly, no significant trends were observed between 2005 and 2006 and 2013 and 2014. The practical implication of these trends is for example in 1988 to 1994, the 95th percentile of BMI among 17-year-old males was 31.5 kg/m² (i.e., 5% of males had a BMI >31.5), and in 2011 to 2014 the 95th percentile was 36.2 kg/m² (i.e., 5% of males had a BMI >36.2). Thus between 1988 and 1994 and 2013 and 2014, the prevalence of obesity increased until 2003 to 2004 and then decreased in children aged 2 to 5 years, increased until 2007 to 2008 and then leveled off in children aged 6 to 11 years, and increased among adolescents aged 12 to 19 years. In 2013 to 2014, 17.4% of children met criteria for class I obesity, including 6.3% for class II and 2.4% for class III. A clear, statistically significant increase in all classes of obesity continued from 1999 through 2014.²⁷¹ In the United States, obesity and severe obesity among children significantly increased with greater age and lower education of household head, and severe obesity increased with lower level of urbanization. Compared with non-Hispanic white youth, obesity and severe obesity prevalence were significantly higher among non-Hispanic black and Hispanic youth. Severe obesity, but not obesity, was significantly lower among non-Hispanic Asian youth than among non-Hispanic white youth.²⁷² Lastly, projections argue that by 2030, 42% of American adults will have obesity.²⁵⁵

Global Prevalence

Obesity has overtaken acquired immunodeficiency syndrome and malnutrition as the number one public health problem in the world.²⁷³ The global prevalence of childhood obesity has been increasing worldwide at an alarming rate during the past 20 years. Rates have increased 2.7 to 3.8-fold over 29 years in the United States,²⁶⁹ 2.0 to 2.8-fold over 10 years in England, 3.4 to 4.6-fold over 10 years in Australia, and 3.4 to 3.6-fold over 23 years in Brazil. European data,²⁷⁴ using slightly different obesity cutoff definitions (a childhood BMI corresponding to >25 and 30 kg/m² in adults signifying overweight and obesity, respectively), suggests that among European countries, prevalence of overweight/obesity combined ranges between 16% and 22% whereas that of obesity ranges between 4% and 6% (corresponding to 2.9–4.4 million children with obesity in the European continent). Rapid increases in the prevalence of overweight schoolchildren are being seen in all European countries for which data are available. The numbers

indicate a lag of 10 to 15 years behind the United States. Using data from the mid-70s to 2016 in 200 countries, it was shown that trends in mean BMI have recently flattened in northwestern Europe and the high-income English-speaking and Asia-Pacific regions for both sexes, southwestern Europe for boys, and central and Andean Latin America for girls. In contrast, the rise in BMI has accelerated in east and south Asia for both sexes, and southeast Asia for boys.¹ In developed countries, the urban poor are more susceptible for developing obesity, presumably because of poor dietary practices and limited opportunity for physical activity.^{275,276} In contrast, obesity is more frequent in upper socioeconomic class of developing countries, probably because of a nutrition transition to a more Western diet with more energy-dense items consisting of higher fats and sugar, which tend to be more palatable at a lower cost.^{277–279} This may be also caused by specific properties of processed food, which may promote leptin resistance.²⁸⁰

Racial and Ethnic Considerations

The NHANES surveys only list prevalence among non-Hispanic whites, non-Hispanic blacks, and Asians, despite the fact that Native Americans, Pacific Islanders, and other racial/ethnic groups are experiencing rapid increases in obesity prevalence as well. Across racial groups, there is a marked dichotomy in the prevalence, and in the rate of increase of childhood obesity.^{281,282} For instance, the prevalence among African American (24.4%), Hispanic (21.7%) and Mexican American adolescents (22.2%) is significantly higher than among white adolescents (15.6%). Importantly, the prevalence of severe obesity (BMI >97th percentile) among African American (18.5%), Hispanic (15.2%) and Mexican American adolescents (15.2%) by far exceeds that of non-Hispanic white adolescents (10.5%). The rate of increase in the prevalence of obesity among African American and Hispanic adolescents almost doubled between 1988 and 1994 and 1999 and 2000, from 13.4% to 23.6% in African Americans, and from 13.8% to 23.4% in Hispanics. The 1994 Pediatric Nutrition Surveillance System (PedNSS) indicated that 12% of 2- to 4-year-old Native American children were overweight, which is similar to Hispanic children at the same age (12%) but much higher than white children (6%). The prevalence of overweight at 5 to 6 years in Native Americans is twice that in US youth in general, and the prevalence of obesity is even 3 times higher.²⁸³ Overall, both American Indian and Alaska Native children and adolescents have a greater prevalence of obesity compared with US children overall.²⁸⁴ Among infants and toddlers less than 2 years of age, the prevalence of obesity is highest in African Americans (18.5%), as compared with 10.1% in non-Hispanic whites and 13.7% in Hispanics. It is possible that different dietary practices may account for some of these differences. For instance, a study of 2-year-old Latino children in California correlated obesity with early consumption of sugar-sweetened beverages.²⁸⁵

Within racial populations, ethnic variability in the prevalence of childhood obesity has also been noted. Only 25% of first-generation Hispanic adolescents were overweight based on BMI in the 85th percentile or higher, as compared with 32% of second- and third-generation Hispanics. The prevalence of overweight in Asian American adolescents in this study was 20.6%, with comparable prevalence among Filipinos (18.5%) and Chinese (15.3%). Again, only 12% of first-generation Asian Americans were overweight, compared with 27% and 28%, of second and third generations, respectively. In Native Americans, there is great variation in the prevalence of obesity from 12% to 77%, based on tribes, age groups, measurement tools, and cut off values, among the studies performed between

1990 and 2000.²⁸³ These studies indicate that obesity in Native Americans begins very early in childhood.

Predictive Factors

The higher the BMI during childhood, the more likely adult obesity will manifest. In general, children with a BMI in the 95th percentile or higher have a very high risk for adult obesity.²⁸⁶ Obesity in adolescence is a primary risk factor for obesity in adulthood, with an increased odds ratio from 1.3 for obesity at 1 to 2 years of age to 17.5 for obesity at 15 to 17 years of age.²⁸⁷ The strongest predictor of adolescent obesity is rapid weight gain between 2 and 6 years of age.²⁸⁸ The change of BMI during and after adolescence is the most important predictive variable for adult obesity.²⁸⁹ Children and adolescents with BMI in the 95th percentile or higher have a 62% to 98% chance of having obesity at 35 years of age, with a 50% chance in males aged 13 years or older and 66% chance in girls age 13 years or older.²⁹⁰ Importantly, an elevated BMI in adolescence (even one that is considered well within the “normal” range) constitutes a substantial risk factor for obesity-related disorders in midlife.^{291,292} Although the risk of diabetes is mainly associated with increased BMI close to the time of diagnosis, the risk of coronary heart disease is associated with an elevated BMI both in adolescence and in adulthood.²⁹³

The age of adiposity rebound, that is, the point of the BMI nadir before body fatness begins to rise (between 5 and 6 years of age), typically more pronounced in girls (see Fig. 24.6A and B), is also an important predictor for adult obesity.²⁹⁴ Children with early adiposity rebound have a fivefold greater chance of having obesity as adults, compared with those with late adiposity rebound. At the age of adiposity rebound, children already overweight have a sixfold greater risk for adult obesity, as compared with lean children. Weight accumulation at an earlier age confers longer exposure to the obesity-related metabolic milieu and thus increases the risk for the development of obesity-related morbidity.²⁵⁷ Therefore the earlier the onset of childhood obesity, the greater is the risk for adult obesity.

Infant overnutrition plays an extremely important role in the future development of obesity. Numerous studies have implicated bottle feeding as a specific risk factor.²⁹⁵ The prevalence of obesity in children who were never breastfed was 4.5%, as compared with 2.8% in breastfed children, and a clear time-response effect was identified for the duration of breastfeeding on the decline in prevalence of obesity as well.²⁹⁶ Early overnutrition has been correlated with elevated leptin concentrations in later life.²⁹⁷ Differences in both volume and composition of commercial formula versus breast milk have been proposed as etiologic factors. An emerging paradigm posits that the gut microbiome plays a critical factor in the development of obesity in childhood as well as in adulthood. Early exposures to maternal factors including breast milk and to other dietary constituents in infancy (such as introduction of solids, exposure to artificial sweeteners etc.) may be key determinants of the profile of the microbiome impacting metabolism and weight balance during childhood and adulthood.²⁹⁸

Parental obesity is also an important predictor of childhood obesity. Children with at least one overweight parent at the age of adiposity rebound have a fourfold to fivefold greater chance of becoming adults with obesity. Lean children aged 5 years or younger have a 13-fold risk of adult obesity if both parents have obesity. Excessive BMI gains of parents during childhood and adulthood are also associated with a higher BMI and risk of obesity in the offspring.²⁹⁹ Conversely, older children with obesity (10–14 years of age) have a 22.3-fold increased risk to become adult with obesity regardless of parental weight,²⁶² suggesting that parental obesity is more important in early childhood weight gain.³⁰⁰ Upon studying associations of

parental and child obesity status, stronger associations were shown in older children than in younger children, in both parents than in father or mother only, in parental obesity and child obesity compared with overweight status of both.³⁰¹ Parental obesity is also related to early adiposity rebound, although it remains unclear whether the relation between parental and childhood obesity is genetic, epigenetic, or environmental.

METABOLIC IMPACT OF CHILDHOOD OBESITY

Many of the metabolic and cardiovascular (CV) complications of obesity are already evident during childhood and are closely related to the development of insulin resistance-hyperinsulinemia, the most common biochemical abnormality seen in obesity.³⁰² The obesity-related comorbidities that emerge early in childhood are alterations in glucose metabolism, dyslipidemia, and hypertension. Although an accelerated atherogenic process is present in children with obesity, thrombotic CV events do not usually appear until adulthood. The clustering of these manifestations is termed the *metabolic syndrome*, or the *insulin resistance syndrome*, suggesting that peripheral insulin resistance may be the driving force of the majority of the obesity-related morbidity.³⁰³

Insulin Resistance

Insulin resistance is defined as the decreased tissue response to insulin-mediated cellular actions and is the inverse of insulin sensitivity. The term insulin resistance, as generally applied, refers to whole-body reduced glucose uptake in response to physiologic insulin levels and its consequent effects on glucose and insulin metabolism. However, it is now clear that not all insulin-responsive tissues are equally sensitive to insulin. Generalized insulin resistance would result in global metabolic dysfunction, such as leprechaunism or Rabson-Mendenhall syndrome. Thus the insulin resistance of obesity must of necessity affect different tissues quantitatively (see Chapter 3 and Chapter 21 on diabetes mellitus and insulin receptor mutations).

Hepatic Insulin Resistance. The liver plays a major role in substrate metabolism and is the primary target of insulin action. After insulin's release from the β -cell following a glucose load, it travels directly to the liver via the portal vein, where it binds to the insulin receptor and elicits two key actions at the level of gene transcription. First, insulin stimulates the phosphorylation of FOXO1, which prevents it from entering the nucleus,^{304,305} and thus diminishes the expression of genes required for gluconeogenesis, mainly phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase. The net effect is diminished hepatic glucose production. Second, insulin activates the transcription factor sterol regulatory element-binding protein (SREBP)-1c, which in turn increases the transcription of genes required for fatty acid and triglyceride (TG) biosynthesis, most notably ATP-citrate lyase, acetyl-coenzyme A carboxylase, and fatty acid synthase; which together constitute the process of de novo lipogenesis (DNL). TGs synthesized by DNL are then packaged with apolipoprotein B (apoB) into very low-density lipoproteins (VLDL) for export to the periphery for storage or utilization by reciprocal activation of lipoprotein lipase (LPL) on the surfaces of endothelial cells in adipose or muscle tissues.³⁰⁶

For reasons that remain unclear, insulin-resistant subjects typically have “selective” or “dissociated” hepatic insulin resistance; that is, they have impaired glucose homeostasis (mediated by the FOXO1 pathway) but normal insulin-mediated hepatic DNL (mediated by the SREBP-1c pathway³⁰⁷ and the GSKR gene³⁰⁸). The increase in FFA flux within the liver, either by DNL or FFA delivery via the portal vein, impairs hepatic

insulin action via fatty acyl-CoA intermediates within the hepatocyte,³⁰⁹ leading to increases in hepatic glucose output, the synthesis of proinflammatory cytokines, and excess TG secretion by the liver, low high-density lipoprotein (HDL) cholesterol levels, and an increase of relatively cholesterol-depleted LDL particles.³¹⁰ Furthermore, the intrahepatic accumulation of FFA and lipid are also detrimental to liver insulin sensitivity as this leads to the generation of toxic lipid-derived metabolites, such as DAG, fatty acyl CoA, and ceramides.³¹¹ These in turn trigger activation of protein kinase C- ϵ (PKC ϵ , and serine/threonine phosphorylation of insulin receptor substrate 1 (IRS-1), which attenuates hepatic insulin signal transduction.³¹² Intrahepatic insulin resistance results in greater first pass insulin clearance in the liver, resulting in lower amounts of insulin reaching the systemic circulation.

Adipose Tissue Insulin Resistance. The expanded adipose tissue mass that accompanies obesity often leads to increased lipolysis and FFA turnover. Normally, insulin inhibits adipose tissue lipolysis; however, in the insulin-resistant state, the lipolytic process is accelerated, leading to increased FFA release into the circulation. Moreover, visceral adipocytes are more sensitive to catecholamine-stimulated lipolysis than subcutaneous adipocytes, further increasing FFA flux.³¹³ Macrophages also infiltrate into adipose tissue and contribute to both adipocyte hypertrophy and cytokine release.^{314–316} These circulating cytokines also affect insulin action in other tissues, such as liver and muscle. Within the normal glucose tolerance range, an increase in adipose insulin resistance is related to an increase in 2-h glucose levels. A tight relation exists between visceral fat ($r = 0.34$; $P < .001$) and the visceral/subcutaneous fat ratio and adipose tissue resistance to insulin. Greater FFA concentration following an oral glucose load is also evident with worsening glucose tolerance indicating reduced suppression of lipolysis and lower FFA clearance.³¹⁷

Muscle Insulin Resistance. Downstream of an insulin-resistant liver, increased plasma FFA flux into skeletal muscle results in fatty acyl-CoA derivatives altering the insulin signal transduction pathway and resulting in reduced insulin-mediated glucose transport in skeletal muscle, facilitating the development of hyperglycemia.³¹¹ The ectopic deposition in skeletal muscle of fat as intramyocellular lipid may also play a direct role in the pathogenesis of whole-body insulin resistance and metabolic syndrome via lipid metabolite-induced activation of PKC ϵ with subsequent impairment of insulin signaling.³¹² Greater intramyocellular lipid deposition is tightly associated with insulin resistance and is typically detected in children with obesity who have altered glucose metabolism.^{318,319} The manifestation of impaired insulin signal transduction in skeletal muscle is reduced translocation of GLUT4 to the cell membrane leading to reduced systemic glucose uptake.

Assessment of Insulin Resistance. The euglycemic hyperinsulinemic clamp is the gold standard for measuring insulin sensitivity; the frequently sampled intravenous glucose tolerance test (FSIVGTT) and steady-state plasma glucose (SSPG) methods are also valid measurements. The clamp is performed by infusing a body-surface-area-adjusted continuous insulin drip while maintaining fasting plasma glucose concentrations by modifying a glucose infusion. Greater glucose infusion rates needed to maintain euglycemia indicate greater insulin sensitivity.³²⁰ Euglycemic hyperinsulinemic clamp studies have shown that insulin resistance is determined primarily by the response of skeletal muscle, with over 75% of infused glucose taken up by muscle and only 2% to 3% by adipose tissue.³²¹ All three methods are generally time consuming, require intravenous infusions and frequent blood sampling, are burdensome for participants, costly, and require a research setting. In an attempt to simplify the measurement of insulin sensitivity, a

number of methods using single simultaneously obtained samples of fasting insulin and glucose have been developed, such as the homeostatic model for assessment of insulin resistance (HOMA-IR). Each of these uses a mathematical formula that adjusts for individual variability in insulin and glucose secretion and clearance. Although the goal for these methods was to improve the accuracy of fasting insulin alone by the addition of fasting glucose, it is now agreed that they yield similar results to fasting insulin. When correlated with gold standard methods in children, fasting insulin is a poor measure of whole-body insulin sensitivity in an individual child.³²¹ Although the primary interest has been in insulin resistance, the adverse effects related to insulin resistance are more likely mediated via compensatory hyperinsulinemia. The fasting triglyceride to HDL-cholesterol ratio, a surrogate of insulin resistance that does not use insulin measurements, has been proposed and correlate quite well with clamp-derived insulin resistance, yet its utilization needs validation in ethnically diverse populations.³²²

The two most important biologic conditions associated with insulin resistance in childhood are ethnicity and puberty. Studies show that African American, Hispanic, Pima Indian, and Asian children are less insulin sensitive compared with non-Hispanic white children with similar anthropometric measures.³²³ Insulin resistance in minority ethnic groups is manifested as lower insulin-stimulated glucose uptake, concomitant with hyperinsulinemia, evidence of increased insulin secretion from the β -cell, and decreased insulin clearance. During puberty there is around 25% to 50% decline in insulin sensitivity with recovery when pubertal development is complete.³²⁴ The compensatory increase in insulin secretion during puberty may be blunted in African American and Hispanic youth, thus increasing their risk for type 2 diabetes (T2DM) around the time of puberty. The development of T2DM is covered in depth in Chapter 21, yet it is worth noting that impaired glucose tolerance (IGT), known as prediabetes, is a relatively common condition in children and adolescents with obesity.³²⁵ IGT in youth with obesity is typically characterized by obesity with an unfavorable pattern of lipid partitioning, with increased deposition of fat in the visceral, hepatic, and intramyocellular compartments.³²⁶

Lipid Partitioning

The term lipid partitioning refers to the distribution of body fat in various organs and compartments. The majority of excess fat is stored in its conventional subcutaneous depot, yet other potential storage sites exist as well, such as the intraabdominal (visceral) fat compartment and insulin-responsive tissues, such as muscle and liver. One hypothesis to explain the relation between obesity and insulin resistance is the “portal-visceral” paradigm.³²⁷ This hypothesis claims that increased adiposity causes accumulation of fat in the visceral depot, leading to an increased portal and systemic FFA flux³²⁸ (Fig. 24.7). Associations between visceral adiposity, insulin resistance, and comorbidities have been demonstrated across most age groups and ethnicities.³²⁹ Of note, studies of in vivo FFA fluxes from the visceral and the subcutaneous truncal and abdominal depots have failed to demonstrate a substantial difference in net fluxes between these depots.

Subcutaneous fat, which does not drain into the portal system, is strongly related to insulin resistance in healthy men with obesity and in men with diabetes.³³⁰ Similarly, truncal subcutaneous fat mass has been demonstrated to independently predict insulin resistance in women with obesity. Visceral and subcutaneous fat differ in their biologic responses³³¹ because visceral fat is more resistant to insulin and has increased sensitivity to catecholamines. These observations emphasize that both visceral and subcutaneous abdominal fat can contribute to insulin resistance, possibly by different mechanisms.³³²

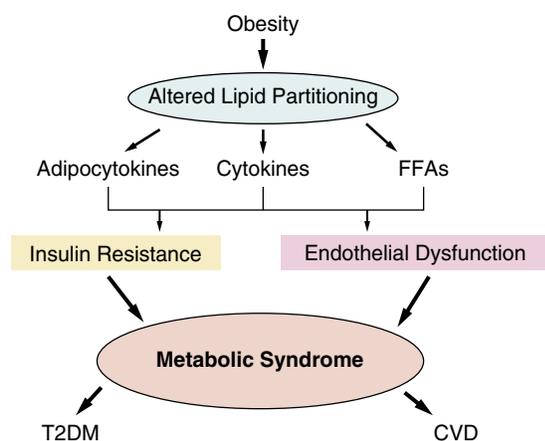


Fig. 24.7 A hypothesis on the relation between obesity and the metabolic syndrome. The metabolic impact of obesity is determined by the pattern of lipid partitioning. Lipid storage in insulin-sensitive tissues, such as liver or muscle, and in the visceral compartment is associated with a typical metabolic profile characterized by elevated free fatty acids and inflammatory cytokines alongside reduced levels of adiponectin. This combination can independently lead to peripheral insulin resistance and to endothelial dysfunction. The combination of insulin resistance and early atherogenesis (manifested as endothelial dysfunction) drives the development of altered glucose metabolism and of cardiovascular disease.

Studies performed in adolescents with obesity highlight the fact that the ratio of visceral to subcutaneous fat may be the determinant of their metabolic impact rather than their absolute quantity of fat. Indeed, adolescents with obesity who have a high visceral to subcutaneous fat ratio, despite having a comparable degree of obesity, demonstrate a markedly adverse metabolic phenotype of severe insulin resistance and alterations in glucose and lipid metabolism.³³³ Moreover, intrahepatic fat, although strongly associated with high levels of visceral fat, is also associated with the insulin-resistant state in adolescents with obesity, independent of all other fat depots.³³⁴

A more unifying paradigm is the “adipose tissue expandability” hypothesis claiming that total body fat is not the culprit of adverse health in obesity rather the relative proportion of lipids in various fat depots is what determines the metabolic phenotype and its derived metabolic risk.³³⁵ This theory claims that as adipose tissue expands, “ectopic lipid deposition” in insulin-responsive tissues is the culprit of adverse effects on metabolism.³³⁶ This theory is based on the observations that lipid content in liver and/or muscle is increased in obesity and in T2DM and is a strong predictor of insulin resistance.^{337,338} Moreover, in conditions such as lipodystrophies, all fat is stored in liver and muscle because of lack of subcutaneous fat tissue, causing severe insulin resistance and diabetes.³³⁹ In adults with obesity (BMI >30), muscle attenuation on computed tomography ([CT]; representing lipid content) is a stronger predictor of insulin resistance than is visceral fat.³⁴⁰ Studies performed in vivo using proton nuclear magnetic resonance (¹H-NMR) spectroscopy demonstrated increased intramyocellular lipid (IMCL) content to be a strong determinant of insulin resistance in adults³⁴¹ and in adolescents with obesity.³⁴² Alternatively, lipid deposition in hepatocytes to produce intrahepatocellular lipid (IHCL) is highly predictive of insulin resistance, even more so than visceral fat.³⁴³ Thus obesity-driven morbidity may begin when the subcutaneous adipose tissue reaches its capacity to store excess fat and begins to shunt lipid to ectopic tissues, such as liver and muscle, leading to peripheral insulin resistance³⁴⁴; or possibly when liver or muscle accumulates

lipid produced de novo in response to dietary manipulation (see later). Another postulated cause of IMCL and IHCL accumulation is a reduction of fat β -oxidation,³⁴⁵ related to low aerobic capacity, a reduced number or malfunction of mitochondria, or reduced SNS tone. The effect of IMCL or IHCL accumulation on peripheral sensitivity is postulated to be caused by an alteration of the insulin signal transduction pathway in muscle, caused by derivatives of fat, such as long-chain fatty acyl-CoA and DAG within the hepatocyte or myocyte. These derivatives activate the serine/threonine kinase cascade and cause serine phosphorylation of IRS-1, which inhibits insulin signaling.³⁴⁶ A comparable mechanism has been demonstrated in the liver, where accumulation of lipids, in particular DAG, activates the inflammatory cascade by inducing c-jun N-terminal kinase 1 (JNK-1), which causes serine rather than tyrosine phosphorylation of IRS-1, leading to inhibition of hepatic insulin signaling.^{347,348}

Vascular Changes

Early stages of the atherosclerotic process may be detected in children with obesity. In recent years, it has become clear that endothelial dysfunction represents a key early step in the development of atherosclerosis.³⁴⁹ The hallmark and cause of endothelial dysfunction is impairment in nitric oxide (NO)-mediated vasodilation.³⁵⁰ This is caused by decreased NO production by endothelial nitric oxide synthase (eNOS), which has been postulated to result from high levels of FFAs and inflammatory cytokines (interleukin [IL]-6, tumor necrosis factor [TNF]- α) in insulin-resistant individuals with obesity, increased reactive oxygen species, or increased uric acid, which inhibit eNOS activity.³⁵¹ Decreased NO bioavailability leads to an imbalance between vasodilating and vasoconstricting factors (such as endothelin), which leads to impaired vascular smooth muscle relaxation, increased adhesion of inflammatory cells to the endothelium, increased expression of plasminogen activator inhibitor-1 (PAI-1; a prothrombotic molecule) and increased vascular smooth muscle cell proliferation. Thus decreased NO bioavailability is thought to create a proinflammatory, prothrombotic environment which promotes atherosclerosis.³⁵² Endothelial function represents an integrated index of the overall CV risk burden in any given individual. During the last decade, noninvasive techniques for the assessment of endothelial function, including high-resolution external vascular ultrasound to measure flow-mediated endothelium-dependent dilatation (FMD) of the brachial artery during hyperemia have been developed.^{353,354} Impaired FMD correlates with arterial wall stiffness, coronary dilatation, and endothelial dysfunction in children with obesity.³⁵⁵ Similarly, anatomic changes in peripheral arterial vessels, such as increased intimal medial thickness (IMT), have also been demonstrated in children and adolescents with obesity,³⁵⁶ which mimics early coronary pathology and predicts adverse CV outcomes.

There are no longitudinal studies that directly measure in vivo insulin sensitivity and its relationship to the development of atherosclerotic abnormalities in children. Very limited observations suggest a relationship between HOMA-IR and arterial stiffness and fasting insulin levels in youth. However, a role for insulin resistance in the early abnormalities of vascular smooth muscle is proposed based on the observation that circulating biomarkers of endothelial dysfunction (intercellular adhesion molecule and E-selectin) are highest, whereas adiponectin, the antiatherogenic adipocytokine, is lowest among the most insulin-resistant youth. The landmark Bogalusa heart study demonstrated that CV risk factors present in childhood are predictive of coronary artery disease in adulthood.^{302,357} Among these risk factors, LDL-cholesterol and BMI measured in childhood were found to predict IMT in young adults.³⁵⁸

There is now substantial evidence that the insulin resistance of childhood obesity creates the metabolic platform for adult CV disease.^{359–361} Obstructive sleep apnea (OSA), typically present in children with obesity, is also tightly associated with the presence of endothelial dysfunction.³⁶² Moreover, the constellation of peripheral insulin resistance, an unfavorable adipocytokine profile, subacute inflammation, and endothelial dysfunction work in parallel to promote the pathologic processes of aging.

Adipocytokines

Leptin. The discovery of leptin in 1994 has dramatically changed the view of adipose tissue in the regulation of energy balance.³⁶³ Adipocytes secrete several proteins that act as regulators of glucose and lipid homeostasis.³⁶⁴ These proteins have been collectively referred to as *adipocytokines* because of their structural similarity with cytokines. Circulating leptin levels correlate with the degree of obesity. As stated earlier, the primary role of leptin is to serve as a long-term energy storage sensor to protect against starvation. Leptin probably has a permissive role in high-energy metabolic processes, such as puberty, ovulation, and pregnancy, but its role in states of energy excess is less known. In obesity, the development of leptin resistance may result in a breakdown of the normal partitioning of surplus lipids in the adipocyte compartment.³⁶⁵

Adiponectin. The cytokine adiponectin is peculiar in obesity because, in contrast with the other adipocytokines, its level is reduced in individuals with obesity.³⁶⁶ The adiponectin gene is expressed exclusively in adipose tissue and codes a protein carboxyl terminal globular head domain and an amino terminal collagen domain, which is structurally reminiscent of the complement factor 1q.³⁶⁷ The gene is located on chromosome 3q27, a location previously linked to the development of type 2 diabetes and the metabolic syndrome. Several single nucleotide polymorphisms (SNPs) in the adiponectin gene have been reported to be associated with the development of type 2 diabetes in populations around the world, suggesting that adiponectin plays a major role in glucose metabolism.³⁶⁸ Adiponectin circulates in plasma in three major forms: a low-molecular-weight trimer, a middle-molecular-weight hexamer, and a high-molecular-weight 12- to 18-mer.³⁶⁹ Circulating plasma high-molecular-weight adiponectin concentrations demonstrate a sexual dimorphism (females have greater concentrations), suggesting a role for sex hormones in the regulation of adiponectin production or clearance.³⁷⁰ Dietary factors, such as linoleic acid or fish oil versus a high carbohydrate diet or increased oxidative stress, have been shown to increase or decrease adiponectin concentrations, respectively. These observations suggest that the circulating levels of adiponectin are regulated by complex interactions between genetic and environmental factors.³⁷¹

The receptors for adiponectin have been characterized in rodent models and cloned. Two receptors, named ADIPOR1 and ADIPOR2, have been characterized. ADIPOR1 is expressed in numerous tissues including muscle, whereas ADIPOR2 is mostly restricted to the liver.³⁷² Both receptors are bound to the cell membrane, yet are unique in comparison to other G-protein-coupled receptors in the fact that the C-terminal is external, whereas the N-terminal is intracellular.³⁷³ Both ADIPOR1 and ADIPOR2 are receptors for the globular head of adiponectin and serve as initiators of signal transduction pathways that lead to increased PPAR α and increased adenosine monophosphate (AMP) kinase activities, which promote glucose uptake and increased fatty acid oxidation. Adiponectin has been shown to have potent antiatherogenic functions, as it accumulates in the subendothelial space of injured vascular walls to reduce the expression of adhesion molecules and the recruitment of macrophages.³⁷⁴

Studies in children and adolescents with obesity have shown that adiponectin is inversely related with the degree of obesity, insulin sensitivity visceral adiposity, IHCL, and IMCL, whereas weight loss increases adiponectin. In adolescents with obesity and type 2 diabetes, low baseline adiponectin and a reduced elevation in response to treatment have been shown to predict treatment failure.³⁷⁵ All of these observations along with human clinical data support a pivotal role for adiponectin in the prevention of the comorbidities of the metabolic syndrome.

Family studies using parent-offspring regressions revealed that most adipocytokines show evidence for significant inheritance. There are three main common axes of variation in the heritability of adipocytokines. The main axis, which explained 21% of the variation, was most strongly loaded on levels of leptin, TNF- α , insulin, and PAI-1, and inversely with adiponectin. This axis was significantly associated with BMI and phenotypically stronger in children, and showed a heritability of 50%, after adjustment for age, gender, and generational effects. Thus adipocytokines are highly heritable and their pattern of covariation is significantly correlated with BMI as early as the preteen years.³⁷⁶

Myokines and Natriuretic Peptides. Skeletal and heart muscles may serve as an endocrine organ as well. Some of the effects of exercise on skeletal muscle are mediated by the transcriptional coactivator PPAR- γ coactivator 1 α (PGC-1 α). In the mouse, PGC-1 α expression in muscle stimulates an increase in expression of FNDC5, a membrane protein that is cleaved and secreted as a newly identified hormone, named *Irisin*. Irisin acts on white adipose cells in culture and in vivo to stimulate UCP1 expression and induces “beiging” of white adipocytes into cells metabolically more active.³⁷⁷ Irisin is induced with exercise in mice and humans, and mildly increased irisin levels in the blood cause an increase in energy expenditure in mice with no changes in movement or food intake, resulting in improvements in obesity and glucose homeostasis. This novel myokine is actually the first hormonal link between exercise and the adipose tissue changes it may induce.³⁷⁸ Importantly, this molecule has a negative correlation with brain executive function³⁷⁹ and thus probably has multiple effects yet to be discovered. Atrial natriuretic peptides (ANP) also have been implicated in fat metabolism. These natriuretic peptides are produced with exercise, cardiac wall stress, weight loss, and cold exposure, and inhibited by obesity and insulin resistance. ANP binds to its natriuretic receptor, facilitating the formation of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). cGMP phosphorylates cGMP-dependent protein kinase, which activates lipolysis, and phosphorylates p38 mitogen-activated protein kinase to enhance mitochondrial biogenesis with increased energy expenditure and increased heat generation, as part of the brown-fat thermogenic program. Thus both skeletal and cardiac muscle may respond to exercise by inducing changes in fat metabolism to enhance caloric expenditure and limit obesity. These new findings are likely to open new avenues of clinical research to limit the consequences of the “obesity epidemic.”^{380,381}

Inflammatory Cytokines. Accumulating evidence indicates that obesity is associated with subclinical chronic inflammation.³⁸² Adipose tissue serves not merely as a simple reservoir of energy stored as TGs, but also as an active secretory organ releasing many peptides, including inflammatory cytokines, into the circulation. This is probably because of infiltration of adipose tissue by cells of the immune system, mainly macrophages. In obesity, the balance between these numerous peptides is altered, such that larger adipocytes and macrophages embedded within them produce more inflammatory cytokines (i.e., TNF- α , IL-6) and less antiinflammatory peptides, such as adiponectin.³⁸³ One hypothesis posits that as energy

accumulates in adipocytes, the perilipin border of the fat vacuole breaks down, causing the adipocyte death.³⁸⁴ Cell death recruits macrophages into the adipose tissue, especially within the visceral compartment, which in the process of clearing debris, also elaborate inflammatory cytokines, initiating a proinflammatory milieu that predates and possibly drives the development of systemic insulin resistance, diabetes, and endothelial dysfunction.^{385,386} Systemic concentrations of C-reactive protein (CRP) and IL-6, two major markers and participants of the inflammatory process, are increased in children and adolescents with obesity. CRP levels within the “high-normal” range have been shown to predict CV disease³⁸⁷ and development of T2DM³⁸⁸ in adults. Elevated levels of CRP correlate with other components of the metabolic syndrome in children with obesity.^{389,390} Thus inflammation may be one of the links between obesity and insulin resistance, potentially driving myocyte and hepatic resistance within the insulin signal transduction pathway.

Reactive Oxygen Species (ROS). The “Free Radical Theory” holds that imbalance between ROS generation and antioxidant defenses is a major factor in the determination of lipid peroxidation and protein misfolding, with resultant deoxyribonucleic acid (DNA) and cellular damage.³⁹¹ Excessive intracellular ROS formation occurs via three pathways: (1) inflammatory cytokines derived from visceral fat accumulation,³⁹² (2) dysfunctional mitochondrial energetics,³⁹³ and (3) glycation. Excessive nutrient processing by mitochondria can result in uncoupling of oxidative phosphorylation and increased generation of ROS; this, in turn, leads to altered mitochondrial function and further ROS generation.³⁹⁴ ROS

accumulation can also impair ER function, causing ER stress and the compensatory unfolded protein response (UPR). The UPR can itself be overwhelmed by persistent excessive nutrient processing and ROS generation, leading to cellular shutdown, defective insulin secretion, and T2DM^{395,396} (Fig. 24.8).

Because ROSs are inherent by-products of cellular metabolism, endogenous cellular antioxidants (e.g., catalase and glutathione) quench the ROS before they have a chance to promote peroxidation. These antioxidants are found primarily in peroxisomes, which collaborate with the mitochondria in ROS processing. Reduction in peroxisomal activity results in mitochondrial dysfunction and ER stress. Furthermore, cytokines, such as TNF- α , can reduce peroxisomal number and function, rendering cells even more vulnerable.^{396,397}

Comorbidities Related to Insulin Resistance

The Metabolic Syndrome

The association and clustering of T2DM, hypertension, dyslipidemia, and CV disease in adults has led to the hypothesis that they may arise from a common antecedent. The WHO argues that this antecedent is insulin resistance, and defines this association as the metabolic syndrome.^{398–401} A consensus definition of the metabolic syndrome for the pediatric age group has been published⁴⁰² and declares that children younger than 10 years of age should not be defined as having this condition. For children older than 10 years of age, the obesity component of the definition is waist circumference and not BMI, indicating the clinical importance of intraabdominal fat. The metabolic

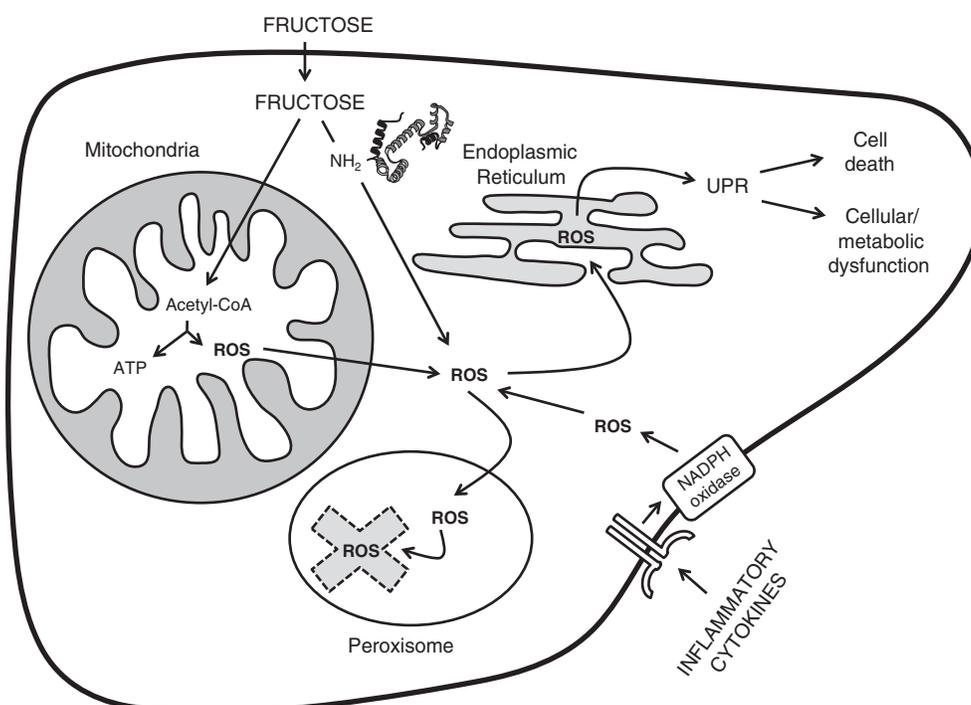


Fig. 24.8 Mechanisms of subcellular metabolic dysfunction, using fructose as an example. The formation of acetyl-CoA leads to lipid deposition and activation of inflammatory pathways, which results in serine phosphorylation of IRS-1, leading to insulin resistance. Furthermore, metabolic processing in the mitochondria, the glycation of protein ϵ -amino groups via the Maillard reaction, and circulating inflammatory cytokines, because of their receptor-mediated activation of NADPH oxidase, all increase intracellular levels of reactive oxygen species (ROS). In the absence of sufficient peroxisomal quenching and degradation, the ROS moieties lead to endoplasmic reticulum (ER) stress, promoting the unfolded protein response (UPR), and cause either cell death (apoptosis) or cellular/metabolic dysfunction. ATP, Adenosine triphosphate; CoA, coenzyme A; NADPH, nicotinamide adenine dinucleotide phosphate. (From Bremer, A.A., Mietus-Snyder, M.L., Lustig, R.H. (2012). Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*, 129(3), 557–570. Courtesy American Academy of Pediatrics.)

syndrome affects approximately 25% of the US adult population.⁴⁰³ Because of its wide prevalence, the metabolic syndrome is of enormous clinical and public health importance, even at its earliest stages. Although still debated, one scheme of the pathophysiology of the metabolic syndrome is shown in Fig. 24.7. According to this paradigm, the impact of obesity is determined by the pattern of lipid partitioning—that is, the specific depots in which excess fat is stored. This pattern of lipid storage determines the adipocytokine secretion profile, on circulating concentrations of inflammatory cytokines and on the flux of FFA. The combined effect of these factors determines the sensitivity of insulin target organs (such as muscle and liver) to insulin and impacts the vascular system by affecting endothelial function. Peripheral insulin resistance and endothelial dysfunction are the early promoters of overt pathology, culminating in T2DM and CV disease. Regardless of the metabolic syndrome definition used, insulin resistance and high insulin levels are associated with the clustering of cardiometabolic risks associated with metabolic syndrome in a variety of ethnic groups. It should be noted that when studying a population (not necessarily an individual), increasing degrees of obesity in childhood are associated with greater risk for the presence of CV risk factors, yet this risk seems to plateau above a threshold of obesity which corresponds to 40 kg/m² in adults.⁴⁰⁴

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) represents fatty infiltration of the liver in the absence of alcohol consumption.⁴⁰⁵ The spectrum of NAFLD ranges from pure fatty infiltration (steatosis) to inflammation (nonalcoholic steatohepatitis, or NASH), to fibrosis and even cirrhosis.⁴⁰⁶ NAFLD was found in the NHANES III survey to be more prevalent in African American and Hispanic males with obesity, T2DM, hypertension, and hyperlipidemia.⁴⁰⁷ These associations have led to the hypothesis that NAFLD is an early marker of the presence of insulin resistance and appears before the development of overt diabetes. NAFLD is now the most common liver disease among children in North America.^{408,409} NAFLD in children is associated with increased visceral fat deposition,⁴¹⁰ and may progress to cirrhosis and related complications.⁴¹¹ The association between abdominal obesity and fatty liver may be partially explained by sustained exposure of the liver to an increased flux of FFA from the visceral depot.³³² NAFLD may represent an early manifestation of ectopic lipid deposition in the liver and represents a challenge to the clinician because of the contrast of its minimal early manifestations and its potential serious outcomes. Studies using the hyperinsulinemic euglycemic clamp methodology demonstrate that NAFLD is associated with hepatic and peripheral insulin resistance.

Insulin plays a key role in regulating transcription factors, such as SREBP-1c, which are abundantly expressed in the liver.⁴¹² SREBP-1c is pivotal in the control of hepatic lipogenesis and is increased in proportion to circulating insulin levels.⁴¹³ These data raise the possibility that fasting hyperinsulinemia may contribute to hepatic steatosis, rather than vice versa. Alternatively, inflammatory cytokines released by visceral fat or by the hepatic immune-reactive cells may contribute to altered hepatic lipid metabolism.⁴⁰⁵ It has been shown that specific SNPs, such as the rs58542926 SNP in the *TM6SF2* gene, are associated with pediatric NAFLD, explaining why some but not all children with obesity develop this phenotype.⁴¹⁴ The majority of patients probably experience NAFLD without progressing on to NASH. It is likely that subsequent inflammation from increased ROS formation without appropriate quenching is necessary to promote progression to NASH (the so-called *second hit* theory).^{411,415} For the time being, NAFLD can be

surmised by an elevated alanine aminotransferase (ALT) in children with obesity. However, ALT does not have to be very elevated; the 95th percentile for ALT in children is 25.8 U/mL for boys and 22.1 U/mL for girls.⁴¹⁶ Importantly, a normal ALT concentration does not rule out the presence of NAFLD.

Polycystic Ovarian Syndrome

The association of hyperandrogenism and oligomenorrhea or amenorrhea in females, termed *polycystic ovarian syndrome* (PCOS), is a frequent comorbidity of obesity, which can extend down to childhood. This disorder is covered in detail in Chapter 16. The diagnosis of PCOS must be based on the presence of at least two of the following three criteria: chronic anovulation, hyperandrogenism (clinical or biologic), and polycystic ovaries.⁴¹⁷ PCOS is the most common cause of infertility because of anovulation and a major risk factor for development of the metabolic syndrome and altered glucose metabolism in females. The antecedents of PCOS have been identified in prepubertal girls, suggesting a developmental lesion.⁴¹⁸

Adolescent girls with PCOS can have moderate to severe insulin resistance with increased risk for altered glucose metabolism and the impairment in insulin sensitivity is more pronounced in those with obesity compared with girls with PCOS who were lean. Typically, girls with PCOS and obesity demonstrate altered lipid partitioning including high visceral and hepatic lipid content, as well as decreased lipid mobilization, diminished fat oxidation, and metabolic inflexibility.⁴¹⁹ In some ethnic groups, girls with premature pubarche, a potential antecedent of PCOS, have relatively increased insulin levels, thus a causal link between hyperinsulinemia and androgen hypersecretion (of adrenal or ovarian origin) has been hypothesized. Population studies of normal girls have shown that rapid weight gain is associated with higher adrenal androgens and body fatness, and that hyperinsulinemia is related to early menarche. Thus the association of higher insulin levels with premature pubarche and subsequent PCOS may be driven, at least in part, by obesity. Obesity characterizes about 50% of women with classic PCOS,⁴²⁰ although it is even more common among adolescents. Increased peripheral insulin resistance occurs in approximately 50% of patients with PCOS, and almost certainly plays a role in the pathogenesis of this condition. On the other hand, almost all forms of severe insulin resistance, such as T2DM or rare lipodystrophy syndromes, are also associated with PCOS. Of note, insulin-resistance has not been included as a diagnostic criterion for PCOS mainly because of the difficulty of its measurement. Fasting hyperinsulinemia and an increased insulin secretory response to an oral glucose load have been demonstrated in girls with PCOS.⁴²¹ Indeed, adolescent girls with PCOS and obesity have been shown to be 50% more insulin resistant than weight-matched controls without PCOS.⁴²² The constellation of metabolic abnormalities typically seen in insulin resistant individuals is commonly encountered in adolescents with PCOS and obesity, including NAFLD⁴²³ and T2DM.⁴²⁴ The increased prevalence of the metabolic syndrome may be related to the hyperandrogenism independent of obesity-related insulin resistance.⁴²⁵ Early markers of accelerated atherogenesis are already present in young females with PCOS,⁴²⁶ indicating that early intervention aimed at reducing CV risk may be beneficial. Importantly, weight loss in women with PCOS is associated with improved menstrual function.⁴²⁷

Metabolic examination of patients with PCOS demonstrates hepatic and muscle resistance, but not ovarian insulin resistance; possibly accounting for insulin stimulation of theca cell androgen production.⁴²⁸ The correlation between insulin resistance and hyperandrogenism begs a unifying hypothesis as to

their pathogenesis, which is proffered by the “serine phosphorylation hypothesis,” which suggests that both P450c17 and the insulin receptor are aberrantly serine phosphorylated; in the case of P450c17, this leads to excess activity and increased androgen production,⁴²⁹ and in the case of the insulin receptor, this leads to tissue-specific insulin resistance.⁴³⁰ However, this hypothesis remains to be proven.

Other Endocrine Comorbidities

Obesity causes changes in other hormonal systems, some of which confer specific morbidities. The age of pubertal initiation has been creeping earlier, particularly in African American girls. This advancement is explained in part by the increasing overnutrition and BMI seen in this population.⁴³¹ Infertility in older adolescents and adult women may occur either as a manifestation of PCOS because of excessive ovarian androgen production in females, or because of excessive aromatization of androgen to estrogen by peripheral adipose tissue with suppression of the hypothalamic-pituitary gonadal axis in both sexes.⁴³² The hyperestrogenemia may also promote gynecomastia in males.⁴³³ In addition, the hypercapnia associated with OSA can suppress hypothalamic gonadotropin hormone (GnRH) function, leading to a syndrome of delayed puberty.⁴³⁴

Obesity is associated with decreased GH secretion, and indeed most subjects with obesity, despite normal or excessive statural growth, fail GH stimulation testing.⁴³⁵ However, caloric restriction for 24 hours can restore normal GH responsiveness.⁴³⁶ Despite the functional GH inadequacy, statural growth is accelerated, bone age is advanced, and peripheral total and free insulin-like growth factor (IGF)-1 levels are normal or elevated in obesity, suggesting normal or accentuated GH sensitivity,⁴³⁷ or possibly because of the suppression of insulin-like growth factor-binding protein (IGFBP)-1, and the effects of hyperinsulinemia on activation of the growth plate IGF-1 receptor.⁴³⁸ Free thyroxine levels tend to be lower and TSH higher in children with obesity, although mostly within the normal range along with some TSH levels within the subclinical hypothyroidism category; the mechanism is unknown. The elevated TSH levels in obesity seem a consequence rather than a cause of obesity. Therefore treatment of hyperthyrotropinemia with thyroxine seems unnecessary in children with obesity.⁴³⁹ Lastly, obesity can be associated with increased cortisol exposure, possibly because of conversion of circulating cortisone to cortisol by the enzyme 11- β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) located within adipocytes.⁴⁴⁰

Other Nonendocrine Comorbidities

Childhood obesity is associated with numerous other comorbidities. Pseudotumor cerebri⁴⁴¹ is a rare and poorly understood condition leading to intracranial hypertension, whose manifestations include papilledema and headache. Treatment includes serial lumbar punctures, acetazolamide to reduce CSF production, in severe cases there is a need for a ventriculoperitoneal shunt, and occasionally optic nerve sheath fenestration is necessary to save eyesight. OSA occurs frequently in children with severe obesity; presumably because of the large amount of retropharyngeal fat that compresses the upper airway during sleep.⁴⁴² Affected patients snore, often stop breathing for more than 20 seconds during sleep, and wake up during the night with headache. Treatment includes nocturnal positive airway pressure and, when appropriate, tonsillectomy; however, symptoms often recur. Children with obesity manifest numerous orthopedic difficulties, including fractures, knee pain, anatomic lower limb malalignment, and impairment in mobility.⁴⁴³ Cholelithiasis occurs in approximately

2.5% of adolescents with obesity, especially in females, but is not usually seen in prepubertal children with obesity.⁴⁴⁴ Lastly, psychologic distress, including clinical depression, is clearly manifested in children with obesity⁴⁴⁵ and specifically in those with severe obesity. These various comorbidities all appear to be associated with BMI z-score in a curvilinear fashion⁴⁴⁶; thus the more their obesity, the more likely patients will manifest comorbidity.

FACTORS ASSOCIATED WITH THE CURRENT EPIDEMIC OF OBESITY

Genetics

The association between obesity and genetics owes to two separate lines of investigation: (1) the discoveries of monogenic disorders of the energy balance pathway (see later) and (2) studies of specific racial and ethnic groups, in which obesity seems to segregate, such as the Pima Indians and Hispanics in the Southwest United States.^{447,448} These observations are combined with an attractive theory on the natural selection of individuals in response to drastic environmental/ecologic pressure (i.e., famine), termed the *thrifty gene hypothesis*,⁴⁴⁹ to yield a very strong driving force for the elucidation of specific genetic loci in the pathogenesis of obesity.⁴⁵⁰ However, the rapid timescale of increased prevalence of childhood obesity cannot possibly reflect a population genetic change. Therefore the current model is that obesity is a result of gene-environment interactions; an ancient genetic selection to deposit fat efficiently may have provided a survival advantage in earlier times but is maladaptive with our current food overabundance. An evolutionary approach to obesity development involves a genomic/anthropologic dimension. For millions of years, the lifestyle of hunter-gatherers comprised intense physical activity and a high-protein/low-carbohydrate diet and the genomes of our ancestors were adapted to low insulin sensitivity. Upon development of farming, a diet high in carbohydrates emerged and since the industrial revolution, our genome is rapidly adapting to such diet. Our current genome represents a mixture of the two. Upon exposure to energy excess induced by the current food environment, these genomes may manifest as development of obesity at different timing and may respond differently to specific diets.⁴⁵¹

In the common forms of obesity, relating SNPs with associated risks for obesity is difficult because the effects are uncertain, and the results not always confirmed. Several SNPs in specific genes have been identified, such as the fat mass and obesity associated (*FTO*) gene. Variation in the *FTO* gene has provided the most robust associations with common obesity to date yet the *FTO* variant that confers a predisposition to obesity does not appear to be involved in the regulation of energy expenditure but may have a role in the control of food intake and food choice, suggesting a link to a hyperphagic phenotype or a preference for energy-dense foods.⁴⁵² The large genome-wide association studies (GWAS) have yielded to date more than 300 loci that may be relevant to the development of adult obesity, yet explain less than 5% of the phenotype.^{453,454}

Epigenetics and Developmental Programming

Follow-up studies of newborns born small-for-gestational age (SGA), large-for-gestational age (LGA), and premature, have noted markedly increased risks for obesity and the metabolic syndrome. The “fetal origins hypothesis”⁴⁵⁵ states that some aspect of the in utero environment contributes to the development of obesity and chronic disease in later life. Importantly, each of these three antenatal conditions is associated with insulin resistance. The “developmental model” of chronic diseases

postulates that early-life events affect individual differences in vulnerability to lifestyle and environment. One possible mechanism for such developmental programming includes epigenetic changes, which may contribute to alterations in gene expression. For instance, the pattern of DNA methylation noted in cord blood predicts the degree of adiposity at age 9 years.⁴⁵⁶

Documentation of the relationship of SGA with adult obesity and CV disease started with studies of the Dutch famine during World War II and its aftermath.⁴⁵⁷ Several studies of newborns born SGA demonstrate that they are hyperinsulinemic and insulin resistant at birth, exhibit rapid catch-up growth in the early postnatal period, and develop obesity in childhood, which remains and promotes persistent insulin resistance. An analysis of Indian newborns born in India versus the United Kingdom⁴⁵⁸ demonstrated that despite those born in India weighing 700 g less at birth, their glucose and insulin levels are markedly elevated. After adjustment for birth weight, the India-born babies demonstrate increased adiposity, 4 times higher insulin, 2 times higher leptin levels than the UK-born babies. Thus these babies are insulin resistant even at birth, which translates into increased adiposity. Following such babies into childhood, there are numerous studies documenting insulin resistance during early childhood.^{459–461}

Babies born LGA are often hyperinsulinemic at birth.⁴⁶² Although most LGA babies are caused by gestational diabetes mellitus (GDM) and exposure to maternal obesity and hyperglycemia throughout the pregnancy, this is not always the cause. Follow-up of LGA babies without GDM demonstrates a doubling of prevalence of insulin resistance and metabolic syndrome, whereas LGA babies resulting from GDM manifest a threefold increase.^{463,464} It has been shown that adolescents with obesity who were exposed in utero to GDM are more insulin resistant and have impaired β -cell function compared with peers with equal degree of obesity without such exposure.⁴⁶⁵ Indeed, the “vertical” transmission of maternal diabetes to the offspring in the form of later obesity and diabetes has been documented in studies of Pima Indians.^{466,467} Lastly, weight gain during pregnancy increases birth weight, the risk for LGA, and obesity in the offspring^{468,469} (Fig. 24.9).

The protective effect of breastfeeding against development of future obesity has long been known,²⁹⁶ and there appears to be a dose–response; the longer the breastfeeding, the more

protective.⁴⁷⁰ However, this may be complicated by confounding factors, such as socioeconomic status, maternal smoking in pregnancy, and maternal BMI.⁴⁷¹ The mechanism of breastfeeding’s antiobesity effect is also unclear. Some think infant feeding self-regulation is most relevant, whereas a recent study suggests that leptin in breast milk may contribute to this protection,⁴⁷² and finally, maternal breastfeeding may shape the offspring intestinal microbiome into a profile different than that induced by consumption of formula.^{298,473}

Environmental Factors

Numerous environmental factors have also been associated with the obesity epidemic, particularly in children. However, most of these associations are derived from cross-sectional rather than longitudinal studies, and in many instances, mechanism remains lacking.⁴⁷⁴ Several longitudinal studies in adults have clearly demonstrated that specific dietary and other lifestyle behaviors are independently associated with long-term weight gain, with a substantial aggregate effect. For instance, on the basis of increased daily servings of individual dietary components, 4-year weight change was most strongly associated with the intake of potato chips (0.767 kg), potatoes (0.58 kg), sugar-sweetened beverages (0.45 kg), unprocessed red meats (0.43 kg), and processed meats (0.42 kg) and was inversely associated with the intake of vegetables (-0.1 kg), whole grains (-0.168 kg), fruits (-0.224 kg), nuts (-0.25 kg), and yogurt (-0.37 kg) ($P \leq .005$ for each comparison).⁴⁷⁵ Similarly, the sociodemographic environment has been shown to affect the chance of having obesity in adulthood. Indeed, the opportunity to move from a neighborhood with a high prevalence of poverty to one with lesser poverty was associated with modest but potentially important reductions in the prevalence of extreme obesity and diabetes.⁴⁷⁶ Similar relationships are likely, but not proven, for children and adolescents.

Stress and Cortisol

In humans, elevated cortisol or markers of the hypothalamus, pituitary, and adrenal (HPA) axis dysregulation correlate with abdominal fat distribution and the metabolic syndrome.⁴⁷⁷ Although circulating cortisol is clearly important in determining visceral adiposity, the recent identification of reduction of circulating cortisone to cortisol within visceral fat tissue by the enzyme 11 β HSD1 has also been linked to the metabolic syndrome.^{440,478} These data suggest that cortisol is important both in increasing visceral adiposity and promoting the metabolic syndrome. The mechanistic link between stress and obesity has not been clarified, partly because of the inherent complexity of evaluating a potentially bidirectional effect of stress on eating and body weight. Studies focusing on brown adipose tissue metabolism support a dichotomous relation to explain the impact of stress on obesity: stress promotes obesity in the presence of hyperphagia and stable brown adipose function, whereas stress results in weight loss or protection from obesity development in the presence of hypophagia or when increased calorie intake is associated with brown adipose recruitment and enhanced thermogenesis (being of white adipocytes).⁴⁷⁹

Evidence of associations between elevated cortisol and psychological distress with abdominal fat distribution in adults is compelling. For instance, urinary glucocorticoid excretion is linked to aspects of the metabolic syndrome, including blood pressure, fasting glucose, insulin, and waist circumference.⁴⁷⁷ It seems that some individuals seem to be “high-responders” to a stress stimulus and demonstrate higher cortisol secretion. These individuals seem more prone for an alteration in satiety recognition and consume larger amounts of calories following the stress exposure (see Fig. 24.5). These data suggest that cortisol

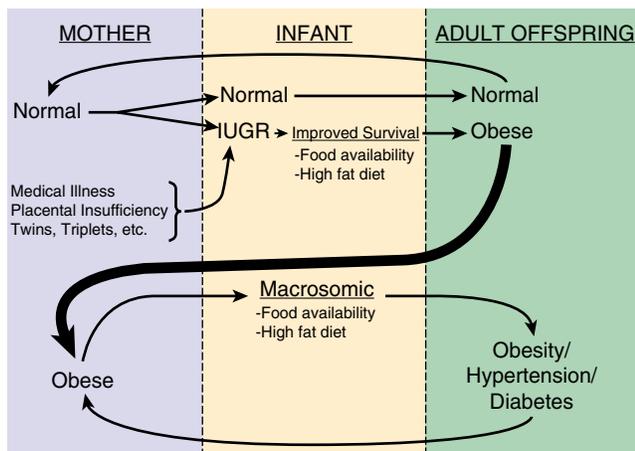


Fig. 24.9 Postulated mechanisms by which developmental programming (either intrauterine growth retardation or neonatal macrosomia) can lead to adult obesity in the offspring. (From Ross, M.G., Huber, I., Desai, M. (2010). Intrauterine growth restriction, small for gestational age, and experimental obesity. In: Lustig, R.H., ed. *Obesity Before Birth: Maternal and Prenatal Effects on the Offspring*. New York, Springer, p. 215–239.⁹⁰⁶ Courtesy Springer, Inc.)

is important both in increasing visceral adiposity and promoting insulin resistance, equivalent to “Cushing syndrome of the abdomen.”⁴⁸⁰

Sleep Deprivation

Adults in the United States currently average less than 7 hours of sleep per night—almost 2 hours less than in 1980—and about one-third of them get less than 6 hours per night.⁴⁸¹ Analyses of data from the NHANES I, revealed that adults (ages 32–49 years) who got less than 7 hours of sleep were more likely to have obesity 5 to 8 years later than those who got 7 or more hours of sleep.⁴⁸² The link between short sleep duration and obesity has also been observed among children.⁴⁸³ Longitudinal studies in children, with subjects from diverse backgrounds, suggested an inverse association between sleep duration and BMI. In 24,821 participants, pediatric subjects sleeping for short duration had twice the risk of having overweight/obesity, compared with subjects sleeping for long duration (odds ratio 2.15; 95% CI, 1.64–2.81).⁴⁸⁴ Like adults, increasing numbers of children are chronically sleep deprived. This is especially true of children with obesity, who have been found to get less sleep than those of normal weight. In addition to its other effects, sleep is one of the most powerful cross-sectional⁴⁸⁵ and longitudinal⁴⁸⁶ predictors of childhood obesity in prepubertal children.

Television Viewing and “Media Time”

Television watching is considered one of the most modifiable causes of childhood obesity.⁴⁸⁷ There are four possible mechanisms linking television watching and obesity. Firstly, television watching may increase stress levels and cortisol (see earlier), causing increased food intake, and promotion of obesity.⁴⁸⁸ Second, television watching displaces physical activity. Most, but not all studies find inverse correlations between television watching and physical activity and fitness.^{489,490} Third, television watching increases calorie consumption from eating during viewing or from the effects of food advertising. Television viewing is also associated with increased high-fat food intake, decreased fruit and vegetable consumption, and increased soft drink intake.⁴⁹¹ “Junk food” is the most frequently advertised product category on children’s television. Lastly, REE and NEAT appear to be decreased during television watching.⁴⁹² According to NHANES III, the prevalence of childhood obesity is lowest among children watching television for 1 hour or less a day, and highest among those watching 4 hour or more a day.⁴⁹³ Several experimental studies of reducing television watching have been conducted and their results support the suggestion that reduced television watching may help to reduce the obesity risk or help promote weight loss in children with obesity.⁴⁹⁴ These studies represent the strongest direct evidence that altering television watching alone is a promising strategy for prevention of childhood obesity. Other forms of screen time, such as cell phones, are also implicated in obesity pathogenesis. It has been shown that parental monitoring of child media exposure predicted lower child BMI z-scores at age 7 years and less steeply increasing child BMI z-scores from 5 to 9 years, thus parental behaviors related to children’s media consumption may have long-term effects on children’s BMI in middle childhood.⁴⁹⁵

Dietary Factors

Calories from any food have the potential to increase risk for obesity and cardiometabolic disease because all calories can directly contribute to positive energy balance and fat gain. However, various dietary components may promote obesity and cardiometabolic disease by additional mechanisms that

are not mediated solely by their caloric content.⁴⁹⁶ Regarding the health effects of specific dietary elements, it has been shown that food-specific saturated fatty acids and sugar-sweetened beverages promote cardiometabolic diseases by mechanisms that are additional to their contribution of calories to positive energy balance. Metabolic effects and responses to certain dietary components are influenced by the individual’s metabolic status, developmental period, or genotype; by the responsiveness of brain regions associated with reward to food cues; and possibly by the microbiome.

Dietary Fat Versus Carbohydrate

Fat is generally considered more obesogenic than other macronutrients, because it is more energy dense, highly palatable, and more effectively converted to body fat.⁴⁹⁷ A high-fat meal induces less thermogenesis and a higher positive fat balance than an isocaloric and isoprotein containing low-fat meal.⁴⁹⁸ Excessive fat intake is believed to cause weight gain,⁴⁹⁹ but the relationships between dietary fat intake and childhood adiposity remain controversial.⁵⁰⁰

The prevalence of overweight in the United States has increased despite a decreased percentage of dietary energy derived from fat. A metaanalysis of 12 studies in adults with overweight or obesity adults who were given dietary advice on low-fat diet and followed for 6 to 18 months suggested that low-fat diets are no better than calorie-restricted diets in long-term weight loss.⁵⁰¹ Similarly, in children, total fat consumption expressed as a percentage of energy intake has decreased.⁵⁰² This decrease in fat consumption is largely caused by increased total energy intake in the form of carbohydrates. Much of this imbalance is attributed to changing beverage consumption patterns, characterized by declining milk intakes and substantial increases in soft-drink consumption,⁵⁰³ which may have its own etiopathogenesis (see later). Most interventions with a low-fat, heart-healthy diet, have not been successful in childhood overweight prevention.⁵⁰⁴

Reduction in carbohydrate intake is taken to the extreme in the ketogenic diets, such as the Atkins diet, which restricts adult subjects to less than 25 g/d of ingested carbohydrate. Adult evaluations of the diet have been disappointing long term,^{505,506} and the popular diet has been abandoned recently. There are currently no data in children or adolescents. However, it should be noted that the ketogenic diet used for seizure control is similar in composition to the Atkins diet. A 2-year study of the ketogenic diet demonstrated persistent decreases in weight z-scores in children who were above average upon diet initiation, without significant compromise in general nutrition or in height.⁵⁰⁷

Trans Unsaturated Fatty Acids (Trans-Fats)

Trans-unsaturated fats in processed foods have been a staple in the Western diet since the early 20th century. This is because the trans-isomerization of the double bond prevents fatty acid breakdown by bacteria, prolonging the shelf life of food products. Like its bacterial predecessors, human mitochondria cannot subject trans-fats to β -oxidation in the liver,⁵⁰⁸ contributing to ectopic intrahepatic lipid accumulation. Fortunately, because of the recognized association between trans-fat consumption and CV disease in the mid-1980s and more stringent labeling requirements since 2006, the percent of calories from trans-fats consumed in the Western diet has been gradually declining.⁵⁰⁹ Trans-fats have no health benefit and cause hepatic steatosis and insulin resistance⁵¹⁰; however, their current consumption trends are temporally disparate with the current increasing prevalence of metabolic syndrome, suggesting that other factors are involved.

Glycemic Index and Fiber

Not all sugars exert the same insulinogenic response. Complex carbohydrates can take two forms: either a combination of α 1-4 linkages and α 1-6 linkages, which gives the starch a globular structure called *amylopectin*, as seen in bread, rice, pasta, potatoes, and glycogen; or a linear polymer of α 1-4 linkages called *amylose*, as seen in beans, lentils, and other legumes. Digestion and absorption of the former in the intestine is rapid because of the simultaneous actions of both α 1-4 and α 1-6 glucosidases, whereas that of the latter is much slower because the α 1-4 glucosidase can only cleave single glucose moieties on either side of the polymer. This phenomenon constitutes the basis of the glycemic index (GI),⁵¹¹ which refers to the relative glucose area under the curve after consumption (in comparison with dextrose). High-GI foods lead to an accentuated insulin response, which can shunt energy substrate to adipose tissue.⁵¹² In children, controlled studies with a high-GI diet demonstrate that energy intake is 53% higher than on low-GI diet.⁵¹³ One adolescent study demonstrated that an *ad libitum* low-GI diet was more effective in promoting weight loss than an energy-restricted low-fat diet.⁵¹⁴ Therefore the GI may be a simple concept to institute, although the “toxic environment” of American food products may make it difficult to maintain.

Dietary fiber consists of the nonstarch, polysaccharide portion of plant foods, including cellulose, hemicellulose, pectins, β -glucans, fructans, gums, and algal polysaccharides. Major sources of dietary fiber include whole grains, fruits, vegetables, legumes, and nuts. Fiber content accounts for 50% of the variability in glycemic load (GL; GI \times volume) between foods. Cohort studies of adults demonstrate that fiber intake is inversely associated with weight gain, fasting insulin levels, and risk of T2DM.^{515,516} Fiber may influence body weight regulation by several mechanisms involving intrinsic, hormonal, and colonic effects, which eventually decrease food intake by promoting satiation (lower meal energy content), satiety (longer duration between meals), or by increasing fat oxidation and decreasing fat storage.⁵¹⁷ A fiber-rich meal is processed more slowly and has less caloric density and lower in fat and added sugars. Fiber-containing foods engender slower glucose absorption, which lessens the postprandial insulin surge and decreases lipogenesis.⁵¹⁸ In addition, high-fiber meals allow for delivery of undigested triglyceride to the colon, where fermentation to short-chain fatty acids and their absorption improve lipids and insulin sensitivity.⁵¹⁹ Archeologists surmise that our ancestors consumed 100 to 300 g of fiber/d.⁵²⁰ However, the dietary fiber intake throughout childhood and adolescence currently averages approximately 12 g/d, and has not changed during the past 30 years.⁵²¹ Therefore parents and school foodservice personnel should strive to offer fiber-rich foods to children so their acceptance and consumption of them will be increased.⁵²²

Fructose

The most commonly used sweetener in the US diet is the disaccharide sucrose (e.g., table sugar), which contains 50% fructose and 50% glucose. However, in North America and many other countries, nondiet soft drinks are sweetened with high-fructose corn syrup (HFCS), which contains up to 55% of the monosaccharide fructose. Thanks to its abundance, sweetness, and low price, HFCS has become the most common sweetener used in processed foods. It is not that HFCS is biologically more ominous than sucrose; rather, it is that its low cost has made it available to everyone, especially low socioeconomic groups. HFCS is found in processed foods ranging from soft drinks and candy bars to crackers to hot dog buns to ketchup. Average daily fructose consumption has increased by over 25% over the past 30 years. The growing dependence on fructose in the Western diet may be fueling the obesity and T2DM epidemics.⁵²³ The highest

fructose loads are soda (1.7 g/30 mL) and juice (1.8 g/30 mL). Although soda has received most of the attention,^{289,524} high fruit juice intake is also associated with childhood obesity, especially by lower income families.⁵²⁵ Animal models demonstrate that high-fructose diets lead to increased energy intake, decreased resting energy expenditure, excess fat deposition, and insulin resistance,⁵²⁶ which suggest that fructose consumption is playing a role in the epidemics of insulin resistance and obesity and T2DM in humans.^{527,528}

Fructose in the gut is transported into the enterocyte via the fructose transporter GLUT5, independent of ATP hydrolysis and sodium absorption. Once inside in the enterocyte, a small portion of the fructose load is converted to lactic acid and released in the portal circulation, another small portion may also be converted to glucose. However, the majority of ingested fructose is secreted into the portal circulation and delivered to the liver. There, fructose is rapidly metabolized to fructose-1-phosphate (F1P) via fructokinase, an insulin-independent process, which also bypasses the negative feedback regulation of phosphofructokinase in the glycolytic pathway. Thus fructose metabolism generates lipogenic substrates (e.g., glyceraldehyde-3-phosphate and acetyl-CoA) in an unregulated fashion, which are delivered directly into the mitochondria. This excessive mitochondrial substrate then drives hepatic DNL, leading to intrahepatic lipid deposition and steatosis.⁴¹⁵ Hepatic DNL also limits further fatty acid oxidation in the liver via excess production of malonyl-CoA, which reduces entry of fatty acids into the mitochondria by inhibiting carnitine palmitoyl transferase 1 (CPT-1). F1P also stimulates SREBP-1c via peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 β ,⁵²⁹ independently of insulin, which activates the genes involved in DNL; moreover, fructose has been shown to induce activation of carbohydrate-response element binding protein (ChREBP), which also increases the expression of all the enzymes of DNL. Furthermore, F1P activates dual-specificity mitogen-activated protein kinase 7 (MKK7), which subsequently stimulates Janus kinase 1 (JAK1), a hepatic enzyme considered to act as a bridge between hepatic metabolism and inflammation.⁵³⁰ In addition, the lipogenic intermediate DAG (formed during fructose metabolism in the liver) activates PKC ϵ , which phosphorylates serine residues on IRS-1, inactivating it, and leading to hepatic insulin resistance.⁴¹⁵ This impairs insulin-mediated phosphorylation of FOXO1, leading to increased expression of the genes required for gluconeogenesis and promoting increased hepatic glucose output, also contributing to hyperglycemia and the development of T2DM. The excess TGs secreted from the liver into the circulation as fat-laden VLDL particles following the ingestion of fructose, coupled with a fructose-induced reduction in LPL activity, cause sustained postprandial dyslipidemia, thereby augmenting the risk for CV disease.^{531,532} (Fig. 24.10A,B).

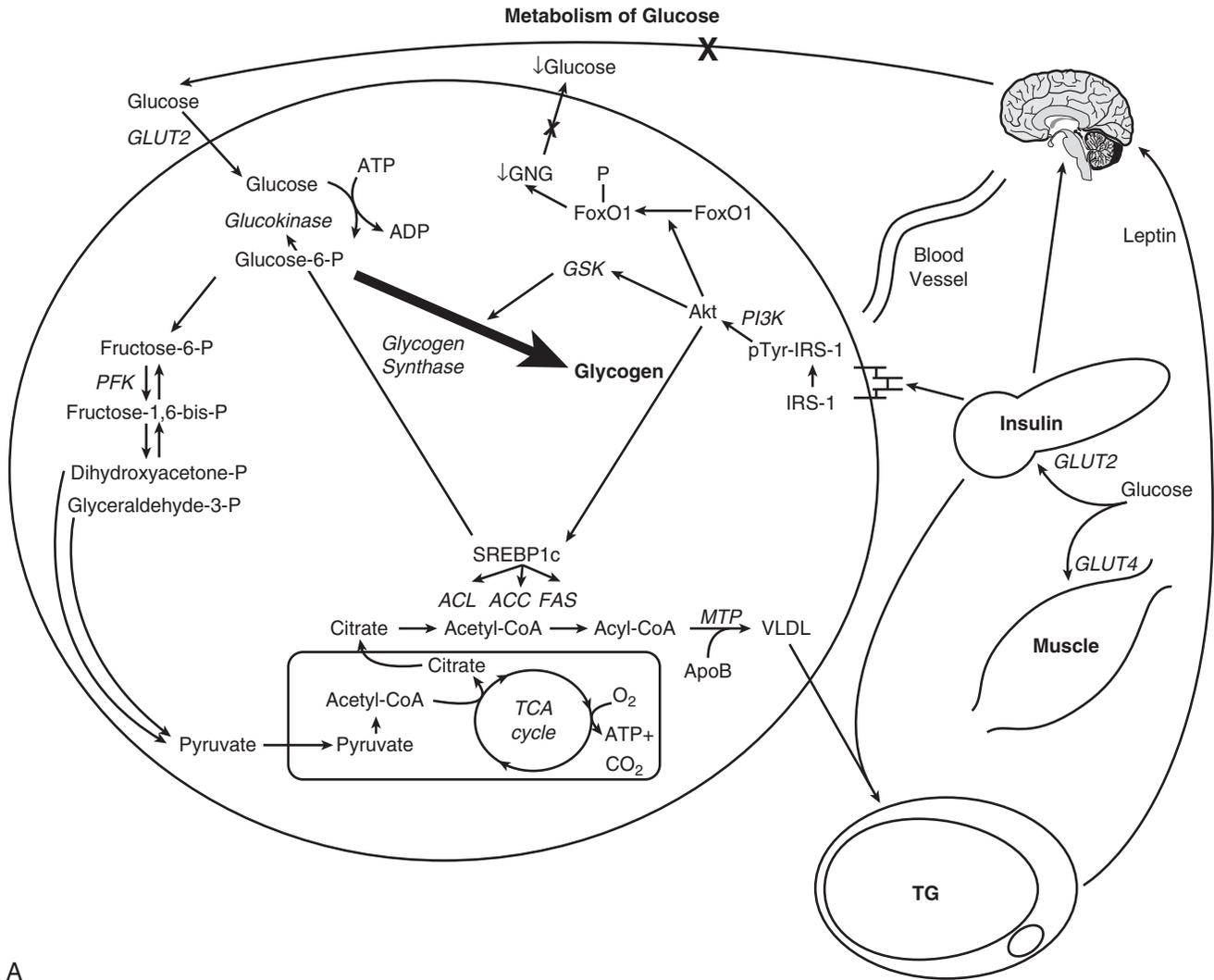
Because of its unique stereochemistry, the ring form of fructose (a five-membered furan with axial hydroxymethyl groups) is under a great deal of ionic strain, which favors the linear form of the molecule, exposing the reactive 2-keto group, which can readily engage in the nonenzymatic fructosylation of exposed amino moieties of proteins via the Maillard reaction, in the same way that the 1-aldehyde position of glucose is reactive.⁴¹⁵ Each Maillard reaction generates one ROS, which must be quenched by an antioxidant or risk cellular damage. In an *in vitro* study, incubation of hepatocytes with fructose yielded no direct damage; however, when these hepatocytes were preincubated with sublethal doses of hydrogen peroxide to reduce their peroxisomal ROS-quenching ability, fructose then became as hepatotoxic as other organic aldehydes.⁵³³ Mild reduction of fructose in an isocaloric diet intervention for a few days was shown to reduce intrahepatic fat, glucose, and insulin levels indicating its unique metabolic impact, independent of its caloric value.⁵³⁴

Branched-Chain Amino Acids

Branched-chain amino acids (BCAAs: valine, leucine, and isoleucine) are essential amino acids that account for more than 20% of the amino acids in the typical “Western diet.”⁵³⁵

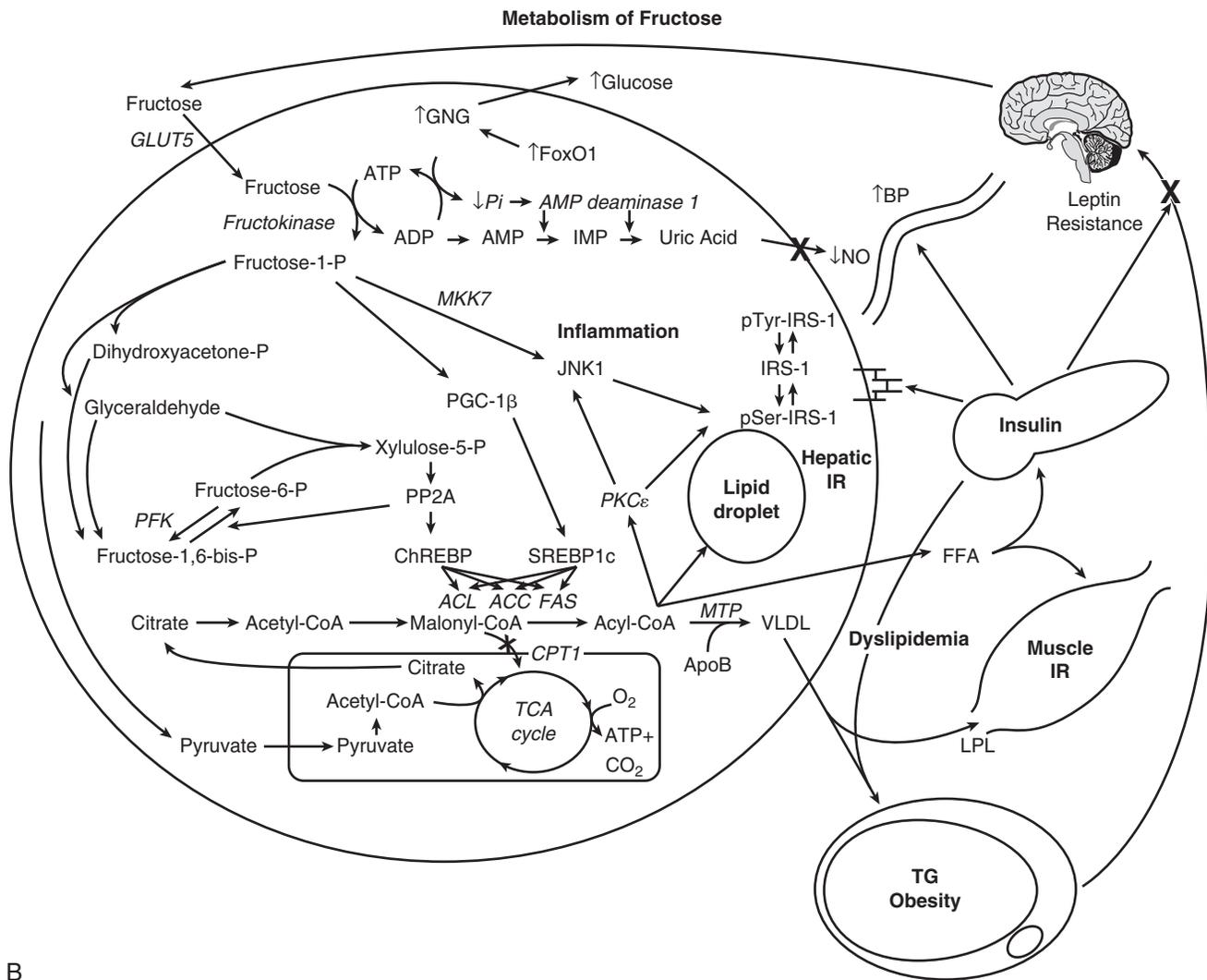
Although normally used for protein biosynthesis and cell growth, when provided in excess they are diverted away from protein synthesis and toward energy utilization.⁵³⁶

In the liver, BCAAs increase transcription of ChREBP and SREBP-1c,⁵³⁷ facilitating DNL. Furthermore, BCAAs limit



A

Fig. 24.10 Hepatic (A) glucose and (B) fructose metabolism. Of an ingested glucose load, 20% is metabolized by the liver; thus in a 120-kCal glucose load (two slices of white bread), 24 calories are hepatically metabolized. Under the action of insulin, glycogen synthase is increased, and the majority of the glucose load is stored as glycogen. Although insulin activation of sterol regulatory element-binding protein 1c (SREBP-1c) activates the lipogenic pathway, there is little citrate formed to act as substrate for lipogenesis. In addition, insulin action on the liver phosphorylates forkhead protein-1 (FOXO1), excluding it from the nucleus, and suppressing the enzymes involved in gluconeogenesis (GNG). In comparison, virtually 100% of a fructose load is hepatically metabolized; thus in a 120-kCal sucrose load (an 8-oz. glass of orange juice), a bolus of 72 calories reach the liver. In contrast to glucose, fructose induces: (1) substrate-dependent hepatocellular phosphate depletion, which increases uric acid and contributes to hypertension through inhibition of endothelial nitric oxide synthase and reduction of nitric oxide (NO); (2) stimulation of de novo lipogenesis and excess production of VLDL and serum triglyceride, promoting dyslipidemia; (3) accumulation of intrahepatic lipid droplets, promoting hepatic steatosis; (4) production of FFA, which promotes muscle insulin resistance; (5) c-jun N-terminal kinase (JNK-1) activation, which serine phosphorylates and the hepatic insulin receptor, rendering it inactive, and contributing to hepatic insulin resistance, which promotes hyperinsulinemia and influences substrate deposition into fat; and (6) central nervous system hyperinsulinemia, which antagonizes leptin signaling (see Fig. 24.5) and promotes continued energy intake. ACC, Acetyl-CoA carboxylase; ACL, ATP citrate lyase; ACSS2, acyl-CoA synthetase short-chain family member 2; ApoB, apolipoprotein B; ChREBP, carbohydrate-response element binding protein; CPT-1, carnitine palmitoyl transferase-1; FAS, fatty acid synthase; FFA, free fatty acids; GLUT2, glucose transporter 2; GLUT4, glucose transporter 4; GLUT5, glucose transporter 5; GSK, glycogen synthase kinase; IR, insulin resistance; IRS-1, insulin receptor substrate-1; LPL, lipoprotein lipase; MKK7, MAP kinase 7; MTP, microsomal transfer protein; PFK, phosphofructokinase; PGC-1 β , peroxisome proliferator-activated receptor- γ coactivator-1 β ; PI3K, phosphatidylinositol-3-kinase; PKC ϵ , protein kinase C- ϵ ; PP2A, protein phosphatase 2a; SREBP-1c, sterol regulatory element binding protein-1c; VLDL, very low-density lipoprotein. (From Lustig, R.H. (2010). Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc*, 110(9), 1307–1321.⁹⁰⁷ Courtesy Elsevier.)



B

Fig. 24.10, cont'd

insulin-induced PI3K signaling and stimulate the activation of the mammalian (aka molecular) target of rapamycin (mTOR), promoting the serine phosphorylation of IRS-1 and impairment of insulin signaling. In addition, just as there are obesity-related changes in adipokines and CV risk markers, there also appear to be obesity-associated changes in BCAA metabolism and subsequent serum levels. In particular, valine and leucine/isoleucine levels have been reported to be 20% and 14% higher, respectively, in subjects with obesity compared with those with normal weight.⁵³⁶ Mechanistically, this appears to be accounted for by a high rate of flux through the BCAA catabolic pathway, resulting in the increased production of alanine. Because alanine is a highly gluconeogenic amino acid, increased BCAA catabolism may thus contribute to increased hepatic glucose output.⁵³⁸ Furthermore, the increased α -ketoacids generated by increased flux of the BCAAs through their catabolic pathways also potentially suppress mitochondrial β -oxidation.

Furthermore, chronic BCAA elevation impairs the transport of aromatic amino acids into the brain; the reduced production of serotonin (derived from tryptophan) and catecholamines (derived from phenylalanine and tyrosine) may drive hunger.⁵³⁶ The "BCAA overload" hypothesis suggests that in the context of a dietary pattern that includes high fat consumption, BCAAs may make an independent contribution to the

development of insulin resistance, a hypothesis supported by metabolomics studies demonstrating high BCAA levels in normoglycemic individuals that subsequently develop insulin resistance and diabetes.^{539,540}

Ethanol

Although adult epidemiologic studies associate light to moderate ethanol consumption with improved insulin sensitivity and red wine consumption with reduced CV risk, other cross-sectional and prospective studies implicate a dose-dependent effect of alcohol in metabolic syndrome, and suggest that chronic consumption of large amounts of ethanol worsen insulin sensitivity. Ethanol bypasses glycolysis by being converted by alcohol dehydrogenase-1B to form acetaldehyde, which promotes ROS formation and must also be quenched by hepatic antioxidants, such as glutathione or ascorbic acid. Acetaldehyde is then metabolized by the enzyme aldehyde dehydrogenase-2 to acetic acid, which in turn is metabolized by the enzyme acyl-CoA synthase short-chain family member 2 to form acetyl-CoA. The acetyl-CoA can then enter the mitochondria, or, in the presence of other caloric substrates, it is preferentially used for the synthesis of fatty acids through DNL. The excess malonyl-CoA produced from ethanol

metabolism inhibits CPT-1 and thus limits mitochondrial fatty acid β -oxidation. Ethanol also blocks fatty acid β -oxidation by inhibiting PPAR- γ , which suppresses microsomal triglyceride transfer protein, thereby altering the liver's lipid export machinery.^{541–543} Buildup of intrahepatic lipid metabolites leads to subsequent activation of the enzyme JNK-1 and serine-phosphorylation of the IRS-1, driving further hepatic insulin resistance. Thus ethanol metabolism results in intrahepatic lipid accumulation and liver injury,^{544,545} driving hepatic insulin resistance and promoting the metabolic syndrome.⁵⁴⁶ However, although clearly a concern in adults, it is unlikely that ethanol contributes significantly to metabolic syndrome in children.

Calcium and Dairy

There have been several reports of an *inverse* relationship between dietary calcium and obesity indices.^{547,548} Dietary calcium plays an important role in energy metabolism regulation. Increased calcitriol (1,25-dihydroxyvitamin D) in response to low-calcium diets stimulates Ca^{2+} influx in human adipocytes, which may lead to stimulation of lipogenic gene expression and lipogenesis, as well as inhibition of lipolysis.⁵⁴⁸ This may result in an expansion of adipocyte triglyceride stores, which can promote adiposity. Increased dietary calcium reduces calcitriol levels and leads to reduction of fat mass without caloric restriction in mice,⁵⁴⁹ and this antiobesity effect of dietary calcium is supported by human clinical and epidemiologic studies.⁵⁵⁰ Vitamin D deficiency correlates with increasing BMI, especially in African Americans⁵⁵¹; however, it is not known if this is caused by substitution of soft drinks for dairy, lactose intolerance, or other factors. One adult study⁵⁵² revealed a consistent effect of higher calcium intake on lower body weight and body fat; however, pediatric studies are lacking.

Intestinal Microbiome

Despite establishing a strong association between the gut microbiota and obesity in humans, a causal relationship and discovery of the underlying mechanisms remain to be identified. Studies have shown that fecal transplants from humans with obesity and normal weight to gnotobiotic mice result in adoption of the donor obesity phenotype by the formerly germ-free rodents. This suggests that the microbiome is indeed implicated in the development of obesity, and perhaps in the development of adiposity-related comorbidities. The gut acquires its microbial profile by colonizing bacteria from birth and during the first year of life. Intestinal crosstalk with colonizing bacteria in the developing intestine affects the infant's adaptation to extrauterine life (immune homeostasis) and may provide protection against disease development (such as obesity) later in life. Disrupted colonization (dysbiosis) caused by maternal dysbiosis, cesarean section delivery, and use of perinatal and neonatal antibiotics may adversely affect the gut development of host defenses and predispose to inflammation rather than to homeostasis, leading to increased susceptibility to development of diseases later in life.⁵⁵³ It has been shown that the gut microbiome responds to diet, antibiotics, and other external stimuli in ways that impact a variety of metabolic conditions, including obesity and NAFLD. The predominance of certain human intestinal flora species (*Firmicutes* vs. *Bacteroides*) may predispose both animals and humans to obesity,⁵⁵⁴ possibly by increasing efficiency of energy absorption⁵⁵⁵; however, factors that determine their predominance are unknown.

Medications

Numerous medications promote excessive weight gain in children. The most commonly prescribed are pharmacologic doses

of glucocorticoids (e.g., prednisone, methylprednisolone, dexamethasone) used for their antiinflammatory and antineoplastic activities. Patients so treated frequently develop obesity,^{556,557} and develop many of the features of Cushing syndrome (e.g., visceral adiposity, hyperlipidemia, hypertension, glucose intolerance), which typify the metabolic syndrome.⁵⁵⁸ Sex hormone administration also promotes excessive weight gain, presumably by inducing insulin resistance.⁵⁵⁹ In patients with type 1 diabetes, strict glycemic control is usually accompanied by slight overinsulinization of the patient, leading to greater occurrence of mild hypoglycemic episodes requiring nonhunger-driven calorie consumption, and potentially to excessive weight gain.⁵⁶⁰ Lastly, more and more children are being placed on the atypical antipsychotics risperidone, olanzapine, quetiapine, clozapine, aripiprazole, and ziprasidone to affect mood and behavior.⁵⁶¹ These antipsychotics are generally associated with weight gain. Studies assessing the weight-protective effects of augmentation therapy with metformin or topiramate demonstrate lower yet still substantial weight gain upon addition of these agents.⁵⁶²

DISORDERS OF OBESITY

The concept that obesity is a phenotype of numerous pathologies is evident from the examination of specific disorders leading to obesity in early childhood (Box 24.1, Fig. 24.11). Some involve neural mechanisms, others involve classic hormonal mechanisms, whereas others involve dysregulation of increased energy intake, decreased energy expenditure, or increased energy storage at the adipocyte. Remember that even in referral centers for pediatric obesity, children with an "organic" cause of their obesity represent a small minority of the population. Less than 1% will have a "classic" endocrinopathy and less than 3% will have an identifiable genetic etiology in general cohorts with obesity, and approximately 7% will have an endocrinopathy or identifiable genetic cause in pediatric cohorts with severe obesity.⁵⁶³

"Classic" Endocrine Disorders With an Obesity Phenotype

In children, linear or statural growth accounts for up to 20% of ingested calories. Endocrine states that allow for normal energy intake for age, but inhibit linear growth, will of necessity lead to excessive energy storage. This is the case for the four "classic" endocrine disorders associated with obesity. These can be distinguished from other causes of pediatric obesity on the basis of their suboptimal growth rate, as opposed to overnutrition, which tends to increase the rate of both growth and skeletal maturation, probably caused, at least in part, by excess insulin cross-reacting with the IGF-1 receptor.⁵⁶⁴

Hypothyroidism. Insufficient triiodothyronine (T_3) hormone causes lower REE and decreased physical activity caused by fatigue. Moreover, T_3 is permissive for the anabolic effects of GH. The decrease in total energy expenditure, despite a relatively low caloric intake, promotes persistent energy storage and increases adiposity. Signs, symptoms, and diagnostic evaluation of hypothyroidism are discussed in Chapter 13. Thyroid hormone replacement is sufficient to increase growth, REE, and physical activity to resolve the obesity over time. Of note, hypothyroidism as a cause of weight gain should not be confused with mild elevations in TSH that occur as a consequence of obesity, thought to be mediated by increased thyrotropin-releasing hormone (TRH) secretion induced by leptin. Thyroid hormone supplementation is generally not indicated in such cases, and TSH usually normalizes with weight loss and reduction in adiposity, which in turn reduces circulating leptin concentrations.

BOX 24.1 Classification of Childhood Obesity Disorders**CLASSIC ENDOCRINE DISORDERS (SHORT STATURE/GROWTH FAILURE PROMINENT)**

- a. Hypothyroidism
 1. Primary
 2. Central
- b. Cushing syndrome (glucocorticoid excess)
 1. Adrenal adenoma/carcinoma
 2. Adrenal micronodular hyperplasia
 3. Pituitary ACTH-secreting tumor
 4. Ectopic ACTH-secreting tumor
 5. Exogenous glucocorticoid administration
- c. Growth hormone deficiency
- d. Pseudohypoparathyroidism 1a
 1. Maternal transmission (AHO + multihormone resistance)
 2. Paternal transmission (Pseudopseudohypoparathyroidism, AHO only)

LEPTIN-MELANOCORTIN PATHWAY DEFECTS (HYPERPHAGIA PROMINENT)

- a. Leptin deficiency
- b. Leptin receptor deficiency
- c. *POMC* mutation (adrenal insufficiency and hypopigmentation/red hair)
- d. Prohormone convertase-1 deficiency (proinsulin excess)
- e. Carboxypeptidase E deficiency
- f. *MC3R* mutation
- g. *MC4R* mutation (tall stature)
- h. *SIM1* mutation (autonomic dysfunction, intellectual disability)
- i. Melanocortin receptor accessory protein 2 (MRAP2) deficiency
- j. Brain-derived neurotrophic factor (BDNF) haploinsufficiency/11p14.1 deletion
- k. *NTRK2* mutation
- l. *SH2B1* haploinsufficiency/16p11.2 deletion

SYNDROMIC OBESITY DISORDERS (MULTIPLE SYSTEM INVOLVEMENT AND DISTINCT PHYSICAL FEATURES)

- a. Prader-Willi syndrome
 - neonatal failure to thrive then rapid weight gain after infancy
 - hypotonia
 - intellectual disability
 - hypogonadism
 - short stature
- b. Bardet-Biedl syndrome
 - retinal dystrophy
 - polydactyly
 - intellectual disability
 - kidney disease
 - male hypogonadism

- c. Alström syndrome
 - retinal dystrophy
 - hearing loss
 - pulmonary fibrosis
 - kidney disease
 - severe insulin resistance
 - hypothyroidism
 - male hypogonadism
- d. Smith-Magenis syndrome
 - intellectual disability
 - maladaptive and self-injurious behaviors
 - sleep disturbance
- e. WAGR syndrome
 - Wilms tumor
 - aniridia
 - genitourinary anomalies
 - range of developmental delays/intellectual disability
 - reduced pain perception
- f. Cohen syndrome
 - microcephaly
 - hypotonia
 - intellectual disability
 - progressive myopia
 - retinal dystrophy
 - joint hypermobility
 - neutropenia
 - prominent incisors
- g. Carpenter syndrome
 - craniosynostosis
 - intellectual disability
 - brachydactyly, polydactyly, syndactyly
 - congenital heart disease
 - cryptorchidism
 - umbilical hernia
 - situs inversus

HYPOTHALAMIC OBESITY INSULIN DYNAMIC DISORDERS

- tumor
- surgery
- radiation
- trauma
- infiltrative disease
- inflammation ROHHAD syndrome (rapid-onset obesity with hypothalamic dysfunction, hypoventilation, autonomic dysregulation, neural crest tumor)

ACTH, Adrenocorticotrophic hormone; *AHO*, Albright hereditary osteodystrophy; *POMC*, proopiomelanocortin.

Whether thyroid hormone supplementation may aid weight loss or ameliorate CV comorbidities and hepatic steatosis is uncertain.⁵⁶⁵

Glucocorticoid Excess. Cushing syndrome is state of glucocorticoid excess that arrests growth and induces hyperphagia,²²⁵ along with a decrease in REE and physical activity caused by muscle wasting. Exogenous glucocorticoid therapy can result in a similar obesity phenotype. Cushing syndrome is discussed further in [Chapter 14](#). A reduction of circulating glucocorticoid through medical or surgical treatment reverses obesity, but central adiposity often persists.⁵⁶⁶ Although Cushing syndrome as a cause of obesity occurs very rarely, functional hypercortisolism (pseudo-Cushing syndrome) is common in

obesity and is thought to be caused by increased HPA-axis activation and greater conversion of inactive cortisone to cortisol by 11 β HSD1 in adipose tissue. This chronically higher glucocorticoid tone in obesity and other pseudo-Cushing conditions, such as depression, diabetes, add sleep disorders, may contribute to metabolic health risk and exacerbate visceral adiposity, leading to a viscous cycle of worsening central obesity.⁵⁶⁷ Transgenic mice that overexpress 11 β HSD1 selectively in adipose tissue develop insulin-resistant diabetes, hyperlipidemia, and hyperphagia.⁵⁶⁸ 11 β HSD1 enzyme activity is higher in visceral versus subcutaneous adipose tissue and correlated with BMI in normal weight prepubertal children.⁵⁶⁹ In adults, however, the associations of 11 β HSD1 polymorphisms and

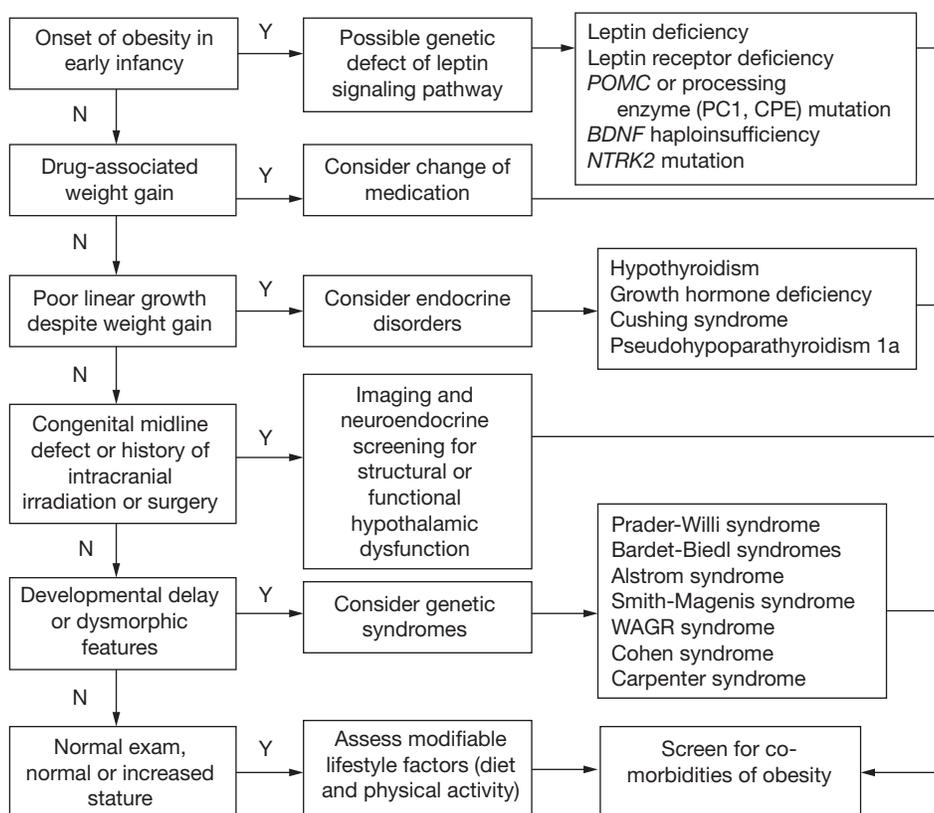


Fig. 24.11 Assessment of childhood-onset obesity. (Figure modified from Han, J.C., Lawlor, D.A., Kimm, S.Y. (2010). Childhood obesity. *Lancet*, 375 (9727), 1737–1748.)

BMI or waist : hip ratio were weak at best,⁵⁷⁰ and enzyme activity was not elevated in obesity.⁵⁷¹ Nonetheless, specific inhibitors of 11 β HSD1 are being investigated as potential drug targets for treatment of obesity and type 2 diabetes.⁵⁷²

Growth Hormone Deficiency. Inadequate GH prevents lipolysis and promotes visceral adiposity, although the severity of obesity is usually mild, such that diagnosis of GH deficiency typically precedes the onset of significant obesity. GH deficiency is also associated with fatigue and decreased physical activity. GH deficiency is often accompanied by other pituitary hormone deficiencies (e.g., central hypothyroidism), which can also decrease REE. GH therapy is able to reverse these energy expenditure deficits, increase muscle mass, and promote weight loss. Diagnostic testing for GH deficiency is discussed in Chapter 11, but it should be mentioned here that obesity itself affects GH secretion such that peak responses during stimulation testing are lower in children with obesity compared with those with normal weight.^{435,573} The mechanism for reduced GH secretion in obesity is not known but postulated contributors include hyperinsulinemia and elevated circulating FFAs.⁵⁷⁴ Therefore interpretation of GH stimulation testing results should account for altered thresholds in obesity. Moreover, the potential benefit of GH supplementation in relative GH insufficiency remains to be determined.

Pseudohypoparathyroidism Type 1a (PHP1a) and Albright Hereditary Osteodystrophy (AHO). PHP1a and AHO are caused by an autosomal dominant mutation of *GNAS1*, which encodes the G α subunit necessary for peptide hormone signal transduction of GPCRs (affected ligands include parathyroid hormone, TSH, growth hormone-releasing hormone, and α -MSH).^{575,576} Maternal transmission of the *GNAS1* mutation leads to PHP1a (multihormone resistance, described further in Chapter 3 and Chapter 20) along with AHO, of which a cardinal feature is obesity, caused by reduced MC3R and MC4R anorexigenic/catabolic signaling within the hypothalamus and reduced

ability to stimulate cAMP in response to β -adrenergic stimulation within adipocytes.⁵⁷⁷ In addition to obesity, other typical manifestations include hypocalcemia, short stature, mild intellectual disability, round face, low nasal bridge, short nose and neck, delayed dental eruption with enamel hypoplasia, short fourth and fifth metacarpals and metatarsals, and short distal phalanges of the thumb. Paternal transmission leads to AHO without multihormone resistance, also known as pseudopseudohypoparathyroidism. Besides hormone replacement and prevention of hypocalcemia, no specific treatments for the obesity aspect of this condition are currently available.

Genetic Obesity Disorders

Approximately 5% to 10% of children with hyperphagia and early-onset (first 3 years of life) obesity are estimated to have an identifiable genetic condition,⁵⁶³ and the prevalence increases to as high as 22% when screening children with syndromic obesity.⁵⁷⁸ Genetic obesity disorders can be categorized as monogenic conditions with obesity as the main phenotype and pleiotropic syndromes that include other characteristics, such as intellectual disability and dysmorphic physical characteristics. There are approximately 80 genetic syndromes that include obesity as a feature, but less than one-fourth of these syndromes have had their genetic etiology fully elucidated.⁵⁷⁹ In this chapter, we will focus primarily on the monogenic disorders of the leptin-melanocortin pathway and on a few of the better elucidated pleiotropic syndromes. Further descriptions of the many other syndromes are reviewed in detail elsewhere.^{580–582}

Leptin-Melanocortin Pathway Defects

Since the 1994 discovery of leptin deficiency as the cause of obesity in the *ob/ob* mouse,² the leptin-melanocortin pathway has been extensively elucidated in animal models, whereas in

parallel, monogenic defects along this same pathway have been identified in human obesity disorders. Each disorder will be described in sequential order based on the sequence of the pathway steps as shown in Fig. 24.3. Several pleiotropic obesity syndromes also appear to converge on the leptin-melanocortin pathway, and these conditions will be listed alongside their monogenic disorder counterpart.

Leptin Deficiency. Autosomal recessive mutations of the leptin gene in humans recapitulate the phenotype of the *ob/ob* leptin-deficient mouse.⁵⁸³ Only a few such patients have been described, primarily of Pakistani and Turkish descent, and for the most part they were born to consanguineous parents. Birth weight is normal followed by rapid weight gain during infancy and obesity as early as the first few months of life. Hyperphagia manifests at birth as insatiable hunger with constant demands to be fed in infancy and aggressive food-seeking behavior in childhood. The lack of leptin induces the starvation response in the form of central hypothyroidism, lower body temperature, hypogonadotropic hypogonadism, and defective T-cell-mediated immunity.⁵⁸⁴ Despite hypothyroidism, normal stature and skeletal maturation are generally preserved until the usual age of puberty because obesity-induced hyperinsulinemia allows excess insulin to cross-react with the IGF-1 receptor to maintain growth. However, because of the important role of leptin in initiating and maintaining puberty, untreated adult patients with leptin deficiency are short because of the lack of a pubertal growth spurt. The diagnosis is made by demonstrating extremely low or unmeasurable serum concentrations of leptin. It should be noted that some mutations of the leptin gene can produce nonfunctional protein that is still detected in standard antibody-based assays so bioactivity measurement may be necessary.^{585,586} In either circumstance of reduced or dysfunctional leptin, treatment with recombinant leptin effectively restores leptin signaling, thereby reducing hyperphagia, resolving obesity, and restoring normal pubertal progression and immune function.⁵⁸⁴ Heterozygous carriers of leptin gene mutations display an intermediate phenotype with lower serum leptin concentrations and predisposition for obesity.⁵⁸⁷

Leptin Receptor (LEPR) Deficiency. Autosomal recessive mutations of the leptin receptor gene produce similar symptoms and signs as leptin deficiency. In a large cohort of patients with severe, early-onset obesity, the prevalence of homozygous or compound heterozygous pathogenic variants of *LEPR* was 3%. Affected individuals had hyperphagia, severe obesity, delayed puberty because of hypogonadotropic hypogonadism and altered immune function. Overall, however, their clinical features were less severe than those of patients with congenital leptin deficiency. Serum leptin concentrations were elevated and correlated with fat mass but shifted higher for the degree of adiposity when compared with control subjects with obesity with normal *LEPR*. The generally higher circulating leptin concentration in *LEPR* deficiency is attributed to disruption in the leptin-SNS feedback loop that connects the CNS with peripheral adipocytes.¹⁰³ However, because of the fairly high degree of overlap in values, leptin concentrations cannot be used to diagnose *LEPR* deficiency reliably, so gene sequencing is required.⁵⁸⁸ Heterozygous carriers had higher adiposity but were otherwise asymptomatic.⁵⁸⁸ Variable presentations depending on type of *LEPR* mutation have been reported. Three members of a family in France of Algerian ancestry with a homozygous truncating mutation of *LEPR* (lacking both the transmembrane and the intracellular domains) had additional symptoms of low IGF-1, IGFBP-3, and growth retardation; the cause of these additional features is not known.⁵⁸⁹ Treatment of *LEPR* deficiency requires targeting downstream mediators of the leptin-melanocortin pathway. Because *LEPR* activation normally would increase POMC and hence the POMC-cleaved

product, α -MSH, which binds MC4R, a logical therapeutic target would be melanocortin receptor agonists. One promising candidate drug is setmelanotide, which functions as a biased agonist for MC4R, favoring $G\alpha_q$ signaling regulated by mitogen-activated protein kinase (MAPK),⁵⁹⁰⁻⁵⁹² thus avoiding the hypertensive side effect of previously developed MC4R agonists that primarily increased $G\alpha_s$ signaling.⁵⁹³ An initial case report of three patients with homozygous *LEPR* mutations who received setmelanotide described substantial weight loss with increased skin and hair pigmentation (because of MC1R activation) as the only major side effect.⁵⁹⁴ Whether this drug may be beneficial in individuals with heterozygous *LEPR* mutations remains to be determined.

POMC Deficiency and Splice Site Mutations. Autosomal recessive mutations in the *POMC* gene that interfere with protein translation or cleavage of the protein into ACTH and α -MSH products results in fair skin and red hair (because of lack of MC1R activation by α -MSH), adrenal hypoplasia and glucocorticoid deficiency (because of lack of MC2R activation by ACTH) with preserved aldosterone and catecholamine production, and obesity (because of lack of MC4R and MC3R activation by α -MSH). Birth weight is normal but hyperphagia drives rapid weight beginning in infancy.⁵⁹⁵ Although red hair is a common feature, a Turkish patient with an early nonsense mutation of *POMC* was reported to have dark hair, indicating that pigmentation can be variable.⁵⁹⁶ The diagnosis can be established based on clinical presentation, ACTH deficiency, and hypocortisolemia. Heterozygous carriers have normal pigmentation and cortisol, but higher predisposition for obesity.⁵⁹⁶ Methylation variants of *POMC* in the general population are correlated with individual body weight, suggesting a wider role of *POMC* in determining obesity risk.⁵⁹⁷ A case report in two patients with *POMC* deficiency showed significant weight loss with setmelanotide treatment, suggesting that MC4R agonism may be beneficial for this condition as well.⁵⁹⁸

Prohormone Convertase-1 Deficiency. Autosomal recessive mutations in the *PCSK1* gene that encodes the PC1 enzyme lead to the inability to process various prohormones to their active ligands, such as POMC to ACTH and α -MSH, proinsulin to insulin, and various gut propeptides to active hormones.^{599,600} Only a handful of patients have been described in the literature, and they are reported as displaying severe early-onset obesity, ACTH deficiency, hypogonadotropic hypogonadism, hyperproinsulinemia, and small intestinal dysfunction because of the inability to cleave intestinal propeptides to their mature form. Hypothyroidism and diabetes insipidus have also been reported.⁶⁰¹ Because of impaired proinsulin cleavage, postprandial hyperglycemia followed by hypoglycemia several hours after a meal is observed. This is caused by initially insufficient insulin secretion followed by reduced clearance of proinsulin, which has mild potency at the insulin receptor, inducing hypoglycemia in the postabsorptive state. The diagnosis can be made by finding extremely high levels of proinsulin, and molecular diagnostics may be required to confirm the gene defect.⁶⁰¹ The potential benefit of using the MC4R agonist setmelanotide to treat obesity in patients with *PCSK1* mutations is currently being investigated.

Carboxypeptidase E Mutation. One adult patient with homozygous truncating mutation of the *CPE* gene has been reported.⁶⁰² *CPE* processes various neuropeptides and prohormones, including POMC and gonadotropins. This patient manifested hyperphagia, severe obesity since childhood, type 2 diabetes, and hypogonadotropic hypogonadism. She also had intellectual disability, which is not a typical characteristic of the other proximal leptin-melanocortin pathway defects discussed thus far. Mice with *CPE* deficiency display hippocampal degeneration and memory deficits, suggesting a role of *CPE* in cognitive function.

Melanocortin-3 Receptor Mutation. α -MSH is the endogenous ligand for both MC3R and MC4R, with MC4R being the more critical receptor for regulation of appetite and energy balance whereas MC3R has only a modest role.¹¹² A heterozygous missense mutation of *MC3R* was identified in two family members of an ethnically Indian family in Singapore, manifesting as severe early-onset obesity in the child but only mild obesity in the father.⁶⁰³ The common MC3R variant T6K+V81I is associated with greater adiposity in children and adults. In vitro, this variant appears to decrease MC3R expression. Humanized knock-in mice display increased feeding efficiency and higher triglyceride storage in adipocytes.⁶⁰⁴ Diagnosis of *MC3R* variants can only be made by gene sequencing. No specific treatment currently exists.

Melanocortin-4 Receptor Mutation. Mutations in the *MC4R* gene appear to account for approximately 5% of severe early-onset obesity cases.^{605–610} Individuals with heterozygous mutations, severe obesity, increased lean mass, increased linear growth, hyperphagia, and severe hyperinsulinemia; individuals with homozygous or compound heterozygous mutations are even more severely affected.^{606,611} Functional severity varies based on the specific mutation, with patients who have mutations retaining residual signaling capacity having a less severe obesity phenotype.^{592,612,613} Thus mutations in *MC4R* result in a distinct obesity syndrome that is inherited in a codominant manner.⁶⁰⁶ Blood pressure is typically lower compared with control subjects with obesity and normal *MC4R*, consistent with the role of the melanocortin system in mediating SNS outflow to the renal and CV systems.⁶¹⁴ Intellectual function is typically normal, although a possible association with attention deficit hyperactivity disorder has been reported in children with homozygous mutations.⁶¹⁵ The diagnosis is made by gene sequencing. There is currently no specific treatment for this disorder, but clustered regularly interspaced short palindromic repeat (CRISPR) activation technologies in mice have successfully upregulated the expression of the remaining intact allele in the heterozygous *Mc4r* knockout to restore normal body weight.⁶¹⁶ Whether this type of gene editing approach can be translatable into humans remains to be determined.

SIM1 Mutation. Single-minded 1 (*SIM1*) is a human homolog of the *Drosophila* single-minded gene that is involved in neurogenesis in both species. In humans, *SIM1* is involved in the development and function of the PVN, the region of the hypothalamus that expresses MC4R and prodynorphin neurons.⁶¹⁷ *SIM1* is believed to function as a downstream mediator of the anorexigenic functions of both these neuronal subpopulations.^{618,619} In a cohort of 2100 individuals with severe obesity, the prevalence of heterozygous *SIM1* mutations was 1.3%,⁶²⁰ and microarray analysis of 279 individuals with syndromic obesity identified one subject with a microdeletion involving *SIM1*.⁵⁷⁸ Patients with *SIM1* haploinsufficiency present with hyperphagia, severe early-onset obesity, normal basal metabolic rate, autonomic dysfunction (lower systolic blood pressure, reduced SNS tone, reduced heart rate variability between sleep and awake states), and varying degrees of neurobehavioral abnormalities and intellectual disability.^{620–624} Increased methylation at the *SIM1* locus is associated with higher BMI in adolescents, suggesting that intermediate phenotypes exist in the general population. The diagnosis of *SIM1* mutation is made by gene testing. There is currently no specific treatment for this disorder, but CRISPR activation technologies in mice have successfully upregulated the expression of the remaining intact allele in the heterozygous *Sim1* knockout to restore normal body weight.⁶¹⁶ Whether this type of gene editing approach can be translatable into humans remains to be determined.

Melanocortin Receptor Accessory Protein 2 (MRAP2) Mutation. MRAP2 protein directly interacts with MC4R and enhances generation of cAMP upon ligand binding of

MC4R.⁶²⁵ Whole-body and brain-specific knockout of *Mrap2* in mice causes severe obesity. Heterozygous pathogenic mutations of *MRAP2* have been identified in four patients with nonsyndromic severe early-onset obesity without additional syndromic features.⁶²³

Brain-Derived Neurotrophic Factor Haploinsufficiency. The *BDNF* gene is located in the 11p14 region. Homozygous loss of function is incompatible with survival but heterozygous loss of *BDNF* is observed in WAGR syndrome, 11p14 microdeletion, and chromosome 11 paracentric inversion, and *BDNF* gene variants.

WAGR/11p Deletion Syndrome: The Wilms tumor, aniridia, genitourinary abnormalities, and range of developmental delays syndrome is a rare genetic disorder (prevalence ~1 in 1,000,000) caused by contiguous gene deletions on the short arm of chromosome 11.¹²⁴ Haploinsufficiency of *WT1* and *PAX6*, which are involved in genitourinary and eye development, respectively, cause the core features of the syndrome. *BDNF* resides at 11p14.1, which is 4 Mb from the 11p13 WAGR critical region that contains *WT1* and *PAX6*, and is included in the deleted segment of approximately half of patients with WAGR syndrome.¹²⁴ In a cohort of 33 patients with WAGR syndrome, *BDNF* haploinsufficiency was associated with higher BMI z-score, fivefold higher frequency of developing childhood obesity (100% vs. 20% for patients with and without *BDNF* deletion, respectively), reduced serum *BDNF* concentrations, and higher scores on a parent-reported hyperphagia questionnaire.⁶²⁶ In this cohort of patients with WAGR syndrome, having *BDNF* haploinsufficiency versus intact *BDNF* was also associated with 20-point lower IQ,⁶²⁷ greater social impairment and higher rate of meeting criteria for autism on the Autism-Diagnostic Interview-Revised,⁶²⁷ lower parent-reported behavioral responses to typically painful stimuli,¹²⁴ and lower self-reported perception of pain in response to hot and cold temperature stimuli.⁶²⁸

11p14 Microdeletion: Microdeletions causing *BDNF* haploinsufficiency while sparing the WAGR region have been reported in 11 individuals with obesity and neurodevelopmental abnormalities.^{130,629,630} Combining the reports, the 1-Mb common region shared by the deletions of all 11 individuals includes only one gene, *BDNF*.

11p Paracentric Inversion: Another form of *BDNF* haploinsufficiency can be caused by loss of expression from one *BDNF* allele even if the allele is still present. Gray et al.¹²⁵ described an 8-year-old girl with a heterozygous paracentric 11p13p15.3 inversion that encompassed *BDNF* without disrupting the sequence of the gene itself but interfered with expression of the inverted *BDNF* allele. Her serum *BDNF* concentration was significantly reduced compared with control subjects with obesity and normal weight, and she displayed hyperphagia, obesity, impaired nociception, and intellectual disability.¹²⁵

***BDNF* gene mutations/variants:** Sequencing of 765 children with early-onset nonsyndromic obesity revealed five heterozygous *BDNF* rare variants predicted to be deleterious in silico that were not present in 480 control subjects without obesity.⁶³¹ The common *BDNF* Val66Met polymorphism, which impairs activity-dependent *BDNF* secretion, is linked to binge episodes in bulimia nervosa and binge eating disorder,¹³² and *BDNF* hypermethylation is associated with bulimia nervosa.¹³³ Associations of *BDNF* Val66Met with BMI and obesity are inconclusive, but the preponderance suggests that the Met allele is protective.^{632–641} The intronic *BDNF* rs12291063 variant (homozygous in ~10% of individuals with African or Latino ancestry; rare in European non-Hispanic whites) is associated with reduced VMH *BDNF* expression and increased adiposity.¹³⁴ Together these observations indicate that *BDNF* insufficiency may underlie common, as well as rare, causes of hyperphagia and obesity.

***NTRK2* Mutation.** The *NTRK2* gene encodes the ligand-specific subunit of TrkB, which is the receptor for BDNF. A case report describes one 8-year-old boy with a heterozygous mutation of *NTRK2* that prevents autophosphorylation of the receptor upon ligand binding. The child manifested normal weight at birth, hyperphagia and severe weight gain beginning at age 6 months, and neurologic abnormalities including seizures, hypotonia, developmental delay, and decreased nociception.^{642,643}

***SH2B1* Haploinsufficiency.** Chromosome 16p11.2 has been shown to account for 0.7% of a large cohort of patients with severe obesity⁶⁴⁴ and 4% of a pediatric cohort that included children with developmental delay in addition to obesity.⁶⁴⁵ A candidate gene for this locus is *SH2B1*, which encodes a signaling adapter protein that is needed for BDNF-stimulated neurite outgrowth.⁶⁴⁶ Heterozygous mutations in *SH2B1* were described in 1.7% of a cohort of patients with severe obesity. The affected patients also displayed neuropsychiatric abnormalities.⁶⁴⁷

Syndromic Obesity Disorders

Several obesity syndromes were included in the earlier section for leptin-melanocortin pathway disorders because the causative genes are thought to be connected with that pathway. Subsequently, additional syndromes with etiologies are not as well delineated.

Prader-Willi syndrome. PWS is a hyperphagic obesity disorder (prevalence ~1 in 20,000) caused by lack of expression of paternally derived genes on chromosome 15q11-13, because of heterozygous deletions of paternal alleles (70%), uniparental disomy in which two copies of maternal alleles are inherited (20%–30%), or imprinting defects in which paternal alleles are silenced because of inappropriate methylation (2%–5%).⁶⁴⁸ In classical PWS, major diagnostic features include neonatal hypotonia, feeding problems in infancy, rapid weight gain after infancy, hyperphagia, developmental delay, hypogonadotropic hypogonadism, and characteristic facial features (narrow face, almond-shaped eyes, small mouth, thin upper lip, and downturned corners of mouth). Minor diagnostic features include decreased fetal movement, weak cry and lethargy in infancy, behavior problems, sleep disturbance, short stature (GH deficiency), hypopigmentation (in the deletion cases that involve *OCA2*, an albinism-related gene in the region), small hands and feet, and skin picking. Other common features include high pain threshold, decreased vomiting, temperature instability, scoliosis, early adrenarche, and unusual skill with jigsaw puzzles.⁶⁴⁹

Attempts to isolate the causative genes for PWS suggest that involvement of more than one gene is likely necessary for the full syndrome to be manifested. Many features of PWS can be observed in patients with inactivating mutations of *MAGEL2*, and screening for *MAGEL2* mutations is recommended for patients presenting with atypical PWS, also known as *Schaaf-Yang syndrome*. This syndrome is characterized by infant hypotonia, feeding difficulties, developmental delay, and autism spectrum disorder, but only one-third of patients develop hyperphagia.^{650–652} *Magel2*-null mice have neonatal growth retardation, excessive weight gain after weaning, and develop increased adiposity, so these mice can be used as a model to study obesity parameters in PWS.⁶⁵³ The cause of hyperphagia in PWS is unknown and likely multifactorial. Hyperghrelinemia is observed in patients with PWS,^{654–656} but pharmacologic suppression of ghrelin has not been therapeutically successful at reducing hyperphagia or body weight, calling into question the pathophysiologic role of ghrelin in PWS.^{657,658} Higher circulating EC have also been observed in patients with PWS.⁶⁵⁹ *Magel2*-null mice have increased

expression of EC receptors and administration of an EC antagonist induces weight loss.⁶⁵⁹

Serum leptin concentrations in patients with PWS are appropriately increased in proportion to their higher fat mass and at levels similar to patients with nonsyndromic forms of obesity.⁶⁶⁰ Therefore leptin production is preserved in PWS. *NPY* and *AgRP* expression appear to be appropriately suppressed in postmortem hypothalamic tissue from patients with PWS, indicating that overexpression of these orexigenic peptides is unlikely to be the cause of hyperphagia in PWS.⁶⁶¹ However, several lines of evidence point to defective signaling along the POMC/CART branch of the leptin-melanocortin pathway. *Magel2*-null mice are resistant to the appetite-suppressing effect of leptin administration,^{662–664} with development of leptin insensitivity occurring between 4 to 6 weeks of age, concomitant with a decline in the number of arcuate POMC neurons, suggesting that the transition from failure to thrive in infancy to hyperphagia in early childhood for patients with PWS may be attributable to a neurodegenerative process. Reduced *PC1* expression has been reported in induced pluripotent stem cells derived from human patients with PWS, which if also true in vivo, would exacerbate any deficiencies in POMC by further reducing processed α -MSH from *PC1* cleavage of POMC.⁶⁶⁵ Consistent with the hypothesis that disruption of POMC signaling could be the etiology of hyperphagia in PWS is the observation that patients with PWS have reduced serum and plasma BDNF concentrations compared with control subjects with obesity and normal weight.¹²⁶ Considering BDNF's role as a downstream mediator of MC4R signaling and its function in neurocognition and pain perception,^{628,666} insufficiency of BDNF could potentially account for the hyperphagia, intellectual disability, behavior abnormalities, and high pain tolerance associated with PWS. Although hyperphagia is the primary driver of weight gain, lower lean muscle mass in PWS reduces REE by 40%, further contributing to energy imbalance.⁶⁶⁷ GH deficiency also leads to defective lipolysis, further promoting adiposity, but this can be reversed with GH supplementation.⁶⁶⁸

Bardet-Biedl Syndrome (BBS). BBS is an autosomal recessive syndrome (prevalence ~1 in 100,000) characterized by hyperphagia, obesity, retinal dystrophy, polydactyly, cognitive impairment, kidney disease, and hypogonadism in males.^{669,670} Over 20 genes encoding proteins involved in the formation, stability, and function of cilia have been implicated in BBS.⁶⁷¹ Mouse models of BBS have defective cilia^{672–675} and impaired leptin receptor trafficking⁶⁷⁵ and signaling.⁶⁷⁶ These mice are unresponsive to leptin administration with lack of STAT-3 phosphorylation and a lack of reduction in food intake. Hyperleptinemia precedes onset of obesity in some but not all studies, calling into question whether leptin resistance develops before or after the onset of obesity, and the role of cilia in adipocyte differentiation suggests that primary fat deposition may also be a contributing cause of obesity in BBS.⁶⁷⁷ In patients with BBS, serum leptin concentrations are higher than those of BMI-matched control subjects, suggesting that in humans, leptin resistance out of proportion to degree of adiposity may play a role in the pathophysiology of ciliopathy-associated obesity.⁶⁷⁸ Supporting the hypothesis that insufficient melanocortin signaling contributes to hyperphagia in BBS, a pilot study of the MC4R agonist setmelanotide showed suppression of hunger and induction of weight loss in patients with BBS.⁶⁷⁹

Alström Syndrome. Alström syndrome (AS) is a rare (<500 reported cases) monogenic form of obesity caused by recessive mutations in the centrosome-body and basal body-associated gene *ALMS1*.⁶⁸⁰ AS is characterized by retinal dystrophy, sensorineural hearing loss, cardiomyopathy, pulmonary fibrosis, renal disease, childhood obesity, severe insulin resistance, higher susceptibility to type 2 diabetes, elevated triglycerides, and steatohepatitis.⁶⁸¹ Frequent endocrinopathies include

hypothyroidism (one-third central, two-thirds primary), central adrenal insufficiency, male hypogonadism (one-third central, two-thirds primary), female hyperandrogenism, and short stature/low IGF-1 concentrations.⁶⁸² In contrast with BBS, patients with AS do not have polydactyly and generally retain normal intellectual functioning. The function of the *ALMS1* protein is not known but is believed to be important for the formation, stability, and function of cilia. *Alms1* knockout mice have reduced number of hypothalamic neuronal cilia,⁶⁸³ and have hyperphagia and obesity.⁶⁸⁴ Because POMC neuronal function is dependent on cilia and intraflagellar transport, reduced POMC function is hypothesized to contribute to the hyperphagia of AS.^{671,685} Consistent with this hypothesis, a pilot study of the MC4R agonist setmelanotide showed suppression of hunger, induction of weight loss, and improved glucose homeostasis in patients with AS.⁶⁸⁶

Smith-Magenis syndrome. SMS (prevalence ~1 in 25,000) is caused by heterozygous retinoic acid–induced 1 (*RAI1*) mutation or deletion. *RAI1* is a transcriptional regulator of *BDNF* involved in craniofacial and nervous system development.⁶⁸⁷ In frogs, knockdown of *Rai1* using antisense morpholinos results in lower *Bdnf* mRNA expression and abnormal brain and face development.⁶⁸⁸ Heterozygous *Rai1* knockout mice have diminished hypothalamic *Bdnf* expression and display hyperphagia and obesity after age 20 weeks on normal chow,⁶⁸⁷ and by 16 weeks on high-fat or high-carbohydrate diets.⁶⁸⁹ SMS in human patients is characterized by intellectual disability, maladaptive and self-injurious behaviors, sleep disturbance, and dysmorphic facial features (brachycephaly, broad face, frontal bossing, synophrys, hypertelorism, upslanting eyes, midface hypoplasia with a depressed nasal bridge, a tented upper lip, prognathism, and low-set or abnormally shaped ears). Hyperphagia and obesity are typically not observed until later childhood or adolescence, mimicking the older onset of these symptoms in mice, and are more pronounced in patients with *Rai1* mutations compared with those with deletions.⁶⁹⁰ Examination of functional effects of mutations found in patients has revealed that the mutated *RAI1* protein fails to localize to the nucleus and does not activate expression of a reporter gene driven by an endogenous *BDNF* promoter.^{127,691} Presence of the mutated protein may have a dominant negative effect on the remaining normal protein, thus leading to a more severe obesity phenotype in patients with mutations.

Cohen Syndrome. Cohen syndrome (<1000 cases reported) is caused by autosomal recessive mutations in *VPS13B* and characterized by microcephaly, hypotonia, intellectual disability, progressive myopia, retinal dystrophy, joint hypermobility, neutropenia, truncal obesity that develops in later childhood, insulin resistance/type 2 diabetes, and dysmorphic features (thick hair and eyebrows, long eyelashes, downslanting and wave-shaped palpebral fissures, bulbous nasal tip, smooth or shortened philtrum, prominent upper central teeth, open mouth, narrow hands and feet, and slender fingers). *VPS13B* encodes a Golgi apparatus protein that is involved in protein glycosylation and intracellular transport in neurons and adipocytes.^{692–697}

Carpenter Syndrome. Carpenter syndrome (<100 cases reported) is caused by autosomal recessive mutations in *RAB23* (involved in vesicle trafficking) or *MEGF8* (involved in cell adhesion) and is characterized by acrocephalic or cloverleaf craniosynostosis, intellectual disability, childhood-onset obesity, flat nasal bridge, downslanting palpebral fissures, low-set and abnormally shaped ears, micrognathia, small primary teeth, cutaneous syndactyly of third and fourth fingers, brachydactyly, polydactyly, umbilical hernia, hearing loss, heart defects, deformed hips, kyphoscoliosis, genu valgum, cryptorchidism, situs inversus, dextrocardia, and transposition of the great arteries.^{698–704}

Hypothalamic Obesity

Hypothalamic damage can occur because of CNS tumor, surgery, radiation, trauma, inflammation, or infiltrative diseases. Injury to the VMH and ARC of the MBH are the critical regions that lead to the phenomenon of hypothalamic obesity, which is characterized by extremely rapid weight gain. For example, it is typical in the first 6 to 12 months after craniopharyngioma surgery for tumors involving the floor of the third ventricle (the hypothalamic homeostatic centers described earlier) for patients to increase weight by greater than 50%. Mechanistically, it has been well known that bilateral electrolytic lesions or deafferentation of the VMH in rats leads to intractable weight gain, even upon food restriction.^{200,201,705–708} Originally, weight gain was thought to be solely a problem with hyperphagia and increased energy storage.⁷⁰⁹ However, we now understand that a dysfunction of leptin-melanocortin pathway signaling can alter both the afferent and efferent pathway of energy balance and lead to severe and intractable weight gain that is caused by more than just overeating.^{710,711} The hypothalamic insult prevents the integration of peripheral afferent signals, and therefore the CNS is unable to detect nutrient sufficiency within the periphery and therefore responds as if in the starvation state. Caloric restriction alone is insufficient because patients also have reduced sympathetic activity, which reduces energy expenditure and lipolysis, and heightened parasympathetic (vagal) tone, which promotes insulin hypersecretion and energy storage.^{710,712–716} In both animals and humans, vagal hyperreactivity can be prevented by pancreatic vagotomy.^{201,717–719} Therapy for this disorder remains extremely problematic, as the brain seems to be “locked” in an orexigenic balance favoring energy consumption and storage. As the crucial period of weight gain is within the first year following the brain insult, it is crucial to focus on preventive measures during this narrow window of opportunity. Comprehensive treatment should include psychologic care to address cognitive, behavior, and sleep disturbances that commonly occur; endocrine hormone replacement for deficiencies; and intensive lifestyle (nutrition and exercise) management. Medical therapies, such as insulin sensitizers, insulin secretion blockers, GLP-1 analogues, stimulants, and oxytocin have all been investigated and each show modest benefits, but the intractable nature of the disease makes management exceedingly challenging.⁷²⁰ It is accepted practice today that all efforts should be made to avoid surgical trauma of the hypothalamic homeostatic centers, even at the price of leaving remnant tumor tissue (in cases of craniopharyngioma which typically involves this region).

ROHHAD (rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysfunction) syndrome is a form of hypothalamic obesity that is not well understood and has a very poor prognosis. Median age of diagnosis is 4 years and presents as extremely rapid weight gain in an otherwise previously healthy child.⁷²¹ ROHHAD appears to be a progressive neurodegenerative condition that evolves from hyperphagic obesity to global hypothalamic dysfunction (including GH deficiency, hypogonadotropic hypogonadism, central hypothyroidism, and ACTH abnormalities), central hypoventilation or altered respiratory control, autonomic dysfunction (bradycardia requiring pacemaker placement), thermal dysregulation, and risk for the development of tumors of neural crest origin (e.g., ganglioneuromas and ganglioneuroblastomas).⁷²² Although *BDNF* and *NTRK2* have been screened as candidate genes for ROHHAD, no mutations in these genes have been identified to date.⁷²³ However, one patient, an 11-year-old boy with BMI of 62 kg/m² with a clinical diagnosis of ROHHAD, was found to have a heterozygous truncating mutation of *RAI1*, which is a transcriptional regulator of *BDNF*

and is implicated as the causative gene for SMS.⁷²⁴ In addition to severe obesity, this patient also exhibited intellectual disability, autism spectrum disorder, dysmorphic facial features (macrocephaly, hypertelorism, flat nasal bridge, prominent forehead, and anteverted nares), high pain tolerance, excessive sweating, lack of fever with infections, obstructive and central sleep apnea, and hypoventilation leading to tracheostomy placement. Pituitary hormone abnormalities and neural crest tumors were lacking in this patient. Thus he could be categorized as atypical ROHHAD or possibly atypical SMS, and screening for *RAI1* haploinsufficiency should be considered in the differential for patients presenting with features of ROHHAD. Without treatment, ROHHAD has a high mortality rate from respiratory failure. Immunomodulator therapies have been attempted and show some benefit in slowing disease progression but there are no curative treatments known.⁷²⁵

EVALUATION AND TREATMENT OF PEDIATRIC OBESITY

Diagnostic Approach

The key to successful obesity therapy is accurate diagnosis. Our diagnostic armamentarium is not yet fully developed, so matching treatment to diagnosis is still uncertain. Specific points in the evaluation and their rationale are listed in Table 24.1. In eliciting the history, birth weight, parent's BMIs, exposure to gestational diabetes, prematurity, history of breast-feeding, and neonatal complications (especially CNS injury) are all relevant. The younger the patient's obesity is noted, the more likely an organic reason will be identified. Neurodevelopmental abnormalities and signs of dysmorphism may signify the need for a genetic referral. The medication list must be reviewed, especially for glucocorticoids and atypical antipsychotics. Orthopedic pain, headache, and snoring must be assessed. Dietary history must include skipping breakfast, daily ingestion of sodas and juices, and frequency and type of snacking. A corollary is the number of caretakers of the child because this increases stress, family chaos, and lack of child supervision.

On physical examination, linear growth is key, as classical endocrine evaluation (e.g., hypothyroidism, Cushing, GH deficiency, PHP) is not necessary if linear growth is not attenuated. However, hyperinsulinemia and insulin resistance may cause accelerated growth, because of cross-reactivity of insulin with the IGF-1 receptor.⁴³⁸ Important physical features to assess include acanthosis nigricans and waist circumference (both of which are associated with insulin resistance and the metabolic syndrome); fundoscopic examination to rule out pseudotumor cerebri if symptoms are suggestive; liver enlargement to suggest hepatic steatosis; hirsutism to suggest PCOS; and muscle tone to evaluate hypotonia and myopathy, which reduce energy expenditure. Early-onset obesity (during infancy and toddlerhood) along with dysmorphic features should raise the suspicion of an underlying genetic disorder.

Laboratory evaluation includes tests of obesity-related morbidity (e.g., aspartate aminotransferase, ALT, lipids, fasting glucose and hemoglobin A1c) and z knee and hip x-rays (in cases of suspected Blount deformity). Specific diagnostic studies must be tailored to the individual patient. For instance, growth attenuation (typically of accelerated weight gain along with deceleration of linear growth) requires endocrine evaluation, including thyroid function tests, IGF-1 and IGFBP-3, 24-hour urinary cortisol or midnight serum cortisol, and possibly MRI of the hypothalamus and pituitary. Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels may be appropriate when evaluating for delayed puberty in males or PCOS in females. Patients with developmental delay will require karyotype and MRI. Severe obesity in a toddler may require a

TABLE 24.1 Diagnostic Evaluation of Childhood Obesity and Its Comorbidities

HISTORY	
Genetic etiology	Parent BMI, race, intellectual disability
Epigenetic etiology	Birth weight, gestational difficulties, prematurity
CNS etiology	CNS insult, intellectual disability or developmental delay
Endocrine etiology	Slowdown in linear growth, red hair
Medication etiology	Medication history, esp. atypical antipsychotics
Dietary etiology	Calorie recall, esp. sugar-containing liquids, breast-feeding Hx
Physical activity etiology	Exercise history, television, computer, and cellphone recall
Stress etiology	Socioeconomic status, number of caregivers, television recall, sleep status, atypical depression
Sleep apnea	History of snoring, headache, waking up with headache
PCOS	Hirsutism, oligomenorrhea, amenorrhea
Type 2 diabetes	Polyuria, polydipsia, nocturia, recent weight loss
Orthopedic morbidity	Knee or hip pain, limitation of motion
Depression	Affect, activity level, school performance
PHYSICAL	
Insulin resistance	Acanthosis nigricans, skin tags, waist circumference
Hypertension	SBP or DBP >90 th percentile for age
Pseudotumor cerebri	Papilledema
Hepatic steatosis	Hepatomegaly
PCOS	Hirsutism
Precocious/delayed puberty	Gonadal and pubic hair status
Sleep apnea	Tonsillar hypertrophy
Myopathy	Decreased muscle tone, hyporeflexia
Syndromic obesity	Specific neurocutaneous stigmata (see Box 24.1), intellectual disability
LABORATORY	
Hepatic steatosis	ALT, hepatic ultrasound
Glucose intolerance	Fasting glucose > 100 or 2-h glucose >140
Type 2 diabetes mellitus	Fasting glucose >125 or 2-h glucose >200; HbA1c >6.5%
Dyslipidemia	Lipid profile with increased VLDL, TG:HDL >2.5
Insulin resistance	Fasting insulin, glucose
Insulin hypersecretion	3-hour OGTT with insulin levels
CNS lesion	MRI, esp. with hypothalamic coned-down views

Also uric acid: >5.5 as a proxy for sugar consumption
 Free thyroxine, thyroid-stimulating hormone to rule out hypothyroidism
 ALT, Alanine aminotransferase; BMI, body mass index; CNS, central nervous system; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; MRI, magnetic resonance imaging; OGTT, oral glucose tolerance test; PCOS, polycystic ovarian syndrome; SBP, systolic blood pressure; TG, triglyceride; VLDL, very low-density lipoprotein.

leptin level and genetic testing for an MC4R or leptin receptor mutation. The correlation between fasting insulin and euglycemic-hyperinsulinemic clamp-derived whole-body insulin sensitivity in children is poor, indicating that it is an inappropriate surrogate of whole-body insulin sensitivity.⁷²⁶ This may have to do with the fact that fasting insulin levels likely reflect hepatic rather than total body insulin sensitivity.⁷²⁷ An oral glucose tolerance test (OGTT) may become necessary in a child with obesity, to evaluate for type 2 diabetes in cases where a suggestive history and the presence of familial risk factors is present.

Lifestyle Modification

Lifestyle modification remains the cornerstone of obesity therapy, especially in children. This approach is based on common sense, and on a handful of early studies demonstrating efficacy of lifestyle in a select handpicked group of children with intensive follow-up.⁷²⁸ Data supporting the long-term efficacy of lifestyle modification in the “real world” is not particularly persuasive. A recent analysis of numerous methodologies concluded that although there are limited quality data to recommend one treatment program to be favored over another, combined behavioral lifestyle interventions compared with standard care or self-help can produce a significant and clinically meaningful reduction in overweight in children and adolescents.⁷²⁹ Importantly, follow-up studies of numerous large-scale interventions often find a rapidly diminishing effect once the study is completed. Finally, a metaanalysis of 39 published intervention studies designed to prevent childhood obesity showed that 40% of the 33,852 participating children had reduction in BMI, whereas the remaining 60% exhibited no effect.⁷³⁰

It has been shown that 1 year after initial weight reduction, levels of the circulating mediators of appetite that encourage weight regain after diet-induced weight loss do not revert to the levels recorded before weight loss. Moreover, following significant weight reduction, whether induced by conservative or invasive measures, the metabolic and hormonal profile maintains a “starvation response” (a metabolic adaptation aiming at returning to the previous weight) even years after the intervention.²⁵⁰ This emphasizes the problem of long-term weight loss maintenance and the difficulties facing those who wish to lose weight and maintain it.⁷³¹ Optimistically, the effectiveness of interventions to prevent, rather than reverse, obesity in childhood (particularly for programs targeted to children aged 6–12 years) seem to be much more effective.⁷³²

Although weight loss is the conventional goal for intervention among adults, weight maintenance is the recommended target for the majority of children. The prevention of weight gain is easier, less expensive, and more effective than treating obesity itself,⁷³³ and the prevention of overweight among children before the presence of risk-related behaviors is crucial to stem the obesity epidemic. The primary goal of obesity prevention should be to promote physical activity and healthy diet with emphasis on improving overall health, rather than weight loss.⁷³⁴ There are at least four reasons to promote interventions to improve nutrition and physical activity in children.⁷³⁵ First, the child may receive immediate benefits, such as better fitness or energy or micronutrient intake. Second, intervention at critical periods may improve adult health. Third, modifying chronic disease risks in childhood may lead to lower rates and risk factors in adults. Lastly, modification of children’s behaviors may lead to improved behaviors in adulthood that would protect against chronic diseases.

Behavioral-cognitive therapy is designed to deal with both parent and patient, with behavior restructuring and reinforcement. Behavior changes include counseling sessions, teaching parenting skills, praise and contracts, self-monitoring tools, stimulus control within the home, role-modeling of behaviors by parents, and vigorous and long-term exercise programs. These programs have been successful in small studies with hand-picked subjects by specific investigators,^{736,737} but have not yet been successful when attempted in clinic populations. One new clinical approach involves motivational interviewing,⁷³⁸ a method for helping patients work through their ambivalence about behavior change. This method has been shown to be effective for substance abuse and in adult diabetes; whether it will be successful in pediatric obesity remains to be seen.⁷³⁹

Dietary Intervention

Dietary intervention is essential to reduce not just caloric intake, but to reduce the insulin response that promotes excessive energy deposition into adipose tissue. A myriad of studies demonstrate an association between consumption of high-calorie, high-fat, high-carbohydrate, low-fiber foods and the development of pediatric obesity.⁵⁰⁴ Specific maneuvers that have been successful in reducing the insulin response and promoting weight loss or stability in children include (1) elimination of sugar-containing beverages (including both soda and juice)^{740,741} and (2) a shift to a low glycemic load diet.⁵¹⁴ Not only does dietary modification to reduce the insulin response assist in promoting weight loss,⁷⁴² but it also promotes increased energy expenditure during the weight maintenance phase,⁷⁴³ presumably by improving leptin sensitivity, which would help prevent weight regain. However, dietary intervention alone is not a successful strategy for reducing pediatric overweight, unless the treatment is very intensive,⁷⁴⁴ in which case, the majority drop out. Furthermore, studies of non-supervised dieting have demonstrated the opposite effect, that is, increased attempts in female adolescents predict a greater increase in weight and risk for obesity.⁷⁴⁵

Importantly, diets are a crucial part of weight maintenance and not only aimed at weight loss. Specifically, following a successful weight loss—a weight maintenance diet is crucial to prevent weight regain because of the metabolic “starvation response” mentioned earlier. Thus the dietary changes proposed to the child should be adapted for life as a “healthy eating” strategy rather than a short-term weight plan. Comparison of dietary approaches to weight maintenance following weight loss in children has shown that among young adults with overweight/obesity compared with preweight-loss energy expenditure, isocaloric feeding following 10% to 15% weight loss resulted in decreases in REE and total energy expenditure (TEE) that were greatest with a low-fat diet, intermediate with the low-glycemic index diet, and least with the very low-carbohydrate diet. This suggests that a low carbohydrate diet may attenuate the “starvation response” induced by weight loss and help sustain the new weight achieved.⁷⁴³

Physical Activity Intervention

In adults, exercise is not an effective means of inducing weight loss unless combined with reduction of caloric intake. The role of exercise may have a greater impact on weight maintenance, rather than weight loss.⁷⁴⁶ Similarly, there is the question as to whether physical activity regimens for children can stabilize or reduce BMI.^{747,748} Short-term studies show that vigorous exercise can result in short-term reductions in adiposity⁷⁴⁹ and improved insulin sensitivity.⁷⁵⁰ This amounts to a minimum of 30 minutes of vigorous exercise, 5 days per week, as recently proposed activity guidelines from the Institute of Medicine.⁷⁵¹ However, such interventions eventually plateau in their effectiveness, and even short-term cessation rapidly reverses any accrued benefit.⁷⁵² To succeed, physical activity interventions must be long-term, sustained, and incorporated into behavioral modifications aimed at the individual, at the family, at the school, and within the community in general.^{749,752} In other words, to make physical activity work, it must become a priority.

Lifestyle programs including supervised exercise can improve fasting insulin levels as quickly as 2 weeks before measurable weight loss occurs. Furthermore, lifestyle intervention improved body composition without a change in body weight. Available studies suggest that fitness may play a more important role than BMI reduction on improvement in insulin sensitivity in adolescents with obesity. Appropriate interventions include (1) making school-based physical education mandatory for

every child and school; (2) increasing access to after-school recreation; (3) increasing culturally appropriate activities; (4) reducing the competitive nature of sports, so more children will participate; (5) increase incorporation of physical activity into daily life (e.g., stairs, walking to school as appropriate); and (6) increase participation of parents in physical recreation, for their own weight management and as role models for their children. Lastly, the effects of reducing sedentary behavior by restricting television viewing has been efficacious in both small and large studies, and in diverse ethnic groups,⁴⁸⁷ although fitness is not improved. Restriction of television also has a secondary effect of reducing caloric intake while watching television.

The results of the TODAY study have shown that the addition of an intensive exercise intervention to pharmacologic and dietary regimens in adolescents with type 2 diabetes had no additional benefit in any clinical outcome.⁷⁵³ This observation probably reflects the “real life” compliance to such interventions among the relevant target populations. Results using either dietary or exercise intervention alone are not encouraging. Those studies that use behavioral, dietary, and exercise components together appear to be more successful,^{504,754} although long-term efficacy is lacking.

Family Intervention

Invariably, the patient is not the only member of the family with obesity. There is frequently a familial environment that promotes obesity in other family members. Various caretakers (e.g., grandmothers, babysitters, etc.) will feed children and allow unrestricted television time as a method of confining their activities indoors, particularly in dangerous neighborhoods. Some parents will not alter their shopping for less healthy food because they believe other sibs should not be deprived of it. Divorced parents will often use food as reward to buy the child's love and loyalty. Thus the family itself must be the target of lifestyle intervention. Parental involvement is critical, and the concept of “food is not love” must be emphasized. Families need training to modify behavior to make healthy dietary choices, increase activity, and to reduce perceived stress. A recent meta-analysis of randomized trials of combined lifestyle interventions for treating pediatric obesity yielded a significant although underwhelming decrease in BMI of 1.5 with targeted family intervention, and a nonsignificant decrease in BMI of 0.4 in those that targeted the patient alone.⁷⁵⁵

Pharmacotherapy

Indications for Pharmacotherapy

Pharmacologic therapies in children must currently be considered adjuncts to standard lifestyle modification. The main problem is that presently most drugs are not approved for use in children. In addition, several limitations preclude physicians from early implementation of drug therapies for the treatment of childhood obesity, including (1) the youngest child for whom any obesity pharmacotherapy is currently US Food and Drug administration (FDA) approved is 10 years; (2) the long-term use of pharmacologic intervention has not always proven to be more efficacious than behavior modification; (3) there exists a limited number of well-controlled studies of safe and effective pharmacologic intervention in children with obesity; (4) the relative risk for the development of adverse events in children must be weighed against the long-term potential for improvement of morbidity and mortality, which is difficult to estimate in children; and (5) targeting the pathology is still in its infancy. Also, we must not forget that many drugs used for treatment of adult obesity resulted in unforeseen complications, which resulted either in their restriction (thyroid hormone, amphetamine) or recall (e.g., sibutramine,

rimonabant, dinitrophenol, fenfluramine, dexfenfluramine, phenylpropranolamine, ephedra).⁷⁵⁶⁻⁷⁶¹ Despite these concerns, the negative health impact of childhood obesity may justify long-term medication to control its progression.

In the current pharmacopoeia of childhood obesity (Table 24.2), only a nonspecific fat absorption reduction approach is available for obesity per se. Targeting obesity-related insulin resistance adds additional agents yet their effect in regard to weight reduction is disappointing.

Reduction of Energy Absorption: Orlistat

This drug is a modified bacterial product that specifically inhibits intestinal lipase and can reduce fat and cholesterol absorption by approximately 30% in subjects eating a 30% fat diet.⁷⁶² Orlistat irreversibly binds to the active site of the lipase, preventing intraluminal deacylation of triglycerides, resulting in an approximate 16 g/d increase in fecal fat excretion.⁷⁶³ Orlistat does not inhibit other intestinal enzymes. It has minimal absorption and exerts no effect on systemic lipases.^{764,765}

Although there have been several open-label trials of orlistat in adolescents, only two randomized control trials (RCTs) have been published.^{766,767} The side effects with orlistat are predictable from its mechanism of action on intestinal lipase.⁷⁵⁶ Orlistat appears to be well tolerated in adults, with the principal complaints being borborygmi, flatus, and abdominal cramps. The most troubling side effects are fecal incontinence, oily spotting, and flatus with discharge, which are highly aversive in the pediatric population. Orlistat does not affect the pharmacokinetic properties of most other pharmaceutical agents. Absorption of vitamins A and E and β -carotene may be slightly reduced, and this may require vitamin therapy in a small number of patients. Orlistat must be taken with each meal, which reduces its attractiveness in children, who are in school during lunchtime. Orlistat is currently approved for treatment of children as young as 12 years. An over-the-counter lower dose preparation recently obtained FDA approval, and should be available soon.

Improvement of Insulin Resistance: Metformin

Metformin is a bisubstituted, short-chain hydrophilic guanidine derivative used for the treatment of children and adults with T2DM.⁷⁶⁸⁻⁷⁷¹ Metformin also decreases fasting hyperinsulinemia, may prevent T2DM⁷⁷² and promotes weight loss in some but not all individuals with obesity,^{773,774} by improving hepatic and muscle insulin sensitivity. Metformin has little effect on energy expenditure.⁷⁶⁹ Although some believe that metformin promotes weight loss through a primary anorectic effect (because initial side effects of nausea and gastrointestinal distress limit caloric intake acutely),⁷⁷⁵ most believe that the decline in caloric intake observed with metformin is related to its enhancement of glucose clearance, through reduction of hepatic glucose output, and reduction in fasting hyperinsulinemia.^{776,777} Metformin improves hepatic insulin resistance by inducing hepatic AMP kinase,⁷⁷⁸ which reduces hepatic gluconeogenesis; therefore pancreatic insulin secretion and peripheral insulin levels fall. Metformin also restores PI3K and MAPK activity in muscle cells, improving muscle insulin sensitivity.⁷⁷⁹ Another possible mechanism of metformin action is through stimulation of GLP-1,^{780,781} which may inhibit food intake through central actions on the VMH.⁷⁸²

Thus far, results of two RCTs in children and adolescents have been reported.^{783,784} Examination of the open-label responses to metformin in a multivariate analysis demonstrated two predictors for efficacy: race (white >black American) and the degree of insulin resistance before therapy (among youth with obesity, those with insulin resistance

TABLE 24.2 Medications for the Treatment of Pediatric Obesity

Drug	Dosage	Efficacy	Side Effects	Monitoring and Contraindications
Orlistat Not FDA approved for <12 years of age	120 mg PO TID	Open-label: Wt–5.4 kg, BMI–2.0 kg/m ² at 6 months; RCT: Wt–2.6 kg, BMI–0.85, W –2.7 cm over placebo at 12 months	Borborygmi, flatus, abdominal cramps, fecal incontinence, oily spotting, vitamin malabsorption	Monitor 25OHD ₃ levels. MVI supplementation is strongly recommended. A lower dose preparation has been approved for over-the-counter sale
Metformin Not FDA approved for treatment of obesity Approved for ≥10 years of age for type 2 diabetes mellitus	250–1000 mg PO BID	RCT: BMI z-score –0.35 SD vs. placebo at 6 months RCT: Wt–2.7% vs. placebo at 6 months; Post-hoc analysis: efficacy dependent on degree of insulin resistance; BMI z-score –0.23 SD in first 4 months, –0.12 SD in next year	Nausea, flatulence, bloating, diarrhea; usually resolves. Lactic acidosis not yet reported in children	Do not use in renal failure or with intravenous contrast MVI supplementation is strongly recommended
Octreotide Not FDA approved for treatment of obesity, otherwise ≥18 years of age	5–15 mcg/kg/d SQ ÷ TID	Open-label: Wt–4.8 kg, BMI–2.0 kg/m ² in 6 months; RCT: –7.6 kg, BMI–2.5 kg/m ² over placebo at 6 months Posthoc analysis: BMI z-score –0.70 SD in 6 months, dependent on insulin secretion and sensitivity	Gallstones, diarrhea, edema, abdominal cramps, nausea, bloating, reduction in thyroxine concentrations	Monitor fasting glucose, FT ₄ , HbA _{1c} . Useful only for hypothalamic obesity Ursodiol coadministration strongly recommended
Leptin Not approved by FDA	Titration of dose to serum levels, SQ	Anecdotal: BMI–19.0 kg/m ² over 4 years	Local reactions	Useful <i>only</i> in leptin deficiency
Topiramate Not FDA approved for treatment of obesity	96–256 mg PO qd	RCT: Wt–8.0% over placebo at 6 months	Paresthesia, difficulty with concentration/attention, depression, difficulty with memory, language problems, nervousness, psychomotor slowing	No pediatric data
Growth hormone Not FDA approved for treatment of obesity	1–3 mg/m ² SQ qd	Decreases in percentage body fat, with increases in absolute lean body mass	Edema, carpal tunnel syndrome, death in patients with preexisting obstructive sleep apnea	Recommended only in Prader-Willi syndrome primarily to increase height velocity. It also decreases fat mass but should only be used in those have been after screening to rule out obstructive sleep apnea. Must closely monitor pulmonary function, glucose, HbA _{1c}

Should be considered only after an unsuccessful 6-month trial of lifestyle intervention.

All drugs effective only when combined with appropriate lifestyle intervention.

BID, Twice a day; BMI, body mass index; FDA, US Food and Drug administration; FT₄, free thyroxine; HbA_{1c}, hemoglobin A1c; MVI, multivitamins; PO, orally; qd, every day; RCT, randomized controlled trial; SD, standard deviation; SQ, subcutaneously; TID, three times a day; wt, weight.

respond better than those who are insulin sensitive).⁷⁸⁵ Metformin has also been used “off-label” for treatment of PCOS and NASH, with varying degrees of success.^{786–791} One particular use for metformin may be to combat the weight gain associated with atypical antipsychotics.⁷⁹² However, cessation of metformin therapy leads to a rebound hyperinsulinemia and rapid weight gain, which may negate any beneficial effects seen during the medication window.

Side effects with metformin include nausea, flatulence, bloating, and diarrhea at initiation of therapy, which appears to be self-limited and resolves within 3 to 4 weeks of initiation of the drug. Approximately 5% of pediatric patients discontinue metformin therapy because of the severity of side effects. The most feared complication of metformin in adults is lactic acidosis, which is estimated to occur at a rate of 3 per 100,000 patient-exposure years, primarily in patients with

contraindications to the use of metformin; however, no documented cases in children have been reported. Metformin increases the urinary excretion of vitamins B₁ and B₆, which are important in the tricarboxylic acid cycle, and which may hasten the lactic acidosis.⁷⁹³ Vitamin B₁₂ deficiency also has been reported in as many as 9% of adult subjects using metformin. Therefore prophylactic multivitamin supplementation is recommended with metformin use. Contraindications to metformin use include renal insufficiency, congestive heart failure or pulmonary insufficiency, acute liver disease, and alcohol use sufficient to cause acute hepatic toxicity. Metformin also should be withheld when patients are hospitalized with any condition that may cause decreased systemic perfusion, or when use of contrast agents is anticipated.⁷⁷⁶ It should be noted that metformin is FDA approved for treatment of T2DM in children but is unlikely to be approved for childhood obesity or insulin resistance.

Suppression of Insulin Hypersecretion: Octreotide

It is well known that bilateral electrolytic lesions or deafferentation of the VMH in rats leads to intractable weight gain,^{794–798} even upon food restriction.⁷⁹⁹ In humans, hypothalamic damage, either caused by CNS tumor, surgery, radiation, or trauma, can alter both the afferent and efferent pathway of energy balance, and lead to severe and intractable weight gain.^{800,801} In this syndrome of “hypothalamic obesity,” hypothalamic insult confers an “organic leptin resistance” as the VMH senses starvation;^{797,802} therefore energy intake is high, and expenditure is low.⁸⁰³ Children with hypothalamic obesity exhibit weight gain, even in response to forced caloric restriction,⁸⁰⁴ secondary to (1) overactivation of the vagus, which promotes an obligate insulin hypersecretion and energy storage and (2) defective activation of the SNS, which retards lipolysis and energy expenditure.^{805,806} Insulin hypersecretion with normal insulin sensitivity is noted on oral glucose tolerance testing in these children.⁸⁰⁷ This same phenomenon of insulin hypersecretion has also been documented in a subset of adults who have obesity without CNS damage.⁸⁰⁸

The voltage-gated calcium channel of the β -cell is coupled to a somatostatin-5 receptor (SSTR₅).^{809,810} Octreotide binds to this receptor, which limits the opening of this calcium channel, reduces influx of calcium into the β -cells, and in turn reduces calmodulin activation and vesicle exocytosis, thereby acutely decreasing the magnitude of insulin response to glucose⁸¹¹ (see Fig. 24.3), which results in weight loss or stabilization. Two RCTs and an observational prediction study using octreotide for obesity have been performed.^{246,812} An examination of BMI responses to octreotide in pediatric hypothalamic obesity in a multivariate analysis demonstrated that insulin hypersecretion with concomitant retention of insulin sensitivity before therapy augured for success.⁷⁸⁵ A larger scale study of patients following a brain insult showed limited efficacy, probably because of the timing of the intervention—the majority of weight gain in cases of hypothalamic obesity occurs early following the insult. Pharmacotherapy that begins too late may fail to reverse this condition whereas early initiation of treatment may prevent excessive weight gain.

Octreotide is usually well tolerated. The most common side effects include diarrhea, abdominal cramps, nausea, and bloating, which are self-limited, and usually resolve in 3 to 4 weeks.^{813,814} Other adverse events include gallstones (which are preventable by coadministration of ursodiol), edema, development of sterile abscess at the injection sites, B₁₂ deficiency, suppression of GH and TSH secretion, and mild hyperglycemia, especially in those with severe insulin resistance.⁸¹⁵

Other Targeted Therapies

Leptin. Mutations of the leptin gene in humans recapitulate the phenotype of the *ob/ob* leptin-deficient mouse.⁸¹⁶ Approximately 11 such patients have been described; they manifest hyperphagia from birth, with obesity documentable as early as 6 months of age. Leptin deficiency induces the starvation response,⁸¹⁷ with increased energy intake and decreased REE. The diagnosis is made by extremely low or unmeasurable serum leptin levels. In children with leptin deficiency, leptin therapy results in extraordinary loss of weight and fat mass,^{818,819} along with reduction in hyperphagia, resolution of obesity, induction of puberty, and improvement in immunity.⁸¹⁷ Although leptin administration in adults did not prove effective by itself because of leptin resistance,⁸²⁰ leptin may serve as an adjunct in combination with other medications after leptin sensitivity is ameliorated through weight loss.^{821,822}

GLP-1 Analogs. Several long-acting GLP-1 analogs have been recently introduced, some with an antiobesity indication.

GLP-1 is a gut-derived incretin that also serves a satiety signal as described earlier. As its half-life in the circulation is brief (~2 minutes) because of degradation by the enzyme dipeptidyl peptidase-4 (DPP-4), stable analogues have been designed, aiming initially at β -cell enhancement but also showing some significant weight loss effects.⁸²³ The safety of GLP-1 analogues has been demonstrated in children with obesity,⁸²⁴ efficacy trials are presently performed and should be published soon.

The Future of Pediatric Obesity Pharmacotherapy

In response to the relative lack of efficacy of lifestyle interventions, the ever-expanding knowledge of the physiology of energy balance, and particularly as a business decision of potential financial reward, many pharmaceutical companies have launched obesity research programs. The following agents are currently in human study; however, use of any of these new agents in children will depend on proof of safety and efficacy based on experience in adults. **Topiramate** is an anticonvulsant that blocks voltage-dependent sodium channels, enhances the activity of the GABA_A receptor, and antagonizes a glutamate receptor other than the *N*-methyl-D-aspartate (NMDA) receptor.⁸²⁵ Topiramate promotes weight loss in a dose-dependent fashion.⁸²⁶ An RCT in adults demonstrated a 9.1% weight loss in subjects taking topiramate 192 mg/d along with significant improvements in blood pressure, waist circumference, and fasting glucose and insulin.⁸²⁷ However, almost 33% of the subjects dropped out because of adverse events, which included paresthesias, somnolence, anorexia, fatigue, nervousness, decreased concentration, difficulty with memory, and aggression. There are currently no studies of topiramate in childhood obesity. **Oxyntomodulin** is an analog of PYY₍₃₋₃₆₎, which has been shown in a 4-week RCT to reduce energy intake and weight in adults.⁸²⁸ There are no studies in children. Current weight-loss formulations that have been approved in adults include combination phentermine-topiramate (*QSymia*[®]); combination naltrexone-bupropion (*Contrave*[®]); lorcaserin, a serotonin 2c-agonist (*Belviq*[®]); and liraglutide, a GLP-1 agonist (*Saxenda*[®]). There are no published clinical efficacy trials in children as of yet.

Bariatric Surgery

In comparison with adults, stricter and more conservative criteria must be applied to adolescents because only 85% of adolescents with obesity will become adults with obesity including the slightly improved rate of lifestyle and pharmacotherapeutic efficacy versus adults; a longer time interval before comorbidities become life-threatening; and their inability to give legal consent. For all these reasons, an expert panel with representation from the American Pediatric Surgical Association and the American Academy of Pediatrics has suggested that bariatric surgery for adolescents should be done only in institutions committed to long-term management of these patients⁸²⁹ and is justified in situations when obesity-related comorbid conditions (like OSA) threaten the child’s health. They provided stringent recommendations that bariatric surgery be limited to those adolescents with BMI over 35 or 120% of the 95th percentile with presence of severe comorbidity (severe OSA, pseudotumor cerebri, type 2 diabetes or steatohepatitis) or BMI over 40 or 140% of the 95th percentile with a less severe comorbidity.^{830–832} Published data support the notion that bariatric surgery is effective in adolescents with obesity in regard to weight loss and improvement of comorbidities similar to adults.^{833,834} The majority also improve psychosocial parameters yet, importantly, some may develop depression, suicidal ideation, and overall lower psychosocial well-being.⁸³⁵ It is not clear what the bariatric procedure of choice should be

in this population and what fashion of preparation and follow-up will allow optimization of long-term results.

Gastric bypass has been performed more widely in children and adolescents since the 1990s, whereas sleeve gastrectomy has been adopted from the adult practice since 2010 and is now performed twice as frequently as gastric bypass.^{835a} These procedures appear to be safe and effective when candidates are carefully selected, and the bariatric surgeon has advanced laparoscopic skills. Thus far, we have very limited data on long-term complications, including those that might be associated with future pregnancy, bone health, and outcomes of repeated surgical procedures in case of weight regain. Nonetheless, the data thus far suggest potential benefit for obesity-driven comorbidities and improvement of quality of life in youth with severe obesity and obesity-related complications. However, how widespread bariatric surgery should be used as a solution remains to be determined until we have additional and longer term outcomes data.

Indications for Bariatric Surgery

An expert panel with representation from the American Pediatric Surgical Association and the American Academy of Pediatrics⁸²⁹ suggested that bariatric surgery in adolescence could be justified in situations when obesity-related comorbid conditions threaten the child's health. They provided stringent recommendations that bariatric surgery be limited to those adolescents with BMI over 35 with presence of severe comorbidity, or BMI over 40 with a less severe comorbidity. Such procedures should be performed only in centers of excellence that include a multidisciplinary team that can provide the endocrine, psychiatric, dietary and psychologic patient and family support before and after the bariatric procedure.

Special consideration should be taken to avoid bariatric surgery at very late stages of obesity, when the presence of obesity-related comorbidities, and the inaccessibility of imaging (most MRI scanners have a weight limit of 450 lbs or 200 kg) may affect surgical outcome. Indeed, a review of eight retrospective studies in adolescents found that bariatric surgery in adolescents can promote durable weight loss in most patients, but there appears to be a significant complication and mortality rate.⁸³⁶ Therefore guidance is needed to determine the ideal circumstances at which the balance of risk versus benefit favors health preservation and reversal of complications with the lowest risk of morbidity and mortality from the procedure.

Bariatric procedures for weight loss can be divided into purely restrictive, those that bypass a segment of the foregut, and combination procedures. Purely malabsorptive procedures aim to decrease the functional length or efficiency of the intestinal mucosa through anatomic rearrangement of the intestine. These procedures include the jejunoileal bypass, and the biliopancreatic diversion with duodenal switch. Because of the high morbidity and mortality of these procedures, they cannot be recommended in children, and will not be discussed further. The restrictive procedures reduce stomach volume to decrease the volume of food ingested. They include the bariatric intragastric balloon (no data in children) and laparoscopic adjustable gastric banding (LAGB). The Roux-en-Y gastric bypass (RYGB) is a combination procedure.⁸³⁷ The sleeve gastrectomy seems to be a restrictive procedure yet it probably shares some of the hormonal effects of the RYGB and is gaining increasing popularity.⁸³⁸

Restrictive: Laparoscopic Adjustable Gastric Banding

LAGB uses a prosthetic band to encircle and compartmentalize the proximal stomach into a small pouch and a large remnant.⁸³⁷ The theoretical advantage of this technique is

decreased risk of staple line dehiscence. The more recent introduction of a new laparoscopic approach and the use of an adjustable band (allowing the stomach size to change) make this procedure more attractive. Finally, this procedure is reversible (at least theoretically; there are some surgeons who scoff at this notion), or it can be modified into the RYGB at a later date. Results vary widely in adults. A single RCT comparing LAGB to conservative obesity management in children with obesity has shown superior weight loss that was maintained over 2 years. Among adolescent participants with obesity, use of gastric banding compared with lifestyle intervention resulted in a greater percentage achieving a loss of 50% of excess weight, corrected for age. There were associated benefits to health and quality of life.⁸³⁹ The significant side effects and limited long-term weight maintenance following LAGB have caused a reduction in its popularity in recent years.

Combination: Roux-en-Y Gastric Bypass

RYGB involves dividing the stomach to create a small (15–30 mL) stomach pouch into which a segment of jejunum approximately 15 to 60 cm inferior to the ligament of Treitz is inserted, whereas the proximal portion of the jejunum that drains the bypassed lower stomach and duodenum is reanastomosed 75 to 150 cm inferior to the gastrojejunostomy.⁸³⁷ This procedure combines the restrictive nature of gastrectomy with the consequences of dumping physiology as a negative conditioning response when high-calorie liquid meals are ingested. In addition, RYGB is associated with decline in the circulating level of ghrelin, which may be in part responsible for the decrease in hunger associated with this procedure.⁸⁴⁰ The procedure can not only lead to extraordinary weight loss, it can reverse type 2 diabetes as well.⁸⁴¹

RYGB appears to result in significant early weight reduction in adults^{837,842}; however, long-term studies demonstrate weight regain in many patients.⁸⁴³ RYGB seems to provide complete remission of T2DM in adolescents with obesity. Importantly, when performed in adolescents with severe obesity, the nadir BMI achieved is typically around 37% from baseline. If one performs the procedure on a patient with a BMI in the low 40s—the result may be a BMI within an acceptable healthy range. In contrast, performing the procedure in patients with BMI over 50 leaves them, even after an optimal result, in the obese BMI range without further reductions.⁸⁴⁴

The most common reported complications of RYGB include iron-deficiency anemia (50%), transient folate deficiency (30%), and events requiring surgical intervention (40%: cholecystectomy in 20%, small bowel obstruction in 10%, and incisional hernia in 10%).⁸³⁷ Because most of the stomach and duodenum is bypassed in this procedure there is an increased risk for deficiencies in vitamin B₁₂, iron, calcium, and thiamine. Although beriberi has been reported in teenagers after RYGB,⁸⁴⁵ compliance with daily lifetime supplementation and regular monitoring of patients can prevent such nutritional deficiencies.

Laparoscopic Sleeve Gastrectomy

The laparoscopic sleeve gastrectomy (LSG) procedure is becoming more widely used. It reduces gastric capacity, by cutting out the greater curvature of the stomach, in other words, turning the stomach into a tube. Sleeve gastrectomy is rising in popularity among children as well as adults with obesity because it is seemingly a simpler procedure technically with clinical outcomes comparable with those of RYGB.⁸³⁴ The limited data in adolescents indeed show similar weight loss in comparison to RYGB. The 5-year failure rate in regard to weight regain and return of comorbidities of this procedure in adults with obesity is about 50%,⁸⁴⁶ yet such data in children is still not available.

Who Should Perform Bariatric Surgery in Children?

Surgical outcomes in adults vary widely between surgeons and institutions.^{847–849} Furthermore, there is a very clear learning curve because the morbidity of bariatric surgery varies inversely with the number of procedures performed.⁸⁵⁰ The increased risk of readmission after bariatric surgery in adults⁸⁵¹ argues for close and careful follow-up and monitoring in adolescents. Therefore it is essential that bariatric surgery in adolescents be performed in regional pediatric academic centers with programs equipped to handle the data acquisition, long-term follow-up, multidisciplinary nature of these difficult patients.⁸⁵²

A multidisciplinary team with medical, surgical, nutritional, and psychologic expertise should carefully select adolescents who are well informed and motivated to become potential candidates for bariatric surgery. Attention to the principles of growth, development, and compliance is essential to avoid adverse physical, cognitive, and psychosocial outcomes following bariatric surgery.⁸⁵² It must be clear to the subject and the parent that bariatric surgery is in fact an adjunct to a sincere commitment to lifestyle, rather than a “magic bullet.” Indeed, evidence of relapse in adults after RYGB is now commonplace.

Subjects and families must be well informed as to the risks and complications of such surgery. The medical team will require endocrine, gastrointestinal, cardiology, pulmonary, and otolaryngologic support. Prophylactic tracheostomy is rarely required to maintain airway patency and to allow for resolution of the hypercapnia before surgery.⁸⁵³ Adolescents undergoing bariatric surgery require lifelong medical and nutritional surveillance postoperatively.⁸⁵⁴ Extensive counseling, education and support are required both before and after bariatric surgery; patients left to their own recognizance tend to regain weight over time. Indeed, studies in adults document an increased risk of hospitalization after RYGB, because of difficulties from the procedure.⁸⁵¹ Monitoring of long-term weight maintenance, improvements in CV morbidity, and longevity are all necessary to determine the cost-effectiveness of bariatric surgery in the pediatric population.

ENERGY INADEQUACY

Starvation Versus Cachexia

Although both are weight loss syndromes, understanding the neuroendocrine mechanisms that distinguish starvation from cachexia is integral to both understanding and treating these disorders properly. In starvation, the negative feedback energy balance pathway is intact. The signal of leptin inadequacy from the weight loss is transduced by the VMH neuron into reduced sympathetic activity (to conserve energy) and increased vagal activity (to store energy). However, in cachexia, this pathway is short-circuited by cytokine action on the hypothalamus. The VMH POMC neuron expresses receptors for various cytokines, including IL-1 and TNF- α .⁸⁵⁵ In response to cytokine exposure, POMC neurons are activated, resulting in anorexia, increased sympathetic activity, decreased vagal activity, and energy wastage.^{856,857} Proinflammatory cytokines increase epinephrine, GH, and cortisol, and reduce insulin. These long-term hormonal changes accelerate muscle proteolysis (cortisol), increase resting energy expenditure (SNS), contribute to insulin resistance (both epinephrine and cortisol), increase catabolism (cortisol), and suppress appetite and intestinal transit (vagus). This is clearly adaptive in the short term during times of infection (to generate body heat to eradicate the organism), but maladaptive in the long term, when chronic cytokine signaling can lead to cachexia. Thus even in the situations of leptin decline or inadequacy, cytokine activation of POMC neurons will promote continued cachexia and weight loss through persistent SNS activation.

Failure to Thrive

Failure to thrive (FTT) is not a disease per se but rather a sign of multiple organic and nonorganic conditions and the interactions between them leading to compromised growth at a young age. FTT still represents a common pediatric medical problem mostly managed in the outpatient setting. Although FTT can rarely be a manifestation of critical illness, the majority of cases are the result of undernutrition because of the combination of biologic, environmental, and psychologic factors. The diagnosis of FTT requires a thorough, prudent, and oriented history taking by the caregiver and is not always simple to establish. Moreover, this diagnosis may carry several legal implications that are not within the scope of this text.

Definition

There is no consensus on a single definition for FTT. The condition reflects inadequate physical growth recorded over time using standard growth charts. The commonly used definitions in clinical practice include length and/or weight below the 5th percentile for age and gender, a downward cross of two major percentile lines of the growth chart over time, or a weight per height below the 10th percentile of expected. The practicality of usually using weight and not length curves evolves from the fact that undernutrition and chronic disease tend to primarily affect weight gain while preserving linear growth. Ultimately, linear growth is also affected if these conditions persist. It is important to emphasize that single measurements without longitudinal follow-up growth points are inadequate to make the diagnosis of FTT, and a wrongful diagnosis may be established in infants who were born small for gestational age or prematurely, and in some healthy growing infants growing along the lower percentiles.

Classification and Etiology

The traditional classification of FTT is to segregate between organic and nonorganic causes. The nonorganic causes refer to environmental and psychologic factors, such as sensory deprivation, parental and emotional deprivation, and feeding difficulties of no organic source that occur in infancy. This traditional classification seems to lack the insight that the majority of cases suffer of a combination of the two reflecting a mixed etiology.⁸⁵⁸ A different approach is to classify the disorder based on the pathophysiology of the disorder, that is, inadequate caloric intake, inadequate absorption, excess metabolic requirements, defective utilization of intake, and reduced growth potential.⁸⁵⁹ Common causes of FTT based on this classification scheme are shown in Box 24.2.

Of note, normal growth variation can confound the diagnosis of FTT because some infants may be born large for gestational age as the result of intrauterine causes (such as gestational diabetes), and later experience a “catch down” pattern of growth during infancy toward the actual growth potential curves. Another cause of such negative crossing of percentiles can be constitutional growth delay. It is estimated that up to 25% of children can cross curves by more than 25 percentile lines (representing a cross of two major growth percentile lines) because of the aforementioned reasons.⁸⁶⁰ These infants reach a new point from which they display a normal growth rate and weight gain pattern, yet they do not have FTT.

Endocrine causes of FTT are uncommon, as typical hormonal deficiencies, such as GH deficiency or hypothyroidism, present as growth failure but with preserved or increased weight gain. Hyperthyroidism (representing a state of increased metabolic demands and characteristically manifested by increased linear growth) and disorders of salt metabolism, such as hypoadosteronism and pseudo-hypoadosteronism, may have

BOX 24.2 Differential Diagnosis of Failure to Thrive

1. Inadequate caloric intake
 - Poverty and low food resources
 - Mechanical feeding difficulties (altered swallowing ability, congenital anomalies, central nervous system damage, severe gastroesophageal reflux)
 - Wrongful preparation of infant formula (too diluted, too concentrated)
 - Unsuitable feeding habits by parent
 - Behavioral problems affecting eating
 - Child neglect
 - Poor parent–child interaction
2. Inadequate absorption of caloric intake
 - Reduced absorption surface area (short bowel syndrome, s/p necrotizing enterocolitis)
 - Chronic liver disease, biliary atresia
 - Celiac disease
 - Cystic fibrosis
 - Cow’s milk allergy
 - Chronic diarrhea
 - Vitamin or mineral deficiencies (acrodermatitis enteropathica)
 - Vomiting because of CNS abnormalities (tumor, raised ICP)
3. Increased metabolism
 - Hyperthyroidism
 - Chronic infection (because of immune deficiency)
 - Occult malignancy
 - Congenital heart defects or acquired heart disease (mainly right to left shunts and heart failure)
 - Chronic lung disease with hypoxemia (bronchopulmonary dysplasia)
 - Burns
4. Defective utilization of calories
 - Renal failure, renal tubular acidosis
 - Inborn errors of metabolism (storage diseases, amino acid disorders)
5. Reduced growth potential
 - Genetic disorders (trisomies, skeletal dysplasias, Russell-Silver syndrome)
 - Specific genetic syndromes
 - Primordial dwarfism

CNS, Central nervous system; ICP, intracranial pressure.

FTT as part of their clinical manifestations.⁸⁶¹ Hypophosphatemic rickets may also present as FTT.

Diagnosis and Evaluation

The key to making the diagnosis of FTT is in plotting anthropometric data (weight and length) during a reasonable follow-up period. Although a prudent and focused history and physical examination are the keys to diagnosis, often the correct diagnosis is made in retrospect. The lack of an organic etiology to explain the findings is not enough to establish a diagnosis of nonorganic FTT. A response to an active intervention, manifested at least as a limited period of adequate growth while altering a behavioral element by the caregiver or child, can help establish a diagnosis of nonorganic FTT.

History should focus on the dietary and feeding history, past and present medical history, social environment, and family history. The dietary history is aimed at assessing as accurately as possible the actual caloric intake of the patient. An important tool for this assessment can be the use of food logs of several days. The important details are regarding actual amounts of food, the way the food is prepared (specifically relevant for the dilution technique of infant formulas and to cereals added to the formula), and beverages consumed with specific emphasis on sweetened juices and formula. These details should allow the practitioner to estimate the caloric intake.

The important details regarding feeding begin with the location of the meals and their timing throughout the day. Who feeds the patient or supervises the feeding process is of major importance. The feeding technique should be appropriate for the developmental stage of the child. The timing is relevant in regard to frequent snacking in between meals that may cause early satiety during mealtimes.

A standard pediatric medical history should be taken from all patients, yet it should be focused on details that may be relevant to the diagnosis of FTT. The pregnancy and birth history are important for differentiation of infants who were born small for gestational age versus those who suffer from FTT. The timing at which poor weight gain began, especially in relation to changes in feeding, is critically important for the

diagnosis. Chronic medical conditions, such as congenital heart disease, asthma, multiple recurrent infections, and anemia can all be causes of organic FTT. Multiple hospitalizations and a history of injuries can raise the suspicion of parental neglect. Gastrointestinal manifestations of relevant medical conditions, such as frequent vomiting (in cases of milk allergy or gastroesophageal reflux) and stool frequency and consistency (to rule out malabsorption, celiac, inflammatory bowel disease or cystic fibrosis), should be elicited in detail.

The social history should focus on identifying the actual caregivers of the patient during the majority of the time and whether there are economic issues that may affect the ability to nourish the patient adequately. Potential external and intrafamilial stressors that may affect the supply of food to the child should be sought (any stressor or life event that can affect the functioning of the caregiver in a way that could compromise the well-being of the child). The family history should focus on the body habitus of parents and siblings to obtain clues regarding genetic potential for height and weight. Medical conditions in siblings and relatives can suggest a predisposition to genetic disorders. The caregivers should be asked about mental illnesses, such as depression, that may hamper their ability to provide adequate care for the child. A family history of previous children who suffered from FTT should be investigated as well. A call to the local Department of Family Services may be warranted.

The physical examination begins with plotting the child’s length, weight, and head circumference on standard growth charts, along with previous measurements (if available). The severity of the FTT can be estimated by assessing the present weight in comparison to the expected weight for age. If the weight is less than 60% of expected by the 50th percentile for age and length, the condition is severe, whereas a weight between the 60th and 75th percentile of expected is considered moderate FTT. Microcephaly accompanied by neurologic signs may suggest a CNS lesion. It should be remembered that head circumference is the last parameter to change in FTT, and only in the severest cases. Detection of dysmorphism may suggest a genetic cause for impaired growth and development. Measures of nutritional status (such as thickness of skin folds and body fat distribution) can be examined. It is important to carefully

observe the interaction of the caregiver and the child during feeding. Impaired parent–child interactions can have a major impact on feeding habits, and their identification is critical to the design of effective behavioral interventions tailored for the patient and family.

The majority of children with FTT have no laboratory abnormalities, and no hormonal alterations. There is minimal literature available about comprehensive laboratory workups in children with FTT, although a classic manuscript about the workup of more than 180 infants in an in-patient setting found laboratory abnormalities in less than 1.4% of tests taken.⁸⁶² The choice of tests that may be beneficial should be based on the history and physical examination and usually is focused on the assessment of malnutrition in severe cases. A minimal workup, although not cost-effective, may include a blood count, chemistry panel (including liver and renal function tests, electrolytes, serum protein and albumin concentrations, as well as blood acid-base status) and a urinalysis with pH. Additional tests should be oriented at specific findings from the history and physical examination. No hormonal tests are warranted initially unless a clinical suspicion of a specific disorder arises. In children older than 6 months, screening for iron deficiency and lead poisoning is warranted. Hospitalization and in-patient workup does not add any yield to the workup,⁸⁶³ unless the degree of FTT is severe, or if there are concerns of child safety and neglect.

Management

The management of FTT is based on the identification of the underlying cause and its correction. The vast majority of cases are handled by a combination of nutritional and behavioral intervention. Importantly, the intervention should begin before the workup is complete, that is, from the first evaluation. All medical problems are treated independently of nutritional and behavioral interventions and should not delay or hamper them. The mainstay of treatment of all infants with FTT is a calorie-rich diet accompanied by frequent and close monitoring of weight response. An effective intervention will document a catch-up weight and height gain that is maintained over time.

Feeding and eating behaviors should be addressed walking the fine line between encouragement and pressure to promote eating. Timing meals and snacks and eating as a family in a pleasant environment of low stress may be important for the acquiring of improved eating and feeding practices. The feeding intervention is dependent on the infants' age at presentation. For breastfeeding infants, it is beneficial to attempt to increase breast-milk supply⁸⁶⁴ by pumping milk, treatment with metoclopramide to induce oxytocin secretion,⁸⁶⁵ improving maternal nutrition and fluid intake, and making adaptations at the home and workplace that can promote and simplify the breastfeeding process. Suckling problems in neurologically impaired infants can be solved by providing expressed human milk via bottle-feeding. Bottle-fed and older infants allow more interventions to promote increased caloric content of the diet. Infants with FTT should receive around 150% of the recommended daily caloric intake based on their expected weight (rather than based on their actual weight).⁸⁶⁶ The enrichment of formula may be achieved by adding cereals, and toddlers may benefit from the addition of palatable high-energy density foods (such as cheese and peanut butter) to their diet. High-calorie milk-based drinks (such as PediaSure that provides 30 calories per ounce, in comparison with whole milk which provides 19 calories per ounce) can be added alongside vitamin supplementation. Zinc supplementation has been shown to increase IGF-1 levels without affecting IGFBP-3 in infants with nonorganic FTT, yet this effect did not actually promote growth.⁸⁶⁷

Prognosis

The vast majority of infants and children with FTT show improvement with intervention. Others may even show progression when they achieve a more independent stage of development where they can attain their own food. Those who require gastrostomy feeding because of neurologic dysfunction may require assisted enteral nutrition for life. The cognitive and intellectual function outcomes of those who suffered from FTT seem worse than their peers, although this association has *only* been well established in cases of iron-deficiency anemia.⁸⁶⁸ It seems conceivable that deficiencies of other elements critical for brain development during infancy may have a similar adverse impact on intellectual properties at later ages, although this has not been studied systematically. The effects of nonorganic factors (such as emotional deprivation) on intellectual development, often coexisting with organic factors, may also contribute to decreased cognitive ability at later ages.

Cancer Cachexia

Cancer cachexia is characterized by a chronic wasting syndrome, involves skeletal muscle and adipose tissue loss, and is typically resistant to conventional nutritional support. Cachexia is responsible for a significant reduction in quality and length of life of patients with cancer, also in the pediatric age group. Similar to cachexia caused by chronic infections or inflammatory diseases, cancer patients may manifest both malnutrition and metabolic stress. Both carbohydrate utilization pathways, as well as amino acid incorporation, are decreased in the muscles of cancer cachexia patients. Tumor cells may affect patient metabolism in two ways: their own metabolism of nutrients into other metabolites and by circulating factors they secrete or induce the host to secrete. Accelerated glycolysis and lactate production, and the resultant increase in Cori cycle activity, are the typically observed metabolic effects.⁸⁶⁹ A large number of procachexia circulating metabolites and factors have been found, such as TNF- α , IL-6, myostatin, and PTH-related protein, yet no single molecule has been shown to be a determinant factor that can be targeted individually to treat cancer cachexia in humans. Cancer activates a complex set of CNS metabolic pathways, which result in cachexia (Box 24.3).⁸⁷⁰

BOX 24.3 Metabolic Changes in Cachexia

EXPRESSION OF CYTOKINES

- Increased production of acute phase proteins (APP)
- Upregulation of transcription factor NF Kappa B and AP-1
- Increased interleukin (IL)-1, IL-6, and tumor necrosis factor- α and interferon- γ
- Increased expression of tumor specific cachexins (proteolysis inducing factor, lipid mobilizing factor, and anemia inducing substance)
- Increased expression of ubiquitin, E₁, E₂, E₃, and proteasome components (cell death and removal)

INCREASED SYMPATHETIC NERVOUS SYSTEM TONE

- Increased expression of hormone-sensitive lipase in adipose tissue
- Upregulation of uncoupling proteins (UCP2 and UCP3) in muscle and adipose tissue
- Increased hepatic gluconeogenesis

REDUCED VAGAL TONE

- Reduced lipoprotein lipase expression in adipose tissue
- Reduced intestinal transit
- Reduced hunger

Peripheral cytokines gain access to the CNS through the central circumventricular organs, which bypass the blood-brain barrier, or by stimulation and amplification of CNS microglial cytokine or eicosanoid production. For instance, TNF- α stimulates VMH POMC neurons, which stimulate the SNS, which in turn increases resting energy expenditure, increases cortisol and glucagon levels, and contributes to insulin resistance. IL-1 decreases neuropeptide Y within the VMH and thus decreases appetite. IL-1 also increases CRH, which indirectly inhibits appetite. IL-6 bears striking similarity to ciliary neurotrophic factor (CNTF), which has been shown to reduce weight by activating VMH POMC neurons through a leptin-independent mechanism.⁸⁷¹ Conversely, because of reduction in vagal activity, gastrointestinal motility is impaired in cancer cachexia and is clinically manifest by early and inappropriate satiety, which occurs in 40% to 60% of cancer patients.

In addition, cytokines have adverse peripheral effects. Uncoupling proteins are upregulated by cytokines and contribute to increased energy expenditure. Cancer cachexia leads to overexpression of UCP1 in brown adipose tissue; UCP2 in brain, skeletal muscle, and liver; and UCP3 in skeletal muscle. Levels of UCP2 and UCP3 in the liver and muscle are regulated by prostaglandins, and UCP3 is also regulated by TGs, all of which are increased in cancer.⁸⁷²

Cytokines cause insulin resistance in skeletal muscle, liver, and adipose tissue. TNF- α decreases insulin receptor and PPAR activity. TNF- α has a direct catabolic effect on skeletal muscle and causes wasting of muscle by the induction of the ubiquitin-proteasome system (UPS). TNF- α drives the increase in liver gluconeogenesis, loss of adipose tissue, and proteolysis, while causing a decrease in protein, lipid, and glycogen synthesis. It has been associated with the formation of IL-1 and increases the UCP2 and UCP3 expression in skeletal muscle in the cachectic state.⁸⁷³ In addition, there is an inverse correlation between IL-6 levels and insulin sensitivity.⁸⁷⁴ Adipose tissue insulin resistance increases fat oxidation, and decreases lipoprotein lipase activity, resulting in continued lipolysis.⁸⁷⁵ The insulin resistance of cancer is not related to defective insulin clearance, and is therefore different than other forms of primary insulin resistance.⁸⁷⁶

Cancers are uniformly anaerobic and depend upon glucose for survival; thus the glucose manufactured from gluconeogenesis secondary to hepatic insulin resistance is essential for tumor growth. Cancers release large amounts of lactate, which is converted in the liver back to glucose. Such gluconeogenesis consumes ATP, which also increases energy expenditure.⁸⁷⁷ Additional raw materials for gluconeogenesis are alanine derived from skeletal muscle proteolysis and glycerol from lipolysis.

Given the complex multifactorial nature of the cachexia-associated hormonal/metabolic profile and the individual differences between cancer patients, targeting any single circulating factor would always be insufficient to treat cachexia for all patients with cancer. There currently is no specific pharmacologic agent that can effectively address cancer cachexia, and treatment relies on dietary supplementation and psychological support.

The Diencephalic Syndrome

The diencephalic syndrome includes clinical characteristics of severe emaciation, normal linear growth, and normal or precocious intellectual development in association with CNS tumors. Originally described by Russell in 1951,⁸⁷⁸ this rare disorder presents in infants younger than age 1 year, and is an indication of a hypothalamic lesion, usually an anterior hypothalamic glioma, or other neoplasm affecting hypothalamic function. Although the clinical spectrum is variable,

emaciation with paucity of subcutaneous fat, but with normal linear growth and head circumference is inviolate. Other frequent features include hyperalertness, hyperkinesis, nystagmus, and vomiting.^{879,880}

Numerous patients have been anecdotally reported and characterized; however, the cause of the emaciation remains unclear. Subjects with diencephalic syndrome have extremely elevated baseline GH levels, but with normal IGF-1 levels, suggesting a variant of GH resistance.⁸⁷⁹ It has been suggested that the high GH leads to lipolysis and accounts for the emaciation, but this finding is not consistent in all patients. Only one evaluation of energy balance has been performed, which demonstrated 30% to 50% greater REE in comparison to normal babies, and 13% greater energy expenditure compared with intake.⁸⁸¹

Treatment recommended is surgical excision of the lesion whenever feasible. Radiation is usually reserved for the very young patient. Frequently, these patients postoperatively manifest hypopituitarism, and ultimately develop hypothalamic obesity.⁸⁸⁰

Anorexia Nervosa

Definition

Anorexia nervosa (AN) is an eating disorder that typically begins during adolescence and consists of persistent dieting and intense physical activity, usually accompanied by compulsive behavioral traits and sometimes with binge eating and purging behaviors. Individuals with AN also manifest a disturbed body image and a persistent fear of weight gain, both of which promote further weight loss. The result of this behavior is a pathologic weight loss, with pathophysiologic consequences. The risk of developing AN among females in Western societies is estimated to be between 1.7% and 3.6%.⁸⁸² There are two subtypes of anorexia: the restrictive type, characterized by very low caloric intake and/or excessive exercise, and the binge-eating/purging type, characterized by varying levels of food purging, usually by way of self-induced vomiting and/or laxative abuse. Alongside the emotional and behavioral elements, the definition in the DSM-5 includes having a significantly low weight (defined as BMI <5th percentile for age and sex in children or BMI \leq 17 in adults).

Medical complications driven by the chronically reduced caloric intake, purging behavior, and excessive exercise may affect several organ systems. Typically, patients develop a marked loss of subcutaneous fat tissue, impaired menstrual function, bradycardia and orthostatic hypotension, hypothermia, and increased hair loss. Importantly, AN that develops during adolescence may create adverse clinical effects that persist into adulthood,⁸⁸³ including osteopenia and osteoporosis,⁸⁸⁴ higher rates of miscarriage, and reduced offspring birth weight.⁸⁸⁵ Anorexia may also alter cognitive abilities as during extreme weight loss in cases where a significant reduction of both gray and white cerebral matter occurs; conversely, during weight restoration cerebral white matter returns to premonitory levels, but gray matter does not.⁸⁸⁶ Anorexia carries an increased mortality risk, specifically for suicide, yet also from medical causes, such as starvation per se, as well as purging-induced, electrolyte-mediated arrhythmias. Full recovery of body habitus and of growth and development occurs in 50% to 70% of treated adolescents; yet achieving a full physiologic and psychologic recovery may take a comprehensive treatment intervention that may last as long as 5 to 7 years.⁸⁸⁷ Greater weight loss, lower sustained weight, and the coexistence of other psychiatric disorders adversely affect the probability of recovery. The outcomes for adults with AN are poorer than those who are diagnosed and treated in adolescence.

Endocrine Manifestations of Anorexia Nervosa

Obesity and malnutrition usually result in opposing effects on normal physiology and are both associated with changes in the hormonal profile. AN is associated with multiple, profound endocrine alterations which may be adaptive, reactive, or perhaps etiologic. The majority of these changes represent an adaptive response yet should be considered as part of the differential diagnosis of specific hormonal excess or deficiency disorders. Dysfunction of the hypothalamic-pituitary axis includes hypogonadotropic hypogonadism with relative estrogen and androgen deficiency, GH resistance, elevated cortisol, "sick euthyroid" syndrome, and reduced oxytocin. Levels of leptin are suppressed in patients with AN reflecting the reduced energy stores and levels of ghrelin, an orexigenic gut peptide, are elevated.⁸⁸⁸ Most, but not all, of these endocrine disturbances are adaptive to the low energy state of chronic starvation and reverse with treatment of the eating disorder. Despite this, these alterations contribute to impaired skeletal integrity, as well as neuropsychiatric comorbidities, in individuals with AN. Importantly, only 5% to 15% of patients with AN are male, and only limited data exist regarding the endocrine impact of the disease in adolescent boys. Fig. 24.12 demonstrates the typical hormonal profile of patients with AN, which reflect the organism's attempts at energy preservation and the cessation of energetically costly and nonvital processes.

Hypothalamic-Pituitary-Thyroid Axis. The starvation status of AN may resemble the sick euthyroid syndrome. In patients with AN, serum total and free T_4 , total and free T_3 , TSH and thyroxine binding globulin are significantly lower than normal, whereas reverse triiodothyronine (rT_3) levels are significantly greater than healthy controls.⁸⁸⁹ Most patients with AN have a hyporesponsive or delayed responsiveness of TSH to TRH stimulation. Of note, weight regain reverses the effects of AN on the hypothalamic-pituitary-thyroid axis to normal; thus the reduction in thyroxinemia may actually be a normal adaptive physiologic response to starvation. The differential diagnosis of hypothyroidism should be considered in patients with AN as both disorders are characterized by low T_4 and T_3 levels.

In primary hypothyroidism, TSH levels will be greater than those seen in patients with AN, although in mild secondary hypothyroidism this distinction may be difficult. Obtaining a serum rT_3 level may be helpful in distinguishing a true thyroid disorder from the euthyroid sick syndrome associated with systemic illness.

Growth Hormone-IGF-1 Axis. Patients with AN typically have GH hypersecretion accompanied by low IGF-1 levels. Whether this profile is caused by a primary hypothalamic dysfunction, peripheral target organ resistance to GH, or an impaired negative IGF-1 central feedback mechanism is unclear. An increased GH response to GHRH has been demonstrated in AN, possibly reflecting an impairment of beta-adrenergic suppression of GH secretion.⁸⁹⁰ The clinical presentation of weight loss, cessation of menses and cold intolerance alongside low levels of pituitary-derived hormones may resemble panhypopituitarism, yet patients with AN present with GH hypersecretion.

Hypothalamic-Pituitary-Adrenal Axis. Patients with AN typically present with elevated cortisol levels in the presence of normal ACTH levels.⁸⁹¹ The elevated cortisol levels are apparently because of increased cortisol secretion alongside a reduction in cortisol clearance. Elevated CRH levels found in CSF of patients with AN suggest that AN represents an overall state of activation of the hypothalamic stress response, manifested peripherally by hypercortisolemia. An abnormal response in the dexamethasone suppression test, mainly of reduced suppression, suggests that an element of decreased feedback sensitivity occurs in this disorder.

Bone Metabolism. Adolescence represents a critical period for the accumulation of bone mineral, thus building bone strength and density for later years. The achievement of peak bone mass is dependent on several hormonal effects characteristic of puberty, such as increased estradiol, and IGF-1 levels, as well as being dependent on adequate nutrition; all of which are compromised in patients with AN. Adolescents and adults with AN have a low bone mineral density. In adults this is caused by increased bone resorption and reduced bone formation; adolescents are characterized by an overall reduced bone

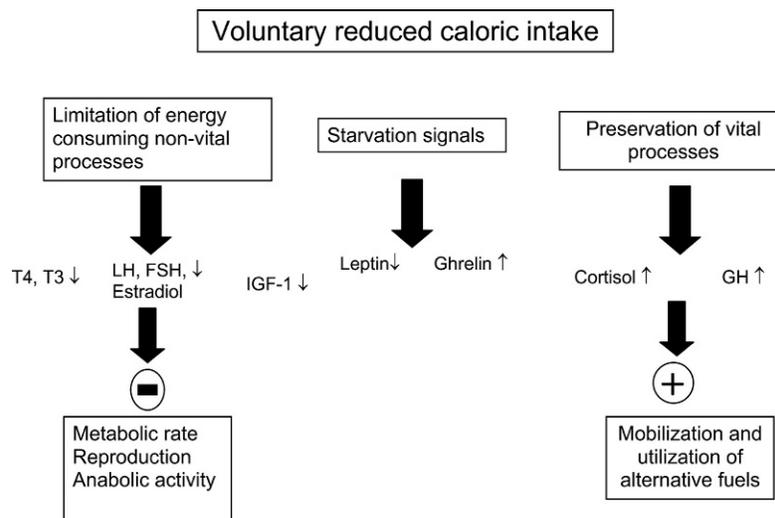


Fig. 24.12 Hormonal changes in anorexia nervosa. The hormonal profile of the patient with anorexia nervosa represents an adaptive response aimed at conserving energy. Processes that require significant energy, such as reproduction and growth, are limited by a complete suppression of the gonadotropic axis and by reduced insulin-like growth factor (IGF)-1 levels, respectively. A seemingly chronic stress response characterized by increased growth hormone (GH) and cortisol is aimed at efficient utilization of the limited energetic sources present. Reduced leptin serves as a signal for down regulating the hypothalamic-pituitary-gonadal axis. Reduced leptin also serves as a starvation signal, although this signal appears to be circumvented in this disorder.

turnover.⁸⁹² The reduced bone density is caused by the typical hormonal profile of AN, that is, reduced estrogens and androgens, low IGF-1, and relative hypercortisolemia. Moreover, the reduced lean body mass and lower mechanical forces acting on long bones may also contribute to the overall reduced BMD. Recent publications also suggest that elevated levels of gut-derived hormones PYY₍₃₋₃₆₎ and ghrelin may also contribute to impaired bone metabolism in AN.⁸⁹³ Importantly, resolution of AN with weight gain often does not bring full recovery of the bone mineral density status. Osteopenia resulting from undernutrition and the typical hormonal dynamics of AN is one of several long-term complications of AN during adolescence, and thus is a major treatment target.

Hypothalamic-Pituitary-Gonadal Axis. Amenorrhea is one of the hallmarks of AN, yet it is not always explained by the severe weight loss; thus hypothalamic amenorrhea in AN often precedes weight loss and may persist after refeeding and achievement of normal weight. Gonadotropin levels are reduced in patients with AN, and GnRH stimulation testing in patients with AN demonstrates a blunted LH response with a preserved FSH response and very low levels of estradiol. The normal pubertal pulsatile pattern of LH levels may revert to prepubertal patterns in adolescents who have previously achieved pubertal status. The amenorrhea of AN is also marked by strikingly reduced leptin levels. Leptin serves as a metabolic signal of energy status and nutritional reserve and thus may have a permissive role for the initiation of the complex hormonal dynamics necessary for normal reproductive function.⁸⁹⁴ Teleologically, conservation of energy for immediate and necessary metabolic demands while suppressing energetically expensive processes, such as reproduction, serves as a protective measure in severely malnourished individuals, as seen in AN. The rise in leptin upon weight gain is associated with increases in gonadotropin secretion, suggesting that leptin serves a permissive role for the activation of the hypothalamic-pituitary-gonadal axis.

Fat-Derived Hormones. Adipocytes secrete a wide array of adipocytokines, the normal profile of which is altered in AN. Patients with AN typically have very low plasma leptin concentrations alongside a marked disturbance of the leptin diurnal secretion profile.⁸⁹⁵ The concentration of leptin-binding protein (the soluble isoform of leptin receptor) has been reported to be increased in patients with AN, contributing to a further reduction in concentration of free leptin.⁸⁹⁶ Leptin has a major role in the neuroendocrine adaptations to the chronic starvation and undernutrition typical of AN, such as reduction in gonadotropins, reduction in thyroid hormone to conserve energy, a modified stress response manifested by hypercortisolemia, and elevated GH aimed at mobilizing and utilizing alternative energy sources.⁸⁹⁷ Weight gain in patients with AN may induce relative hyperleptinemia in comparison to controls matched for BMI; circulating leptin concentrations in AN patients thus traverse from subnormal to supranormal levels within a few weeks.

Adiponectin is the only adipocytokine whose plasma concentrations are inversely related to fat mass. Conflicting results regarding adiponectin concentration in AN, ranging from hyperadiponectinemia⁸⁹⁸ to hypo-adiponectinemia,⁸⁹⁹ have been reported. Interestingly, weight gain in AN is not necessarily associated with reciprocal changes in adiponectin concentration.⁹⁰⁰

Treatment

Indications for hospitalization and in-patient treatment of AN include dehydration, electrolyte disturbances (mainly hypokalemia), arrhythmias, CV instability (significant bradycardia, hypotension, and orthostatic changes), hypothermia, acute

food refusal, acute complications of malnutrition (seizures, pancreatitis, cardiac failure), and psychiatric emergencies.

Along with psychological interventions that may consist of psychotherapy and/or pharmacotherapy (based on the spectrum of psychiatric pathology), an increase in caloric intake must be part of the treatment protocol. Starting with 1200 to 1500 kcal per day and increasing by 500 kcal is aimed at a gain of 0.5 to 1 kg per week.⁹⁰¹ There is no single superior feeding regimen, as long as adequate caloric intake is consumed. Extreme cases may be handled through hospitalization in dedicated in-patient units; caloric intake in this setting may initially be provided by nasogastric tube and in extreme cases via parenteral feeding.

One major treatment decision facing the caregiver of patients with AN is whether to use estrogen replacement therapy to treat amenorrhea and protect the skeleton from osteopenia. There is a lack of a proven efficacious beneficial effect of estrogen replacement therapy in regards to improved bone mass in AN in comparison to placebo,⁹⁰² although this treatment modality is still commonly used.⁹⁰³ This treatment approach with estrogens may have several adverse effects on the treatment of adolescents as it masks the beneficial effect of weight gain on the resumption of menses and may provide an erroneous sense of security in patients that are still at critically low weight status. Increased calcium intake alongside 400 IU of vitamin D to accelerate absorption should be encouraged in all patients with AN as another potential protective measure of bone status.

CONCLUSIONS

Energy balance is complex and regulated by intricate mechanisms that evolved to finely tune and maintain body weight within accepted normal limits. Some of these mechanisms evolved to sustain and preserve life even in the face of famine. The impact of even relatively small imbalances in intake relative to expenditure can have appreciable cumulative effects on body weight over time, leading to obesity or underweight. Although important to consider in the differential diagnosis of childhood obesity, a classic endocrinopathy (glucocorticoid excess or deficiencies in thyroid or GH), genetic obesity syndrome, or hypothalamic lesion is seldom the etiology. Nonetheless, in an era of abundant nutrient surfeit, other subtle endocrine abnormalities, such as leptin concentration or action and insulin resistance, are hallmark features of obesity, and common variants of genes involved in the leptin signaling pathway may contribute to obesity risk in a sizable portion of the population. Furthermore, obesity, by virtue of its presence, indicates some degree of dysfunction in the neuroendocrine homeostatic mechanisms of the CNS that regulate energy balance. Therefore obesity and its consequences should be recognized as part of a spectrum of chronic endocrine dysfunction. The notion that obesity is caused by excessive caloric intake with concomitant inadequate caloric expenditure via work and exercise is simplistic and fails to address the underlying problem of a mismatch in regulatory mechanisms of energy intake and dissipation. It is this mismatch, unmasked in our era of abundant energy intake, that perpetuates the state of energy imbalance, and stigmatizes obesity as being merely caused by lack of discipline or will power. In fact, obesity is a pathologic condition that, in most patients, requires lifelong management. Similarly, in cachexia, investigation must extend beyond the lack of appetite to understand the reasons for the wasting and illness. Understanding the energy balance pathway, and where these various disorders impair its regulation, together with public health education of the consequences of obesity, are the keys to further research, and successful prevention and treatment.

ABBREVIATIONS

Abbreviation	Definition
α -MSH	alpha-melanocyte-stimulating hormone
11 β HSD1	11-beta-hydroxysteroid dehydrogenase type 1
5-HT	5-hydroxytryptamine (serotonin)
A ^y -a	Agouti yellow mouse
ACTH	adrenocorticotrophic hormone
AgRP	Agouti-related peptide
AHO	Albright hereditary osteodystrophy
ALT	alanine aminotransferase
AMP	adenosine monophosphate
AN	anorexia nervosa
ANP	atrial natriuretic peptides
AP	area postrema
apoB	apolipoprotein B
APP	acute phase proteins
ARC	arcuate nucleus
ARC ^{POMC}	proopiomelanocortin-expressing neurons of the arcuate nucleus
AS	Alström syndrome
ATP	adenosine triphosphate
BBB	blood-brain barrier
BBS	Bardet-Biedl syndrome
BCAA	branched-chain amino acids
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BMR	basal metabolic rate
cAMP	cyclic adenosine monophosphate
CART	cocaine-amphetamine-related transcript
CCK	cholecystokinin
cGMP	cyclic guanosine monophosphate
ChREBP	carbohydrate-response element binding protein
CI	confidence interval
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CoA	coenzyme A
CPE	carboxypeptidase E
CPT-1	carnitine palmitoyl transferase 1
CREB	cyclic adenosine monophosphate (AMP) response element binding protein
CRH	corticotropin-releasing hormone
CRISPR	clustered regulary interspaced short palindromic repeat
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	computed tomography
CV	cardiovascular
DAG	diacylglycerol
DIT	diet-induced thermogenesis
DMH	dorsomedial hypothalamic nucleus
DMV	dorsal motor nucleus of the vagus nerve
DNA	deoxyribonucleic acid
DNL	de novo lipogenesis
DSM-5	<i>Diagnostic and Statistical Manual of Mental Disorders, fifth edition</i>
DVC	dorsal vagal complex
EC	endocannabinoid
eNOS	endothelial nitric oxide synthase
ER	endoplasmic reticulum
F1P	fructose-1-phosphate
FDA	US Food and Drug administration
FFA	free fatty acid
FGF19	fibroblast growth factor 19
FMD	flow-mediated endothelium-dependent dilatation
FOXO1	forkhead protein-1
FSH	follicle-stimulating hormone
FSIVGTT	frequently sampled intravenous glucose tolerance test
FTO	fat mass and obesity associated gene
FTT	failure to thrive
FXR	farnesoid X-activated receptor
GABA	gamma-amino butyric acid
GDM	gestational diabetes mellitus
GH	growth hormone
GHSR	growth hormone secretagogue receptor
GI	gastrointestinal
GIx	glycemic index
GL	glycemic load
GLP-1	glucagon-like peptide 1
GLUT4	glucose transporter 4
GNG	gluconeogenesis
GnRH	gonadotropin hormone
GOAT	ghrelin O-acetyltransferase
GPCR19	G-protein-coupled receptor 19
GTP	guanosine triphosphate
GWAS	genome-wide association studies
HDL	high-density lipoprotein
HFCS	high-fructose corn syrup
HGP	Hepatic glucose production
HOMA-IR	homeostatic model for assessment of insulin resistance
HPA	hypothalamus, pituitary, and adrenal
HSL	hormone-sensitive lipase
ICV	intracerebroventricular
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor-binding protein
IGT	impaired glucose tolerance
IHCL	Intrahepatic lipid
IHCL	intrahepatocellular lipid
IL-6	interleukin-6
IMCL	intramyocellular lipid
IMT	intimal medial thickness
IP ₃	inositol triphosphate
IRS-1	insulin receptor substrate 1
IRS-2	insulin receptor substrate 2
JAK1	Janus kinase 1
JAK2	Janus kinase 2
JNK-1	c-jun N-terminal kinase 1
LAGB	laparoscopic adjustable gastric banding
LEPR	leptin receptor
LGA	large-for-gestational age
LH	luteinizing hormone
LHA	lateral hypothalamus
LPL	lipoprotein lipase
LSG	laparoscopic sleeve gastrectomy
MAPK	mitogen-activated protein kinase
MARCKS	myristoylated alanine-rich protein kinase C substrate
MBH	mediobasal hypothalamus
MC3R	melanocortin-3 receptor
MC4R	melanocortin-4 receptor
MCH	melanin-concentrating hormone
MCR	melanocortin receptor
ME	median eminence
MKK7	mitogen-activated protein kinase 7
MRAP2	melanocortin receptor accessory protein 2
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
NA	nucleus accumbens
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NE	norepinephrine
NEAT	nonexercise activity thermogenesis

NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
OGTT	oral glucose tolerance test
OSA	obstructive sleep apnea
PAI-1	plasminogen activator inhibitor-1
PC1	prohormone convertase-1
PCOS	polycystic ovarian syndrome
PedNSS	Pediatric Nutrition Surveillance System
PEPCK	phosphoenolpyruvate carboxykinase
PGC	peroxisome proliferator-activated receptor gamma coactivator
PGC-1 α	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PHP1a	pseudohypoparathyroidism type 1a
PI3K	phosphatidyl inositol-3-kinase
PKA	protein kinase A
PKC	protein kinase C
PKC ϵ	protein kinase C epsilon
POMC	proopiomelanocortin
PP	pancreatic polypeptide
PPAR	peroxisome proliferator-activated receptor
PVN	paraventricular hypothalamic nucleus
PWS	Prader-Willi syndrome
PYY	peptide YY
RAI1	retinoic acid-induced 1
RCT	randomized controlled trial
REE	resting energy expenditure
ROHHAD	Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysfunction
ROS	reactive oxygen species
rT ₃	reverse triiodothyronine
RYGB	Roux-en-Y gastric bypass
SGA	small-for-gestational age
SIM1	single-minded 1
SMS	Smith-Magenis syndrome
SNP	single nucleotide polymorphism
SNS	sympathetic nervous system
SOCS-3	suppressor of cytokine signaling 3
SREBP-1c	sterol regulatory element-binding protein 1c
SSPG	steady-state plasma glucose
SSTR ₅	somatostatin-5 receptor
STAT-3	signal transducers and activators of transcription
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TEE	total energy expenditure
TG	triglyceride
TNF- α	tumor necrosis factor alpha
trans-fats	trans-unsaturated fatty acids
TRH	thyrotropin-releasing hormone
TrkB	tropomyosin-related kinase B
TSH	thyroid-stimulating hormone
UCP	uncoupling protein
UPR	unfolded protein response
UPS	ubiquitin-proteasome system
VLDL	very low-density lipoproteins
VMH	ventromedial hypothalamic nucleus
VTA	ventral tegmental area
WHO	World Health Organization
WT	wild-type
Y2R	type-2 neuropeptide Y receptor
Y4R	type-4 neuropeptide Y receptor

REFERENCES

1. Collaboration NCDRF. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* (London, England), 2017;390(10113):2627–2642.
2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372(6505):425–432.
3. Mayer-Davis EJ, Lawrence JM, Dabelea D, et al. Incidence trends of Type 1 and Type 2 diabetes among youths, 2002–2012. *N Engl J Med*. 2017;376(15):1419–1429. 2017.
4. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. *Am J Clin Nutr*. 2012;95(4):989–994.
5. Rogers PJ, Brunstrom JM. Appetite and energy balancing. *Physiol Behav*. 2016;164:465–471.
6. Speakman JR, Levitsky DA, Allison DB, et al. Set points, settling points and some alternative models: theoretical options to understand how genes and environments combine to regulate body adiposity. *Dis Model Mech*. 2011;4(6):733–745.
7. Lowell BB. New neuroscience of homeostasis and drives for food, water, and salt. *N Engl J Med*. 2019;380(5):459–471.
8. Hall KD, Sacks G, Chandramohan D, et al. Quantification of the effect of energy imbalance on bodyweight. *Lancet* (London, England), 2011;378(9793):826–837.
9. Liu CM, Kanoski SE. Homeostatic and non-homeostatic controls of feeding behavior: Distinct vs. common neural systems. *Physiol Behav*. 2018;193(Pt B):223–231.
10. Anderson GH, Hunschede S, Akilen R, Kubant R. Physiology of food intake control in children. *Adv Nutr*. 2016;7(1):232S–240S.
11. Howick K, Griffin BT, Cryan JF, Schellekens H. From belly to brain: targeting the ghrelin receptor in appetite and food intake regulation. *Int J Mol Sci*. 2017;18(2):273.
12. Kim KS, Seelye RJ, Sandoval DA. Signalling from the periphery to the brain that regulates energy homeostasis. *Nat Rev Neurosci*. 2018;19(4):185–196.
13. Browning KN, Verheijden S, Boeckxstaens GE. The vagus nerve in appetite regulation, mood, and intestinal inflammation. *Gastroenterology*. 2017;152(4):730–744.
14. Tremblay A, Bellisle F. Nutrients, satiety, and control of energy intake. *Appl Physiol Nutr Metab*. 2015;40(10):971–979.
15. de Castro JM, Bellisle F, Dalix AM, Pearcey SM. Palatability and intake relationships in free-living humans. Characterization and independence of influence in North Americans. *Physiol Behav*. 2000;70(3-4):343–350.
16. Rebello CJ, Greenway FL. Reward-induced eating: therapeutic approaches to addressing food cravings. *Adv Ther*. 2016;33(11):1853–1866.
17. Porte Jr D, Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes*. 2005;54(5):1264–1276.
18. Dodd GT, Tiganis T. Insulin action in the brain: roles in energy and glucose homeostasis. *J Neuroendocrinol*. 2017;29(10).
19. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. *N Engl J Med*. 2017;376(3):254–266.
20. Han JC, Lawlor DA, Kimm SY. Childhood obesity. *Lancet* (London, England), 2010;375(9727):1737–1748.
21. Ren X, Zhou L, Terwilliger R, Newton SS, de Araujo IE. Sweet taste signaling functions as a hypothalamic glucose sensor. *Front Integr Neurosci*. 2009;3:12.
22. Claret M, Smith MA, Batterham RL, et al. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *J Clin Invest*. 2007;117(8):2325–2336.
23. Langlet F, Mullier A, Bouret SG, Prevot V, Dehouck B. Tanycyte-like cells form a blood-cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. *J Comp Neurol*. 2013;521(15):3389–3405.
24. Gross PM, Wainman DS, Shaver SW, Wall KM, Ferguson AV. Metabolic activation of efferent pathways from the rat area postrema. *Am J Physiol*. 1990;258(3 Pt 2):R788–R797.
25. Kiecker C. The origins of the circumventricular organs. *J Anat*. 2018;232(4):540–553.

26. Schaeffer M, Langlet F, Lafont C, et al. Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc Natl Acad Sci USA*. 2013;110(4):1512–1517.
27. Kentish SJ, Page AJ. The role of gastrointestinal vagal afferent fibres in obesity. *J Physiol*. 2015;593(4):775–786.
28. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656–660.
29. Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol*. 2006;494(3):528–548.
30. Perello M, Scott MM, Sakata I, et al. Functional implications of limited leptin receptor and ghrelin receptor coexpression in the brain. *J Comp Neurol*. 2012;520(2):281–294.
31. Rhea EM, Salameh TS, Gray S, Niu J, Banks WA, Tong J. Ghrelin transport across the blood-brain barrier can occur independently of the growth hormone secretagogue receptor. *Mol Metab*. 2018;18:88–96.
32. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology*. 2000;141(12):4797–4800.
33. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature*. 2000;407(6806):908–913.
34. Tang-Christensen M, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL, Tschöp M. Central administration of ghrelin and agouti-related protein (83-132) increases food intake and decreases spontaneous locomotor activity in rats. *Endocrinology*. 2004;145(10):4645–4652.
35. St-Pierre DH, Karelis AD, Cianflone K, et al. Relationship between ghrelin and energy expenditure in healthy young women. *J Clin Endocrinol Metab*. 2004;89(12):5993–5997.
36. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714–1719.
37. Druce MR, Neary NM, Small CJ, et al. Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *Int J Obes (2005)*. 2006;30(2):293–296.
38. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med*. 2002;346(21):1623–1630.
39. Castañeda TR, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol*. 2010;31(1):44–60.
40. Ahmed M, Gannon MC, Nuttall FQ. Postprandial plasma glucose, insulin, glucagon and triglyceride responses to a standard diet in normal subjects. *Diabetologia*. 1976;12(1):61–67.
41. Murphy BA, Fakira KA, Song Z, Beuve A, Routh VH. AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. *Am J Physiol Cell Physiol*. 2009;297(3):C750–C758.
42. Shimazu T, Minokoshi Y. Systemic glucoregulation by glucose-sensing neurons in the ventromedial hypothalamic nucleus (VMH). *J Endocr Soc*. 2017;1(5):449–459.
43. Adachi A, Kobashi M, Funahashi M. Glucose-responsive neurons in the brainstem. *Obes Res*. 1995;3(Suppl. 5):735S–740S.
44. Murphy BA, Fioramonti X, Jochnowitz N, et al. Fasting enhances the response of arcuate neuropeptide Y-glucose-inhibited neurons to decreased extracellular glucose. *Am J Physiol Cell Physiol*. 2009;296(4):C746–C756.
45. Moore CX, Cooper GJ. Co-secretion of amylin and insulin from cultured islet beta-cells: modulation by nutrient secretagogues, islet hormones and hypoglycemic agents. *Biochem Biophys Res Commun*. 1991;179(1):1–9.
46. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract*. 2011;93(Suppl. 1):S52–S59.
47. Baura GD, Foster DM, Porte Jr D, et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *J Clin Invest*. 1993;92(4):1824–1830.
48. Schwartz MW, Sipols AJ, Marks JL, et al. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology*. 1992;130(6):3608–3616.
49. Gedulin BR, Jodka CM, Herrmann K, Young AA. Role of endogenous amylin in glucagon secretion and gastric emptying in rats demonstrated with the selective antagonist, AC187. *Regul Pept*. 2006;137(3):121–127.
50. Zuger D, Forster K, Lutz TA, Riediger T. Amylin and GLP-1 target different populations of area postrema neurons that are both modulated by nutrient stimuli. *Physiol Behav*. 2013;112–113:61–69.
51. Lo CC, Langhans W, Georgievsky M, et al. Apolipoprotein AIV requires cholecystokinin and vagal nerves to suppress food intake. *Endocrinology*. 2012;153(12):5857–5865.
52. Nilaweera KN, Giblin L, Ross RP. Nutrient regulation of enteroendocrine cellular activity linked to cholecystokinin gene expression and secretion. *J Physiol Biochem*. 2010;66(1):85–92.
53. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. *Int J Exp Pathol*. 2011;92(4):219–231.
54. Batterham RL, Heffron H, Kapoor S, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab*. 2006;4(3):223–233.
55. Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*. 2002;418(6898):650–654.
56. Ueno H, Yamaguchi H, Mizuta M, Nakazato M. The role of PYY in feeding regulation. *Regul Pept*. 2008;145(1-3):12–16.
57. Koda S, Date Y, Murakami N, et al. The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. *Endocrinology*. 2005;146(5):2369–2375.
58. Andersen A, Lund A, Knop FK, Vilsboll T. Glucagon-like peptide 1 in health and disease. *Nat Rev Endocrinol*. 2018;14(7):390–403.
59. Pocai A. Action and therapeutic potential of oxyntomodulin. *Mol Metab*. 2014;3(3):241–251.
60. Jorgensen R, Kubale V, Vred M, Schwartz TW, Elling CE. Oxyntomodulin differentially affects glucagon-like peptide-1 receptor beta-arrestin recruitment and signaling through Galphas. *J Pharmacol Exp Ther*. 2007;322(1):148–154.
61. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. *Nat Rev Endocrinol*. 2010;6(12):689–697.
62. Adrian TE. Pancreatic polypeptide. *J Clin Pathol Suppl (Assoc Clin Pathol)*. 1978;8:43–50.
63. Schwartz TW, Holst JJ, Fahrenkrug J, et al. Vagal, cholinergic regulation of pancreatic polypeptide secretion. *J Clin Invest*. 1978;61(3):781–789.
64. Batterham RL, Le Roux CW, Cohen MA, et al. Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab*. 2003;88(8):3989–3992.
65. Schmidt PT, Naslund E, Gryback P, et al. A role for pancreatic polypeptide in the regulation of gastric emptying and short-term metabolic control. *J Clin Endocrinol Metab*. 2005;90(9):5241–5246.
66. Khandekar N, Berning BA, Sainsbury A, Lin S. The role of pancreatic polypeptide in the regulation of energy homeostasis. *Mol Cell Endocrinol*. 2015;418(Pt 1):33–41.
67. Adrian TE, Bloom SR, Bryant MG, Polak JM, Heitz PH, Barnes AJ. Distribution and release of human pancreatic polypeptide. *Gut*. 1976;17(12):940–944.
68. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med*. 1996;334(5):292–295.
69. Lonnqvist F, Wennlund A, Arner P. Relationship between circulating leptin and peripheral fat distribution in obese subjects. *Int J Obes Relat Metab Disord*. 1997;21(4):255–260.
70. Heptulla R, Smitten A, Teague B, Tamborlane WV, Ma YZ, Caprio S. Temporal patterns of circulating leptin levels in lean and obese adolescents: relationships to insulin, growth hormone, and free fatty acids rhythmicity. *J Clin Endocrinol Metab*. 2001;86(1):90–96.
71. Boden G, Chen X, Mozzoli M, Ryan I. Effect of fasting on serum leptin in normal human subjects. *J Clin Endocrinol Metab*. 1996;81(9):3419–3423.
72. Kolarzyski JW, Nyce MR, Considine RV, et al. Acute and chronic effects of insulin on leptin production in humans: studies in vivo and in vitro. *Diabetes*. 1996;45(5):699–701.
73. Grinspoon SK, Askari H, Landt ML, et al. Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. *Am J Clin Nutr*. 1997;66(6):1352–1356.

74. Keim NL, Stern JS, Havel PJ. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am J Clin Nutr*. 1998;68(4):794–801.
75. Perry RJ, Wang Y, Cline GW, et al. Leptin mediates a glucose-fatty acid cycle to maintain glucose homeostasis in starvation. *Cell*. 2018;172(1-2):234–248. e217.
76. Perry RJ. Leptin revisited: the role of leptin in starvation. *Mol Cell Oncol*. 2018;5(5). e1435185.
77. Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab*. 1998;83(5):1407–1413.
78. Malmstrom R, Taskinen MR, Karonen SL, Yki-Jarvinen H. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. *Diabetologia*. 1996;39(8):993–996.
79. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature*. 1998;393(6686):684–688.
80. Zakrzewska KE, Cusin I, Sainsbury A, Rohner-Jeanrenaud F, Jeanrenaud B. Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. *Diabetes*. 1997;46(4):717–719.
81. Ricci MR, Fried SK. Isoproterenol decreases leptin expression in adipose tissue of obese humans. *Obes Res*. 1999;7(3):233–240.
82. Chehab FF, Mounzih K, Lu R, Lim ME. Early onset of reproductive function in normal female mice treated with leptin. *Science* (New York, NY). 1997;275(5296):88–90.
83. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab*. 1997;82(4):1066–1070.
84. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations in later life. *Am J Clin Nutr*. 2002;75(6):993–999.
85. Banks WA, Coon AB, Robinson SM, et al. Triglycerides induce leptin resistance at the blood-brain barrier. *Diabetes*. 2004;53(5):1253–1260.
86. Di Spiezio A, Sandin ES, Dore R, et al. The LepR-mediated leptin transport across brain barriers controls food reward. *Mol Metab*. 2018;8:13–22.
87. Priego T, Sanchez J, Palou A, Pico C. Effect of high-fat diet feeding on leptin receptor expression in white adipose tissue in rats: depot- and sex-related differential response. *Genes Nutr*. 2009;4(2):151–156.
88. Cohen P, Yang G, Yu X, et al. Induction of leptin receptor expression in the liver by leptin and food deprivation. *J Biol Chem*. 2005;280(11):10034–10039.
89. Morioka T, Asilmaz E, Hu J, et al. Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *J Clin Invest*. 2007;117(10):2860–2868.
90. Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. *J Comp Neurol*. 1998;395(4):535–547.
91. Bjorbaek C, Elmquist JK, Michl P, et al. Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology*. 1998;139(8):3485–3491.
92. Yuan X, Caron A, Wu H, Gautron L. Leptin receptor expression in mouse intracranial perivascular cells. *Front Neuroanat*. 2018;12:4.
93. Lee GH, Proenca R, Montez JM, et al. Abnormal splicing of the leptin receptor in diabetic mice. *Nature*. 1996;379(6566):632–635.
94. Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford ML. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*. 1997;390(6659):521–525.
95. Kishimoto T, Taga T, Akira S. Cytokine signal transduction. *Cell*. 1994;76(2):253–262.
96. Banks AS, Davis SM, Bates SH, Myers Jr MG. Activation of downstream signals by the long form of the leptin receptor. *J Biol Chem*. 2000;275(19):14563–14572.
97. Niswender KD, Schwartz MW. Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol*. 2003;24(1):1–10.
98. Balthasar N, Coppari R, McMinn J, et al. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron*. 2004;42(6):983–991.
99. Dhillon H, Zigman JM, Ye C, et al. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*. 2006;49(2):191–203.
100. Garfield AS, Patterson C, Skora S, et al. Neurochemical characterization of body weight-regulating leptin receptor neurons in the nucleus of the solitary tract. *Endocrinology*. 2012;153(10):4600–4607.
101. Kanoski SE, Zhao S, Guarnieri DJ, et al. Endogenous leptin receptor signaling in the medial nucleus tractus solitarius affects meal size and potentiates intestinal satiation signals. *Am J Physiol Endocrinol Metab*. 2012;303(4):E496–E503.
102. Leininger GM, Jo YH, Leshan RL, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab*. 2009;10(2):89–98.
103. Mark AL, Rahmouni K, Correia M, Haynes WG. A leptin-sympathetic-leptin feedback loop: potential implications for regulation of arterial pressure and body fat. *Acta Physiol Scand*. 2003;177(3):345–349.
104. Kaiyala KJ, Ogimoto K, Nelson JT, Muta K, Morton GJ. Physiological role for leptin in the control of thermal conductance. *Mol Metab*. 2016;5(10):892–902.
105. Fischer AW, Hoefig CS, Abreu-Vieira G, et al. Leptin raises defended body temperature without activating thermogenesis. *Cell Rep*. 2016;14(7):1621–1631.
106. Thornton JE, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. *Endocrinology*. 1997;138(11):5063–5066.
107. Sohn JW, Williams KW. Functional heterogeneity of arcuate nucleus pro-opiomelanocortin neurons: implications for diverging melanocortin pathways. *Mol Neurobiol*. 2012;45(2):225–233.
108. Cowley MA, Smith RG, Diano S, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron*. 2003;37(4):649–661.
109. Kristensen P, Judge ME, Thim L, et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature*. 1998;393(6680):72–76.
110. Krashes MJ, Lowell BB, Garfield AS. Melanocortin-4 receptor-regulated energy homeostasis. *Nat Neurosci*. 2016;19(2):206–219.
111. Chen AS, Marsh DJ, Trumbauer ME, et al. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet*. 2000;26(1):97–102.
112. Butler AA, Cone RD. The melanocortin receptors: lessons from knockout models. *Neuropeptides*. 2002;36(2-3):77–84.
113. Baldini G, Phelan KD. The melanocortin pathway and control of appetite-progress and therapeutic implications. *J Endocrinol*. 2019;241(1):R1–R33.
114. Lu B. BDNF and activity-dependent synaptic modulation. *Learn Mem*. 2003;10(2):86–98.
115. Noble EE, Billington CJ, Kotz CM, Wang C. The lighter side of BDNF. *Am J Physiol Regul Integ Comp Physiol*. 2011;300(5):R1053–R1069.
116. Unger TJ, Calderon GA, Bradley LC, Sena-Esteves M, Rios M. Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *J Neurosci*. 2007;27(52):14265–14274.
117. Xu B, Goulding EH, Zang K, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci*. 2003;6(7):736–742.
118. Wang C, Bomberg E, Levine A, Billington C, Kotz CM. Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake. *Am J Physiol Regul Integ Comp Physiol*. 2007;293(3):R1037–R1045.
119. Wang C, Bomberg E, Billington CJ, Levine AS, Kotz CM. Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure. *Brain Res*. 2010;1336:66–77.
120. An JJ, Liao GY, Kinney CE, Sahibzada N, Xu B. Discrete BDNF neurons in the paraventricular hypothalamus control feeding and energy expenditure. *Cell Metab*. 2015;22(1):175–188.
121. Lyons WE, Mamounas LA, Ricaurte GA, et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci USA*. 1999;96(26):15239–15244.
122. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. *EMBO J*. 2000;19(6):1290–1300.
123. Coppola V, Tessarollo L. Control of hyperphagia prevents obesity in BDNF heterozygous mice. *Neuroreport*. 2004;15(17):2665–2668.

124. Han JC, Liu QR, Jones M, et al. Brain-derived neurotrophic factor and obesity in the WAGR syndrome. *N Engl J Med.* 2008;359(9): 918–927.
125. Gray J, Yeo GS, Cox JJ, et al. Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes.* 2006;55(12):3366–3371.
126. Han JC, Muehlbauer MJ, Cui HN, Newgard CB, Haqq AM. Lower brain-derived neurotrophic factor in patients with prader-will syndrome compared to obese and lean control subjects. *J Clin Endocrinol Metab.* 2010;95(7):3532–3536.
127. Carmona-Mora P, Canales CP, Cao L, et al. RAI1 transcription factor activity is impaired in mutants associated with Smith-Magenis Syndrome. *PLoS One.* 2012;7(9): e45155.
128. Han JC. Rare syndromes and common variants of the brain-derived neurotrophic factor gene in human obesity. *Prog Mol Biol Transl Sci.* 2016;140:75–95.
129. Hashimoto K, Koizumi H, Nakazato M, Shimizu E, Iyo M. Role of brain-derived neurotrophic factor in eating disorders: recent findings and its pathophysiological implications. *Prog Neuropsychopharmacol Biol Psychiatry.* 2005;29(4):499–504.
130. Ernst C, Marshall CR, Shen Y, et al. Highly penetrant alterations of a critical region including BDNF in human psychopathology and obesity. *Arch Gen Psychiatry.* 2012;69(12):1238–1246.
131. Nakazato M, Hashimoto K, Shimizu E, Niitsu T, Iyo M. Possible involvement of brain-derived neurotrophic factor in eating disorders. *IUBMB Life.* 2012;64(5):355–361.
132. Monteleone P, Zanardini R, Tortorella A, et al. The 196G/A (val66-met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder. *Neurosci Lett.* 2006;406(1-2):133–137.
133. Thaler L, Gauvin L, Joobor R, et al. Methylation of BDNF in women with bulimic eating syndromes: associations with childhood abuse and borderline personality disorder. *Prog Neuropsychopharmacol Biol Psychiatry.* 2014;54:43–49.
134. Mou Z, Hyde TM, Lipska BK, et al. Human obesity associated with an intronic snp in the brain-derived neurotrophic factor locus. *Cell Rep.* 2015;13(6):1073–1080.
135. Liao GY, Kinney CE, An JJ, Xu B. TrkB-expressing neurons in the dorsomedial hypothalamus are necessary and sufficient to suppress homeostatic feeding. *Proc Natl Acad Sci USA.* 2019; 116(8):3256–3261.
136. Wellman PJ. Modulation of eating by central catecholamine systems. *Curr Drug Target.* 2005;6(2):191–199.
137. Leibowitz SF, Roossin P, Rosenn M. Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. *Pharmacol Biochem Behav.* 1984;21(5):801–808.
138. Wellman PJ, Davies BT. Reversal of cirazoline- and phenylpropranolamine-induced anorexia by the α 1-receptor antagonist prazosin. *Pharmacol Biochem Behav.* 1992;42(1):97–100.
139. Bresch A, Rullmann M, Luthardt J, et al. Hunger and disinhibition but not cognitive restraint are associated with central norepinephrine transporter availability. *Appetite.* 2017;117:270–274.
140. Leibowitz SF, Alexander JT, Cheung WK, Weiss GF. Effects of serotonin and the serotonin blocker metergoline on meal patterns and macronutrient selection. *Pharmacol Biochem Behav.* 1993;45(1):185–194.
141. Wong DT, Reid LR, Threlkeld PG. Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. *Pharmacol Biochem Behav.* 1988;31(2):475–479.
142. Garattini S, Bizzi A, Caccia S, Mennini T. Progress report on the anorectic effects of dexfenfluramine, fluoxetine and sertraline. *Int J Obes Relat Metab Disord.* 1992;16(Suppl. 3):S43–S50.
143. Calapai G, Corica F, Corsonello A, et al. Leptin increases serotonin turnover by inhibition of brain nitric oxide synthesis. *J Clin Invest.* 1999;104(7):975–982.
144. Nonogaki K, Strack AM, Dallman MF, Tecott LH. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT_{2C} receptor gene. *Nat Med.* 1998;4(10):1152–1156.
145. Simansky KJ. Serotonergic control of the organization of feeding and satiety. *Behav Brain Res.* 1996;73(1-2):37–42.
146. van Galen KA, Ter Horst KW, Booij J, la Fleur SE, Serlie MJ. The role of central dopamine and serotonin in human obesity: lessons learned from molecular neuroimaging studies. *Metab Clin Exp.* 2018;85:325–339.
147. Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci USA.* 1998;95(25):15043–15048.
148. Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Gene Dev.* 1997;11(5):593–602.
149. Graham M, Shutter JR, Sarmiento U, Sarosi I, Stark KL. Overexpression of AgRP leads to obesity in transgenic mice. *Nat Genet.* 1997;17(3):273–274.
150. Dodd GT, Andrews ZB, Simonds SE, et al. A hypothalamic phosphatase switch coordinates energy expenditure with feeding. *Cell Metab.* 2017;26(2):375–393. e377.
151. Ruan HB, Dietrich MO, Liu ZW, et al. O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat. *Cell.* 2014;159(2):306–317.
152. Wang Q, Liu C, Uchida A, et al. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Mol Metab.* 2014;3(1):64–72.
153. Zhang X, van den Pol AN. Hypothalamic arcuate nucleus tyrosine hydroxylase neurons play orexigenic role in energy homeostasis. *Nat Neurosci.* 2016;19(10):1341–1347.
154. Krashes MJ, Shah BP, Madara JC, et al. An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. *Nature.* 2014;507(7491):238–242.
155. Baver SB, Hope K, Guyot S, Bjorbaek C, Kaczorowski C, O'Connell KM. Leptin modulates the intrinsic excitability of AgRP/NPY neurons in the arcuate nucleus of the hypothalamus. *J Neurosci.* 2014;34(16):5486–5496.
156. Huang Y, He Z, Gao Y, et al. Phosphoinositide 3-kinase is integral for the acute activity of leptin and insulin in male arcuate NPY/AgRP neurons. *J Endocr Soc.* 2018;2(6):518–532.
157. Garfield AS, Shah BP, Burgess CR, et al. Dynamic GABAergic afferent modulation of AgRP neurons. *Nat Neurosci.* 2016;19(12): 1628–1635.
158. Leibowitz SF. Brain peptides and obesity: pharmacologic treatment. *Obes Res.* 1995;3(Suppl. 4):573s–589s.
159. Kalra SP, Kalra PS. Nutritional infertility: the role of the interconnected hypothalamic neuropeptide Y-galanin-opioid network. *Front Neuroendocrinol.* 1996;17(4):371–401.
160. Shipp SL, Cline MA, Gilbert ER. Recent advances in the understanding of how neuropeptide Y and alpha-melanocyte stimulating hormone function in adipose physiology. *Adipocyte.* 2016; 5(4):333–350.
161. Beck B, Stricker-Krongrad A, Nicolas JP, Burlet C. Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behaviour of obese Zucker rats. *Int J Obes Relat Metab Disord.* 1992;16(4):295–302.
162. Stephens TW, Basinski M, Bristow PK, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature.* 1995;377(6549):530–532.
163. Broberger C, Landry M, Wong H, Walsh JN, Hokfelt T. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology.* 1997;66(6):393–408.
164. Masako S, Nicholas AT, Bradford BL, Jeffrey SF, Eleftheria M-F. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature.* 1998;396(6712):670.
165. Ludwig DS, Tritos NA, Mastaitis JW, et al. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest.* 2001;107(3):379.
166. Gomori A, Ishihara A, Ito M, et al. Chronic intracerebroventricular infusion of MCH causes obesity in mice. Melanin-concentrating hormone. *Am J Physiol Endocrinol Metab.* 2003;284(3): E583–E588.
167. Noble EE, Hahn JD, Konanur VR, et al. Control of feeding behavior by cerebral ventricular volume transmission of melanin-concentrating hormone. *Cell Metab.* 2018;28(1):55–68. e57.
168. Taylor MM, Samson WK. The other side of the orexins: endocrine and metabolic actions. *Am J Physiol Endocrinol Metab.* 2003; 284(1):E13–E17.
169. Hara J, Beuckmann CT, Nambu T, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron.* 2001;30(2):345–354.

170. Imperatore R, Palomba L, Cristino L. Role of orexin-A in hypertension and obesity. *Curr Hypertens Rep.* 2017;19(4):34.
171. Mieda M, Yanagisawa M. Sleep, feeding, and neuropeptides: roles of orexins and orexin receptors. *Curr Opin Neurobiol.* 2002;12(3):339–345.
172. Harris GC, Aston-Jones G. Arousal and reward: a dichotomy in orexin function. *Trend Neurosci.* 2006;29(10):571–577.
173. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev.* 2006;27(1):73–100.
174. Malcher-Lopes R, Di S, Marcheselli VS, et al. Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci.* 2006;26(24):6643–6650.
175. Koch M. Cannabinoid receptor signaling in central regulation of feeding behavior: a mini-review. *Front Neurosci.* 2017;11:293.
176. Rahmouni K, Haynes WG, Morgan DA, Mark AL. Role of melanocortin-4 receptors in mediating renal sympathoactivation to leptin and insulin. *J Neurosci.* 2003;23(14):5998–6004.
177. Collins S, Kuhn CM, Petro AE, Swick AG, Chrnyk BA, Surwit RS. Role of leptin in fat regulation. *Nature.* 1996;380(6576):677.
178. Muntzel MS, Morgan DA, Mark AL, Johnson AK. Intracerebroventricular insulin produces nonuniform regional increases in sympathetic nerve activity. *Am J Physiol.* 1994;267(5 Pt 2):R1350.
179. Vollenweider L, Tappy L, Owlya R, Jequier E, Nicod P, Scherrer U. Insulin-induced sympathetic activation and vasodilation in skeletal muscle. Effects of insulin resistance in lean subjects. *Diabetes.* 1995;44(6):641–645.
180. Blaak EE, Saris WH, van Baak MA. Adrenoceptor subtypes mediating catecholamine-induced thermogenesis in man. *Int J Obes Relat Metab Disord.* 1993;17(Suppl. 3):S78–S81. discussion S82.
181. Viguerie SN, Clement SK, Barbe SP, et al. In vivo epinephrine-mediated regulation of gene expression in human skeletal muscle. *J Clin Endocrinol Metab.* 2004;89(5):2000–2014.
182. Navegantes LC, Migliorini RH, do Carmo Kettelhut I. Adrenergic control of protein metabolism in skeletal muscle. *Curr Opin Clin Nutr Metab Care.* 2002;5(3):281–286.
183. Susulic VS, Frederick RC, Lawitts J, et al. Targeted disruption of the beta 3-adrenergic receptor gene. *J Biol Chem.* 1995;270(49):29483–29492.
184. Boss O, Bachman E, Vidal-Puig A, et al. Role of the β 3-adrenergic receptor and/or a putative β 3-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor-g coactivator-1. *Biochem Biophys Res Commun.* 1999;261:870–876.
185. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature.* 2004;404(6778):652–660.
186. Klingenberg M, Huang SG. Structure and function of the uncoupling protein from brown adipose tissue. *Biochim Biophys Acta.* 1999;1415(2):271–296.
187. Powley TL, Laughton W. Neural pathways involved in the hypothalamic integration of autonomic responses. *Diabetologia.* 1981;20(Suppl. 1):378–387.
188. Peles E, Goldstein DS, Akselrod S, et al. Interrelationships among measures of autonomic activity and cardiovascular risk factors during orthostasis and the oral glucose tolerance test. *Clin Auton Res.* 1995;5(5):271–278.
189. Rohner-Jeanrenaud F, Jeanrenaud B. Involvement of the cholinergic system in insulin and glucagon oversecretion of genetic obesity. *Endocrinology.* 1985;116(2):830–834.
190. Lustig RH. Autonomic dysfunction of the beta-cell and the pathogenesis of obesity. *Rev Endocr Metab Disord.* 2003;4(1):23–32.
191. Kreier F, Fliers E, Voshol PJ, et al. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. *J Clin Invest.* 2002;110(9):1243–1250.
192. Boden G, Hoeldtke RD. Nerves, fat, and insulin resistance. *N Engl J Med.* 2003;349(20):1966–1967.
193. D'Alessio DA, Kieffer TJ, Taborsky Jr GJ, Havel PJ. Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. *J Clin Endocrinol Metab.* 2001;86(3):1253–1259.
194. Ahren B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. *Diabetes.* 2001;50(5):1030–1038.
195. Gilon P, Henquin JC. Mechanisms and physiological significance of the cholinergic control of pancreatic beta-cell function. *Endocr Rev.* 2001;22(5):565–604.
196. Miura Y, Gilon P, Henquin J-C. Muscarinic stimulation increases Na⁺ entry in pancreatic B-cells by a mechanism other than the emptying of intracellular Ca²⁺ pools. *Biochem Biophys Res Commun.* 1996;224(1):67–73.
197. Zawulich WS, Zawulich KC, Rasmussen H. Cholinergic agonists prime the beta-cell to glucose stimulation. *Endocrinology.* 1989;125(5):2400–2406.
198. Nishi S, Seino Y, Ishida H, et al. Vagal regulation of insulin, glucagon, and somatostatin secretion in vitro in the rat. *J Clin Invest.* 1987;79(4):1191–1196.
199. Komeda K, Yokote M, Oki Y. Diabetic syndrome in the Chinese hamster induced with monosodium glutamate. *Experientia.* 1980;36(2):232–234.
200. Rohner-Jeanrenaud F, Jeanrenaud B. Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *J Clin Invest.* 1980;65(4):902–910.
201. Berthoud HR, Jeanrenaud B. Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology.* 1979;105(1):146.
202. Tian YM, Urquidi V, Ashcroft SJ. Protein kinase C in beta-cells: expression of multiple isoforms and involvement in cholinergic stimulation of insulin secretion. *Mol Cell Endocrinol.* 1996;119(2):185–193.
203. Arbusova A, Murray D, McLaughlin S. MARCKS, membranes, and calmodulin: kinetics of their interaction. *Biochim Biophys Acta.* 1998;1376(3):369–379.
204. Blondel O, Bell GI, Moody M, Miller RJ, Gibbons SJ. Creation of an inositol 1,4,5-trisphosphate-sensitive Ca²⁺ store in secretory granules of insulin-producing cells. *J Biol Chem.* 1994;269(44):27167.
205. Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev.* 1999;20(6):876–913.
206. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology.* 1999;140(4):1687–1694.
207. Mårin P, Rebuffé-Scrive M, Smith U, Björntorp P. Glucose uptake in human adipose tissue. *Metab Clin Exp.* 1987;36(12):1154–1160.
208. Ramsay TG. Fat cells. *Endocrinol Metabol Clin North Am.* 1996;25(4):847–870.
209. Rossi MA, Stuber GD. Overlapping brain circuits for homeostatic and hedonic feeding. *Cell Metab.* 2018;27(1):42–56.
210. Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M. Opioid modulation of taste hedonics within the ventral striatum. *Physiol Behav.* 2002;76(3):365–377.
211. Hommel JD, Trinko R, Sears RM, et al. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron.* 2006;51(6):801–810.
212. Shalev U, Yap J, Shaham Y. Leptin attenuates acute food deprivation-induced relapse to heroin seeking. *J Neurosci.* 2001;21(4):Rc129.
213. Carr KD, Tsimberg Y, Berman Y, Yamamoto N. Evidence of increased dopamine receptor signaling in food-restricted rats. *Neuroscience.* 2003;119(4):1157–1167.
214. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science (New York, NY).* 2007;317(5843):1355.
215. Figlewicz DP, Szot P, Chavez M, Woods SC, Veith RC. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res.* 1994;644(2):331–334.
216. Sipols AJ, Bayer J, Bennett R, Figlewicz DP. Intraventricular insulin decreases kappa opioid-mediated sucrose intake in rats. *Peptides.* 2002;23(12):2181–2187.
217. Figlewicz DP. Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. *Am J Physiol Regul Integ Comp Physiol.* 2003;284(4):R882–R892.
218. Avena NM, Rada P, Hoebel BG. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci Biobehav Rev.* 2008;32(1):20–39.
219. Garber AK, Lustig RH. Is fast food addictive? *Curr Drug Abuse Rev.* 2011;4(3):146–162.
220. Ziauddeen H, Farooqi IS, Fletcher PC. Obesity and the brain: how convincing is the addiction model? *Nat Rev Neurosci.* 2012;13(4):279–286.

221. Gordon EL, Ariel-Donges AH, Bauman V, Merlo LJ. What is the evidence for "food addiction?" a systematic review. *Nutrients*. 2018;10(4).
222. Dallman MF, Pecoraro NC, la Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun*. 2005;19(4):275–280.
223. la Fleur SE, Akana SF, Manalo SL, Dallman MF. Interaction between corticosterone and insulin in obesity: regulation of lipid intake and fat stores. *Endocrinology*. 2004;145(5):2174–2185.
224. Dallman MF, Pecoraro N, Akana SF, et al. Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci USA*. 2003;100(20):11696–11701.
225. Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol*. 1996;271(2 Pt 1):E317–E325.
226. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav*. 2007;91(4):449–458.
227. Tomiyama AJ, Dallman MF, Epel ES. Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women. *Psychoneuroendocrinology*. 2011;36(10):1513–1519.
228. Johnson JG, Cohen P, Kasen S, Brook JS. Childhood adversities associated with risk for eating disorders or weight problems during adolescence or early adulthood. *Am J Psychiatry*. 2002;159(3):394–400.
229. Roemmich JN, Wright SM, Epstein LH. Dietary restraint and stress-induced snacking in youth. *Obes Res*. 2002;10(11):1120–1126.
230. Suglia SF, Koenen KC, Boynton-Jarrett R, et al. Childhood and adolescent adversity and cardiometabolic outcomes: a scientific statement from the American Heart Association. *Circulation*. 2018;137(5):e15–e28.
231. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*. 1999;282(16):1568–1575.
232. Rosenbaum M, Nicolson M, Hirsch J, Murphy E, Chu F, Leibel RL. Effects of weight change on plasma leptin concentrations and energy expenditure. *J Clin Endocrinol Metab*. 1997;82(11):3647–3654.
233. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med*. 1995;332(10):621–628.
234. Pan WW, Myers Jr MG. Leptin and the maintenance of elevated body weight. *Nat Rev Neurosci*. 2018;19(2):95–105.
235. Caro JF, Kolaczynski JW, Nyce MR, et al. Decreased cerebrospinal fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* (London, England), 1996;348(9021):159–161.
236. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. *Pep-tides*. 1996;17(2):305–311.
237. Banks WA. The blood-brain barrier as a cause of obesity. *Curr Pharm*. 2008;14(16):1606–1614.
238. Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell*. 1998;1(4):619–625.
239. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest*. 2000;105(12):1827–1832.
240. Xu Y, Hill JW, Fukuda M, et al. PI3K signaling in the ventromedial hypothalamic nucleus is required for normal energy homeostasis. *Cell Metab*. 2010;12(1):88–95.
241. Klockener T, Hess S, Belgardt BF, et al. High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat Neurosci*. 2011;14(7):911–918.
242. Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab*. 2002;87(5):2391–2394.
243. Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest*. 2005;115(12):3579–3586.
244. Lustig RH, Sen S, Soberman JE, Velasquez-Mieyer PA. Obesity, leptin resistance, and the effects of insulin reduction. *Int J Obes Relat Metab Disord*. 2004;28(10):1344–1348.
245. Isganaitis HE, Lustig HR. Fast food, central nervous system insulin resistance, and obesity. *Arter Thromb Vasc Biol*. 2005;25(12):2451–2462.
246. Lustig RH, Mietus-Snyder ML, Bacchetti P, Lazar AA, Velasquez-Mieyer PA, Christensen ML. Insulin dynamics predict body mass index and z-score response to insulin suppression or sensitization pharmacotherapy in obese children. *J Pediatr*. 2006;148(1):23–29.
247. Polidori D, Sanghvi A, Seeley RJ, Hall KD. How strongly does appetite counter weight loss? quantification of the feedback control of human energy intake. *Obesity* (Silver Spring, Md), 2016; 24(11):2289–2295.
248. Champigny O, Ricquier D. Effects of fasting and refeeding on the level of uncoupling protein mRNA in rat brown adipose tissue: evidence for diet-induced and cold-induced responses. *J Nutr*. 1990;120(12):1730–1736.
249. Arone LJ, Mackintosh R, Rosenbaum M, Leibel RL, Hirsch J. Autonomic nervous system activity in weight gain and weight loss. *Am J Physiol*. 1995;269(1 Pt 2):R222–R225.
250. Fothergill E, Guo J, Howard L, et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. *Obesity* (Silver Spring, Md), 2016;24(8):1612–1619.
251. Russell SJ, Croker H, Viner RM. The effect of screen advertising on children's dietary intake: a systematic review and meta-analysis. *Obes Rev*. 2019;20(4):554–568.
252. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999–2010. *JAMA*. 2012;307(5):483–490.
253. Murphy M, Robertson W, Oyebode O. Obesity in international migrant populations. *Curr Obes Rep*. 2017;6(3):314–323.
254. Dietz WH. Health consequences of obesity in youth: childhood predictors of adulthood disease. *Pediatrics*. 1998;101(suppl. 3):518–525.
255. Finkelstein EA, Khavjou MA, Thompson H, et al. Obesity and severe obesity forecasts through 2030. *Am J Prev Med*. 2012;42(6):563–570.
256. Must A, Strauss RS. Risks and consequences of childhood and adolescent obesity. *Int J Obes*. 1999;23:S2–S11.
257. Zamrazilova H, Weiss R, Hainer V, Aldhoon-Hainerová I. Cardiometabolic health in obese adolescents is related to length of obesity exposure: a pilot study. *J Clin Endocrinol Metab*. 2016;101(8):3088–3095.
258. Juonala M, Magnussen CG, Berenson GS, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med*. 2011;365(20):1876–1885.
259. Hubbard VS. Defining overweight and obesity: what are the issues. *Am J Clin Nutr*. 2000;72:1067–1068.
260. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW. Body-mass index and mortality in a prospective cohort of U.S. adults. *N Engl J Med*. 1999;341:1097–1105.
261. Troiano RP, Flegal KM, Kuczmarski RJ, Campbell SM, Johnson CL. Overweight prevalence and trends for children and adolescents. *Arch Pediatr Adolesc Med*. 1995;149:1085–1091.
262. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*. 1997;337:869–873.
263. Dietz WH, Robinson TN. Use of the body mass index (BMI) as a measure of overweight in children and adolescents. *J Pediatr*. 1998;132:191–193.
264. Curves B. <https://www.cdc.gov/growthcharts>. 2001.
265. Organization, W.H. *Report of a WHO consultation on obesity. Obesity: preventing and managing the global epidemic*. Geneva: World Health Organization; 1998.
266. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med*. 2015;373(14):1307–1317.
267. Fernández JR, Redden DT, Pietrobello A, Allison DB. Waist circumference percentiles in nationally representative samples of African-American, European-American, and Mexican-American children and adolescents. *J Pediatr*. 2004;145(4):439–444.
268. Gortmaker SL, Dietz WH, Sobol AM, Wehler CA. Increasing pediatric obesity in the United States. *Am J Dis Child*. 1987;141:535–540.
269. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA*. 2002;288:1728–1732.

270. Ogden CL, Carroll MD, Lawman HG, et al. Trends in obesity prevalence among children and adolescents in the United States, 1988-1994 Through 2013-2014. *JAMA*. 2016;315(21):2292-2299.
271. Skinner AC, Perrin EM, Skelton JA. Prevalence of obesity and severe obesity in US children, 1999-2014. *Obesity* (Silver Spring, Md). 2016;24(5):1116-1123.
272. Ogden CL, Fryar CD, Hales CM, Carroll MD, Aoki Y, Freedman DS. Differences in obesity prevalence by demographics and urbanization in US children and adolescents, 2013-2016. *JAMA*. 2018;319(23):2410-2418.
273. Brown LR. *Obesity epidemic threatens health in exercise-deprived societies*; 2000. Worldwatch Institute internet release, www.worldwatch.org/chairman/issue/001219.html.
274. Obesity EEAftSo, (COTF). COTF. 2016; <http://easo.org/task-forces/childhood-obesity-cotf/facts-statistics/>, 2018.
275. Gordon-Larsen P, Adair LS, Popkin BM. Ethnic differences in physical activity and inactivity patterns and overweight status. *Obes Res*. 2002;10:141-149.
276. James WPT, Nelson M, Ralph A, Leather S. Socioeconomic determinants of health. The contribution of nutrition to inequalities in health. *BMJ (Clinical research ed)*. 1997;314:1545-1549.
277. Drewnowski A. Energy density, palatability, and satiety: implications for weight control. *Nutr Rev*. 1998;56:347-353.
278. Martorell R, Khan LK, Hughes ML, Grummer-Strawn LM. Obesity in Latin-American women and children. *J Nutr*. 1998;128:1464-1473.
279. Jayawardena R, Ranasinghe P, Wijayabandara M, Hills AP, Misra A. Nutrition transition and obesity among teenagers and young adults in South Asia. *Curr Diabetes Rev*. 2017;13(5):444-451.
280. Isganaitis E, Lustig RH. Fast food, central nervous system insulin resistance, and obesity. *Arterioscler Thromb Vasc Biol*. 2005;25(12):2451-2462.
281. Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA*. 2010;303(3):242-249.
282. Strauss RS, Pollack HA. Epidemic increase in childhood overweight, 1986-1998. *JAMA*. 2001;286:2845-2848.
283. Crawford PB, Story M, Wang MC, Ritchie LD, Sabry ZI. Ethnic issues in the epidemiology of childhood obesity. *Pediatr Clin North Am*. 2001;48:855-878.
284. Bullock A, Sheff K, Moore K, Manson S. Obesity and overweight in American Indian and Alaska native children, 2006-2015. *Am J Public Health*. 2017;107(9):1502-1507.
285. Warner ML, Harley K, Bradman A, Vargas G, Eskenazi B. Soda consumption and overweight status of 2-year-old Mexican-American children in California. *Obesity*. 2006;14:1966-1974.
286. Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics*. 1998;101:518-525.
287. Styne DM. Childhood and adolescent obesity: prevalence and significance. *Pediatr Clin North Am*. 2001;48:823-854.
288. Geserick M, Vogel M, Gausche R, et al. Acceleration of BMI in early childhood and risk of sustained obesity. *N Engl J Med*. 2018;379(14):1303-1312.
289. Guo SS, Huang C, Maynard LM, et al. Body mass index during childhood, adolescence and young adulthood in relation to adult overweight and adiposity: the Fels Longitudinal Study. *Int J Obes*. 2000;24:1628-1635.
290. Guo SS, Wu W, Chumlea WC, Roche AF. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr*. 2002;76:653-658.
291. Tirosh A, Shai I, Afek A, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Engl J Med*. 2011;364(14):1315-1325.
292. Baker JL, Olsen LW, Sørensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. *N Engl J Med*. 2007;357(23):2329-2337.
293. Tirosh A, Shai I, Afek A, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Engl J Med*. 2011;364(14):1315-1325.
294. Whitaker RC, Pepe MS, Wright JA, Seidel KD, Dietz WH. Early adiposity rebound and the risk of adult obesity. *Pediatrics*. 1998;101:1-6.
295. Toschke AM, Vignerova J, Lhotska L, Oscanova K, Kolestzko B, von Kries R. Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast feeding. *J Pediatr*. 2002;141:764-769.
296. von Kries R, Koletzko B, Sauerwald T, et al. Breast feeding and obesity: cross sectional study. *BMJ*. 1999;319:147-150.
297. Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. Early nutrition and leptin concentrations later in life. *Am J Clin Nutr*. 2002;75:993-999.
298. Lemas DJ, Yee S, Cacho N, et al. Exploring the contribution of maternal antibiotics and breastfeeding to development of the infant microbiome and pediatric obesity. *Semin Fetal Neonatal Med*. 2016;21(6):406-409.
299. Li L, Law C, Lo Conte R, Power C. Intergenerational influences on childhood body mass index: the effect of parental body mass index trajectories. *Am J Clin Nutr*. 2009;89(2):551-557.
300. Dorosty AR, Emmett PM, Cowin S, Reilly JJ. Factors associated with early adiposity rebound. *ALSPAC study team*. *Pediatrics*. 2000;105:1115-1118.
301. Wang Y, Min J, Khuri J, Li M. A Systematic Examination of the Association between Parental and Child Obesity across Countries. *Adv Nutr*. 2017;8(3):436-448.
302. Li S, Chen W, Srinivasan SR, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood. *JAMA*. 2003;290:2271-2276.
303. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev*. 1995;75(3):473-486.
304. Matsumoto M, Han S, Kitamura T, Accili D. Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest*. 2006;116(9):2464-2472.
305. Naïmi M, Gautier N, Chaussade C, Valverde AM, Accili D, Van Obberghen E. Nuclear forkhead box O1 controls and integrates key signaling pathways in hepatocytes. *Endocrinology*. 2007;148(5):2424-2434.
306. Lewis GF, Uffelman KD, Szeto LW, Steiner G. Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes*. 1993;42(6):833-842.
307. Brown MS, Goldstein JL. Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab*. 2008;7(2):95-96.
308. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*. 2014;510(7503):84-91.
309. Kim SP, Catalano KJ, Hsu IR, Chiu JD, Richey JM, Bergman RN. Nocturnal free fatty acids are uniquely elevated in the longitudinal development of diet-induced insulin resistance and hyperinsulinemia. *Am J Physiol Endocrinol Metab*. 2007;292(6):E1590-E1598.
310. Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*. 2005;36:232-240.
311. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest*. 2016;126(1):12-22.
312. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010;375:2267-2277.
313. Large V, Arner P. Regulation of lipolysis in humans. Pathophysiological modulation in obesity, diabetes, and hyperlipidaemia. *Diabetes Metab Res*. 1998;24:409-418.
314. Gustafson B, Hammarstedt A, Andersson CX, Smith U. Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2007;27(11):2276-2283.
315. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des*. 2008;14:1225-1230.
316. Bremer AA, Devaraj S, Afify A, Jialal I. Adipose tissue dysregulation in patients with metabolic syndrome. *J Clin Endocrinol Metab*. 2011;96:E1782-E1788.
317. Hershkop K, Besor O, Santoro N, Pierpont B, Caprio S, Weiss R. Adipose insulin resistance in obese adolescents across the spectrum of glucose tolerance. *J Clin Endocrinol Metab*. 2016;101(6):2423-2431.
318. Sinha R, Dufour S, Petersen KF, et al. Assessment of skeletal muscle triglyceride content by (1)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes*. 2002;51(4):1022-1027.
319. Weiss R, Dufour S, Taksali SE, et al. Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. *Lancet* (London, England). 2003;362(9388):951-957.

320. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237(3):E214–E223.
321. Levy-Marchal C, Arslanian S, Cutfield W, et al. Insulin Resistance in Children Consensus Conference Group. Insulin resistance in children: consensus, perspective, and future directions. *J Clin Endocrinol Metab.* 2010;95(12):5189–5198.
322. Giannini C, Santoro N, Caprio S, et al. The triglyceride-to-HDL cholesterol ratio: association with insulin resistance in obese youths of different ethnic backgrounds. *Diabetes Care.* 2011; 34(8):1869–1874.
323. Whincup PH, Gilg JA, Papacosta O, et al. Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. *BMJ.* 2002;324:635.
324. Goran MI, Gower BA. Longitudinal study on pubertal insulin resistance. *Diabetes.* 2001;50(11):2444–2450.
325. Sinha R, Fisch G, Teague B, et al. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med.* 2002;346(11):802–810.
326. Weiss R, Dufour S, Taksali SE, et al. Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. *Lancet.* 2003;362:951–957.
327. Bjorntorp P. Portal adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis.* 1990;10(4):493–496.
328. Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. *Diab Metab Res Rev.* 2002;18(Suppl. 2):S5–S9.
329. Bonora E. Relationship between regional fat distribution and insulin resistance. *Int J Obes.* 2000;24(Suppl. 2):S32–S35.
330. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Adams-Huet B, Grundy SM. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. *Diabetes.* 1996;45(12):1684–1693.
331. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev.* 2000;21(6):697–738.
332. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. *Diabetes.* 2000;49(6):883–888.
333. Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes.* 2008;57(2):367–371.
334. D'Adamo E, Cali AM, Weiss R, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. *Diabetes Care.* 2010;33(8):1817–1822.
335. Caprio S, Pierpont B, Kursawe R. The "adipose tissue expandability" hypothesis: a potential mechanism for insulin resistance in obese youth. *Horm Mol Biol Clin Investig.* 2018;33(2).
336. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. *Ann NY Acad Sci.* 2002;967:363–378.
337. Sung KC, Kim SH. Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. *J Clin Endocrinol Metab.* 2011;96:1093–1097.
338. Krssak M, Falk, Petersen K, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia.* 1999;42(1):113–116.
339. Garg A. Acquired and inherited lipodystrophies. *N Engl J Med.* 2004;350(12):1220–1234.
340. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes.* 1997;46(10):1579–1585.
341. Perseghin G, Scifo P, De Cobelli F, et al. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes.* 1999;48(8):1600–1606.
342. Sinha R, Dufour S, Petersen KF, et al. Assessment of skeletal muscle triglyceride content by (¹H) nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes.* 2002;51:1022–1027.
343. Fabbri E, Magkos F, Mohammed BS, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA.* 2009;106:15430–15435.
344. Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology.* 2003;144(12):5159–5165.
345. Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD. Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. *Diabetes.* 2001;50(1):123–130.
346. Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes.* 2006;55(Suppl. 2):S9–S15.
347. Samuel VT, Liu ZX, Qu X, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem.* 2004;279(31):32345–32353.
348. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. *Nature.* 2002;420:333–336.
349. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. *Endocr Rev.* 2001;22:36–52.
350. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev.* 1991;43:109–142.
351. Nakagawa T, Tuttle KR, Short R, Johnson RJ. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clin Pract Nephrol.* 2006;1(2):80–86.
352. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arter Thromb Vasc Biol.* 2003;23:168–175.
353. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol.* 1995;26:1235–1241.
354. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002;39(257–265).
355. Tounian P, Aggoun Y, Dubern BP, et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet (London, England).* 2001;358:1400–1404.
356. Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. *Pediatrics.* 2006;117(5):1560–1567.
357. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med.* 1998;338:1650–1656.
358. Raitakari TO, Juonala M, Kahonen M, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood. *JAMA.* 2003;290:2277–2283.
359. Group PDoAiYPR. Relationships in young men to serum lipoprotein cholesterol concentrations and smoking. *JAMA.* 1990;264:3018–3024.
360. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal media thickness is related to cardiovascular risk factors measured from childhood through middle age. *Circulation.* 2001;104:2815–2819.
361. Mahoney LT, Burns TL, Stanford W, et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol.* 1996;27(2):277–284.
362. Katz SL, Pillar G. Obstructive sleep apnea, obesity, and endothelial dysfunction in children. *Am J Respir Crit Care Med.* 2016; 194(9):1046–1047.
363. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;393:72–75.
364. Saltiel AR. You are what you secrete. *Nat Med.* 2001;7: 887–880.
365. Unger RH. The hyperleptinemia of obesity-regulator of caloric surpluses. *Cell.* 2004;117(2):145–146.
366. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Comm.* 1999;257:79–83.

367. Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol*. 1998;8(6):335–338.
368. Kondo H, Shimomura I, Matsukawa Y, et al. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes*. 2002;51(7):2325–2328.
369. Pajvani UB, Du X, Combs TP, et al. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. *J Biol Chem*. 2003;278(11):9073–9085.
370. Capllonch-Amer G, Sbert-Roig M, Galmes-Pascual BM, et al. Estradiol stimulates mitochondrial biogenesis and adiponectin expression in skeletal muscle. *J Endocrinol*. 2014;221(3):391–403.
371. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev*. 2005;26(3):439–451.
372. Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev*. 2005;26(3):439–451.
373. Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*. 2003;423:762–769.
374. Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- κ B signaling through a cAMP-dependent pathway. *Circulation*. 2000;102(11):1296–1301.
375. Arslanian S, El, Ghormli L, Bacha F, et al. Adiponectin, insulin sensitivity, β -cell function, and racial/ethnic disparity in treatment failure rates in TODAY. *Diabetes Care*. 2017;40(1):85–93.
376. Al-Daghri NM, Al-Attas OS, Alokail MS, et al. Parent-offspring transmission of adipocytokine levels and their associations with metabolic traits. *PLoS One*. 2011;6(4). e18182.
377. Pyrzak B, Demkow U, Kucharska AM. Brown adipose tissue and browning agents: irisin and fgf21 in the development of obesity in children and adolescents. *Adv Exp Med Biol*. 2015;866:25–34.
378. Boström P, Wu J, Jedrychowski MP, et al. A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481(7382):463–468.
379. Fagundo AB, Jiménez-Murcia S, Giner-Bartolomé C, et al. Modulation of irisin and physical activity on executive functions in obesity and morbid obesity. *Sci Rep*. 2016;6:30820.
380. Bordicchia M, Liu D, Amri EZ, et al. Cardiac natriuretic peptides ac viap38MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J Clin Invest*. 2012;122(3):1022–1036. Erratum in: 1122(1024),1584.
381. Wang TJ. The natriuretic peptides and fat metabolism. *N Engl J Med*. 2012;367:377–378.
382. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112:1673–1808.
383. Matsuzawa Y, Funahashi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann NY Acad Sci*. 1999;892:146–154.
384. Cinti S, Mitchell G, Barbatelli G, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res*. 2005;46(11):2347–2355.
385. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148:209–214.
386. Pickup JC, Crook MA. Is type 2 diabetes mellitus a disease of the innate immune system? *Diabetologia*. 1998;41:1241–1248.
387. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000;342(12):836–843.
388. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286(3):327–334.
389. Cook DG, Mendall MA, Whincup PH, et al. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis*. 2000;149(1):139–150.
390. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. *J Pediatr*. 2001;138(4):486–492.
391. Civitarese AE, Ravussin E. Mitochondrial energetics and insulin resistance. *Endocrinology*. 2008;149(3):950–954.
392. Subasinghe W, Syed I, Kowluru A. Phagocyte-like NADPH oxidase promotes cytokine-induced mitochondrial dysfunction in pancreatic beta-cells: evidence for regulation by Rac1. *Am J Physiol Regul Integr Comp Physiol*. 2011;300(1):R12–R20.
393. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552:335–344.
394. Fan X, Hussien R, Brooks GA. H₂O₂-induced mitochondrial fragmentation in C2C12 myocytes. *Free Radic Biol Med*. 2010;49(11):1646–1654.
395. de Ferranti S, Mozaffarian D. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem*. 2008; 54(945–955).
396. Dirks R, Vanhorebeek I, Martens K, et al. Absence of peroxisomes in mouse hepatocytes causes mitochondrial and ER abnormalities. *Hepatology*. 2005;41(4):868–878.
397. Schrader M, Fahimi HD. Peroxisomes and oxidative stress. *Biochim Biophys Acta*. 2006;1763(12):1755–1766.
398. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37(12):1595–1607.
399. Haffner SM. Epidemiology of insulin resistance and its relation to coronary artery disease. *Am J Cardiol*. 1999;84:11J–14J.
400. Hu FB, Stampfer JM, Haffner SM, Solomon CG, Willett WC, Manson JE. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care*. 2002;25:1129–1134.
401. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetes Med*. 1998;15:539–553.
402. Zimmet P, Alberti G, Kaufman F, et al. International Diabetes Federation Task Force on Epidemiology and Prevention of Diabetes. The metabolic syndrome in children and adolescents. *Lancet* (London, England), 2007;369:2059–2061.
403. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination survey. *JAMA*. 2002;287:356–359.
404. Zabarsky G, Beek C, Hagman E, Pierpont B, Caprio S, Weiss R. Impact of severe obesity on cardiovascular risk factors in youth. *J Pediatr*. 2018;192:105–114.
405. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346(16):1221–1231.
406. Ludwig J, Viggiano TR, McGill DB, Ott BJ. Nonalcoholic steatohepatitis: Mayo clinic experience with a hitherto unnamed disease. *Mayo Clin Proc*. 1980;55:434–438.
407. Meltzer AA, Everhart JE. Association between diabetes and elevated serum alanine aminotransferase activity among Mexican Americans. *Am J Epidemiol*. 1997;146:565–571.
408. Lavine JE, Schwimmer JB. Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis*. 2004;8(3):549–558.
409. Roberts E. Nonalcoholic steatohepatitis in children. *Curr Gastroenterol Rep*. 2003;5:253–259.
410. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab*. 2006;91:4287–4294.
411. Day CP, James OF. Steatohepatitis: a tale of two “hits”? *Gastroenterology*. 1998;114:842–845.
412. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein 1 in human and mouse organs and cultured cells. *J Clin Invest*. 1997;99:838–845.
413. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL. Decreased IRS2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in liver dystrophic and ob/ob mice. *Mol Cell*. 2000;6:77–86.
414. Goffredo M, Caprio S, Feldstein AE, et al. Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: a multiethnic study. *Hepatology* (Baltimore, Md). 2016;63(1):117–125.
415. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol*. 2010;7(5):251–264.
416. Schwimmer JB, Dunn W, Norman GJ, et al. SAFETY study: Alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology*. 2010; 138(4):1357–1364.

417. Goodman NF, Cobin RH, Futterweit W, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society Disease State Clinical Review: Guide to the best practices in the evaluation and treatment of polycystic ovary syndrome—Part 1. *Endocr Pract.* 2015;21(11):1291–1300.
418. Ibanez L, Potau N, Francois I, de Zegher F. Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab.* 1999;84(8):2691–2695.
419. Kim JY, Tfayli H, Michaliszyn SF, Arslanian S. Impaired lipolysis, diminished fat oxidation, and metabolic inflexibility in obese girls with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2018;103(2):546–554.
420. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab.* 2004;89:2745–2749.
421. Apter D, Butzow T, Laughlin GA, Yen SS. Metabolic features of polycystic ovary syndrome are found in adolescent girls with hyperandrogenism. *J Clin Endocrinol Metab.* 1995;80(10):2966–2973.
422. Lewy VD, Danadian K, Witchel SF, Arslanian S. Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. *J Pediatr.* 2001;138(1):38–44.
423. Setji TL, Holland ND, Sanders LL, Pereira KC, Diehl AM, Brown AJ. Nonalcoholic steatohepatitis and nonalcoholic fatty liver disease in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2006;91(5):1741–1747.
424. Gambineri A, Pelusi C, Manicardi E, et al. Glucose intolerance in a large cohort of Mediterranean women with polycystic ovary syndrome: phenotype and associated factors. *Diabetes.* 2004;53(9):2353–2358.
425. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. *J Clin Endocrinol Metab.* 2006;91(2):492–497.
426. Vryonidou A, Papatheodorou A, Tavridou A, et al. Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab.* 2005;90(5):2740–2746.
427. Ornstein RM, Copperman NM, Jacobson MS. Effect of weight loss on menstrual function in adolescents with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol.* 2011;24(3):161–165.
428. Venkatsen AM, Dunaif A, Corbould A. Insulin resistance in polycystic ovary syndrome: progress and paradoxes. *Recent Prog Horm Res.* 2001;56:295–308.
429. Zhang LH, Rodriguez H, Ohno S, Miller WL. Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. *Proc Natl Acad Sci USA.* 1995;92:10619–10623.
430. Dunaif A, Xia J, Book CB, Schenker E, Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. *J Clin Invest.* 1995;96:801–810.
431. Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. Earlier onset of puberty in girls: relation to increased body mass index and race. *Pediatrics.* 2001;108:347–353.
432. Lustig RH, Hershcopf RJ, Bradlow HL. The effects of body weight and diet on estrogen metabolism and estrogen-dependent disease. In: Frisch RE, ed. *Adipose Tissue and Reproduction.* Basel: Karger; 1990:107–124.
433. Braunstein GD. Aromatase and gynecomastia. *Endoc Relat Cancer.* 1999;6:315–324.
434. Kaplowitz P. Delayed puberty in obese boys: comparison with constitutional delayed puberty and response to testosterone therapy. *J Pediatr.* 1998;133:745–749.
435. Stanley TL, Levitsky LL, Grinspoon SK, Misra M. Effect of body mass index on peak growth hormone response to provocative testing in children with short stature. *J Clin Endocrinol Metab.* 2009;94(12):4875–4881.
436. Rose SR, Burstein S, Burghen GA, Pitukcheewanont P, Shope S, Hodnickak V. Caloric restriction for 24 hours increases mean night growth hormone. *J Pediatr Endocrinol Metab.* 1999;12:175–183.
437. Coutant R, Boux de Casson F, Rouleau S, et al. Body composition, fasting leptin, and sex steroid administration determine GH sensitivity in peripubertal short children. *J Clin Endocrinol Metab.* 2001;86:5805–5812.
438. Olney RC, Mougey EB. Expression of the components of the insulin-like growth factor axis across the growth plate. *Mol Cell Endocrinol.* 1999;156:63–71.
439. Reinehr T. Thyroid function in the nutritionally obese child and adolescent. *Curr Opin Pediatr.* 2011;23(4):415–420.
440. Masuzaki H, Paterson J, Shinyama H, et al. A transgenic model of visceral obesity and the metabolic syndrome. *Science.* 2001;294:2166–2170.
441. Kesler A, Fattal-Valevski A. Idiopathic intracranial hypertension in the pediatric population. *J Child Neurol.* 2002;17(10):745–748.
442. de la Eva R, Baur LA, Donaghue KC, Waters KA. Metabolic correlates with obstructive sleep apnea in obese subjects. *J Pediatr.* 2002;140:654–659.
443. Taylor ED, Theim KR, Mirch MC, et al. Orthopedic complications of overweight children and adolescents. *Pediatrics.* 2006;117(6):2167–2174.
444. Kaechele V, Wabitsch M, Thiere D, et al. Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. *J Pediatr Gastroenterol Nutr.* 2006;42(1):66–70.
445. Young-Hyman D, Tanofsky-Kraff M, Yanovski SZ, et al. Psychological status and weight-related distress in overweight or at-risk-for-overweight children. *Obesity.* 2006;14(12):2249–2258.
446. Bell LM, Byrne S, Thompson A, et al. Increasing body mass index z-score is continuously associated with complications of overweight in children, even in the healthy weight range. *J Clin Endocrinol Metab.* 2007;92(2):517–522.
447. Baier LJ, Hanson RL. Genetic studies of the etiology of type 2 diabetes in Pima Indians. *Diabetes.* 2004;53:1181–1186.
448. Butte NF, Cai G, Cole SA, Comuzzie AG. Viva la Familia Study: genetic and environmental contributions to childhood obesity and its comorbidities in the Hispanic population. *Am J Clin Nutr.* 2006;84(3):646–654.
449. Neel JV. *Diabetes mellitus: a thrifty genotype rendered detrimental by "progress"* Am J Hum Genet. 14;1962:353–362.
450. Mutch DM, Clement K. Genetics of human obesity. *Best Pract Res Clin Endocrinol Metab.* 2006;20(4):647–664.
451. Hochberg Z. An evolutionary perspective on the obesity epidemic. *Trends Endocrinol Metab.* 2018;29(12):819–826.
452. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med.* 2008;359(24):2558–2566.
453. Goodarzi MO. Genetics of obesity: what genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol.* 2018;6(3):223–236.
454. Loos RJF, Kilpelainen TO. Genome-wide association studies and human population obesity. In: Lustig RH, ed. *Obesity Before Birth.* New York: Springer; 2010:95–112.
455. Barker DJ. The developmental origins of chronic adult disease. *Acta Paediatr Suppl.* 2004;93:26–33.
456. Godfrey KM, Sheppard A, Gluckman PD, Lillycrop KA, Burdge GC, McLean C, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. *Diabetes.* 2011;60:1528–1534.
457. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol.* 2001;185:93–98.
458. Yajnik CS, Lubree HG, Rege SS, et al. Adiposity and hyperinsulinemia in Indians are present at birth. *J Clin Endocrinol Metab.* 2002;87:5575–5580.
459. Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC. Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). *Clin Endocrinol.* 2005; 62(1):44–50.
460. Potau N, Gussinye M, Sanchez, Ufarte C, Rique S, Vicens-Calvet E, Carrascosa A. Hyperinsulinemia in pre- and post-pubertal children born small for gestational age. *Horm Res.* 2001;56:146–150.
461. Yajnik CS, Fall CH, Vaidya U, et al. Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabetes Med.* 1995;12(4):330–336.
462. Silverman BL, Landsberg L, Metzger BE. Fetal hyperinsulinism in offspring of diabetic mothers: association with the subsequent development of childhood obesity. *Ann NY Acad Sci.* 1993;699:36–45.

463. Silverman BL, Rizzo TA, Cho NH, Metzger BE. Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes Care*. 1998;21(suppl. 2):B142–B149.
464. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes. *Pediatrics*. 2005;115(3):e290–e296.
465. Holder T, Giannini C, Santoro N, et al. A low disposition index in adolescent offspring of mothers with gestational diabetes: a risk marker for the development of impaired glucose tolerance in youth. *Diabetologia*. 2014;57(11):2413–2420.
466. Knowler WC, Pettitt DJ, Savage PJ, Bennett PH. Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. *Am J Epidemiol*. 1981;113:144–156.
467. Dabelea D, Hanson RL, Lindsay RS, et al. Intrauterine exposure to diabetes conveys risk for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes*. 2000;49:2208–2211.
468. Caughey AB. Obesity, weight loss, and pregnancy outcomes. *Lancet*. 2006;368:1136–1138.
469. Ludwig DS, Currie J. The association between pregnancy weight gain and birth weight: a within family comparison. *Lancet* (London, England). 2010;376:984–990.
470. Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol*. 2005;162:397–403.
471. Owen CG, Martin RM, Whincup PH, Davey-Smith G, Gillman MW, Cook DG. The effect of breastfeeding on mean body mass index throughout life: a quantitative review of published and unpublished observational evidence. *Am J Clin Nutr*. 2005;82(6):1298–1307.
472. Miralles O, Sanchez J, Palou A, Pico C. A physiological role of breast milk leptin in body weight control in developing infants. *Obesity*. 2006;14(8):1371–1377.
473. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. *Nat Med*. 2016;22(7):713–722.
474. Keith SW, Redden DT, Katzmaryk PT, et al. Putative contributors to the secular increase in obesity: exploring the roads less traveled. *Int J Obes*. 2006;30:1585–1594.
475. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med*. 2011;364:2392–2404.
476. Ludwig J, Sanbonmatsu L, Genetian L, et al. Neighborhoods, obesity, and diabetes—a randomized social experiment. *N Engl J Med*. 2011;365(16):1509–1519.
477. Andrew R, Gale CR, Walker BR, Seckl JR, Martyn CN. Glucocorticoid metabolism and the Metabolic Syndrome: associations in an elderly cohort. *Exp Clin Endocrinol Diabetes*. 2002;110:284–290.
478. Kotelevtsev YV, Holmes MC, Burchell A, et al. 11 β -hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity and stress. *Proc Natl Acad Sci*. 1997;94:14924–14929.
479. Razzoli M, Bartolomucci A. The dichotomous effect of chronic stress on obesity. *Trends Endocrinol Metab*. 2016;27(7):504–515.
480. Bujalska JJ, Kumar S, Stewart PM. Does central obesity reflect “Cushing’s disease of the omentum”? *Lancet*. 1997;349(9060):1210–1213.
481. Spiegel K, Sheridan JF, Van, Cauter E. Effect of sleep deprivation on response to immunization. *JAMA*. 2002;288:1471–1472.
482. Gangwisch JE, Malaspina D, Boden-Albala B, Heymsfield SB. Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep*. 2005;28:1217–1220.
483. Taheri S. The link between short sleep duration and obesity: we should recommend more sleep to prevent obesity. *Arch Dis Child*. 2006;91:881–884.
484. Fatima Y, Doi SA, Mamun AA. Longitudinal impact of sleep on overweight and obesity in children and adolescents: a systematic review and bias-adjusted meta-analysis. *Obes Rev*. 2015;16(2):137–149.
485. Sekine M, Yamagami T, Hamanishi S, et al. Parental obesity, lifestyle factors and obesity in preschool children: results of the Toyama birth cohort study. *J Epidemiol*. 2002;12:33–39.
486. Reilly JJ, Armstrong J, Dorosty AR, et al. Early life risk factors for obesity in childhood: cohort study. *BMJ*. 2005;330:1357.
487. Robinson TN. Television viewing and childhood obesity. *Pediatr Clin North Am*. 2001;48:1017–1025.
488. Sgoifo A, Braglia F, Costoli T, et al. Cardiac autonomic reactivity and salivary cortisol in men and women exposed to social stressors: relationship with individual ethological profile. *Neurosci Biobehav Rev*. 2003;27(1-2):179–188.
489. Taras HL, Sallis JF, Patterson TL, Nader PR, Nelson JA. Television’s influence on children’s diet and physical activity. *J Dev Behav Pediatr*. 1989;10:176–180.
490. DuRant RH, Baranowski T, Johnson M, Thompson WO. The relationship among television watching, physical activity, and body composition of young children. *Pediatrics*. 1994;94:449–455.
491. Coon KA, Goldberg J, Rogers BL, Tucker KL. Relationships between use of television during meals and children’s food consumption patterns. *Pediatrics*. 2001;107. e7.
492. Dietz WH, et al. Effect of sedentary activities on resting metabolic rate [computer program]. *Am J Clin Nutr*. 1994;59(3):556–559.
493. Crespo CJ, Smit E, Troiano RP, Bartlett SJ, Macera CA, Andersen RE. Television watching, energy intake, and obesity in US children: results from the third National Health and Nutrition Examination Survey, 1988–1994. *Arch Pediatr Adolesc Med*. 2001;155:360–365.
494. Robinson TN. Reducing children’s television viewing to prevent obesity: a randomized controlled trial. *JAMA*. 1999;282:1561–1567.
495. Tiberio SS, Kerr DC, Capaldi DM, Pears KC, Kim HK, Nowicka P. Parental monitoring of children’s media consumption: the long-term influences on body mass index in children. *JAMA Pediatr*. 2014;168(5):414–421.
496. Stanhope KL, Goran MI, Bosy-Westphal A, et al. Pathways and mechanisms linking dietary components to cardiometabolic disease: thinking beyond calories. *Obes Rev*. 2018;19(9):1205–1235.
497. Parsons TJ, Power C, Logan S, Summerbell CD. Childhood predictors of adult obesity: a systematic review. *Int J Obes*. 1999;23(suppl. 8):S1–S107.
498. Maffei C, Schutz Y, Grezzani A, Provera S, Piacentini G, Tato L. Meal-induced thermogenesis and obesity: is a fat meal a risk factor for fat gain in children? *J Clin Endocrinol Metab*. 2001;86:214–219.
499. Jequier E. Is fat intake a risk factor for fat gain in children? *J Clin Endocrinol Metab*. 2001;86:980–983.
500. Tucker LA, Seljaas GT, Hager RL. Body fat percentage of children varies according to their diet composition. *J Am Diet Assoc*. 1997;97:981–986.
501. Pirozzo S, Summerbell C, Cameron C, Glasziou P. Advice on low-fat diets for obesity. *Cochrane Database Syst Rev*. 2002, CD003640.
502. Kennedy E, Powell R. Changing eating patterns of American children: a view from 1996. *J Am Coll Nutr*. 1997;16:524–529.
503. Harnack L, Stang J, Story M. Soft drink consumption among US children and adolescents: nutritional consequences. *J Am Diet Assoc*. 1999;99:436–441.
504. Health CfWa. Pediatric overweight: a review of the literature June 2001. http://www.cnr.berkeley.edu/cwh/news/announcements.shtml#lit_review; 2001.
505. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*. 2003;348:2082–2090.
506. Stern L, Iqbal N, Seshadri P, et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Int Med*. 2004;140(10):778–785.
507. Vining EP, Pyzik P, McGrogan J, et al. Growth of children on the ketogenic diet. *Dev Med Child Neurol*. 2002;44:796–802.
508. Rasooly R, Kelley DS, Greg J, Mackey BE. Dietary trans 10, cis 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver. *Br J Nutr*. 2007;97(1):58–66.
509. Chantumugam P, Guthrie JF, Cecilio S, Morton JF, Basiotis PP, Anand R. Did fat intake in the United States really decline between 1989–1991 and 1994–1996? *J Am Diet Assoc*. 2003;103(7):867–872.
510. Dorfman SE, Laurent D, Gounarides JS, et al. Metabolic implications of dietary trans-fatty acids. *Obesity*. 2009;17:1200–1207.
511. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA*. 2002;287:2414–2423.

512. Pawlak DB, Bryson JM, Denyer JS, Brand-Miller JC. High glycemic index starch promotes hypersecretion of insulin and higher body fat in rats without affecting insulin sensitivity. *J Nutr.* 2001;131:99–104.
513. Ludwig DS, Majzoub JA, Al-Zahrani A, Dallal GE, Blanco I, Roberts SB. High glycemic index foods, overeating, and obesity. *Pediatrics.* 1999;103:e261–e266.
514. Ebbeling CB, Leidig MM, Sinclair KB, Hangen JP, Ludwig DS. A reduced-glycemic load diet in the treatment of adolescent obesity. *Arch Ped Adolesc Med.* 2003;157(8):773–779.
515. Ludwig DS, Pereira MA, Kroenke CH, et al. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA.* 1999;282(16):1539–1546.
516. Liese AD, Schulz M, Fang F, et al. Dietary glycemic index and glycemic load, carbohydrate and fiber intake, and measures of insulin sensitivity, secretion, and adiposity in the Insulin Resistance Atherosclerosis Study. *Diabetes Care.* 2005;28(12):2832–2838.
517. Pereira MA, Ludwig DS. Dietary fiber and body weight regulation. Observations and mechanisms. *Pediatr Clin North Am.* 2001;48:969–980.
518. Rigaud D, Paycha F, Meulemans A, Merrouche M, Mignon M. Effect of psyllium on gastric emptying, hunger feeling and food intake in normal volunteers: a double blind study. *Eur J Clin Nutr.* 1998;52:239–245.
519. Slavin J. Why whole grains are protective: biological mechanisms. *Proc Nutr Soc.* 2003;62(1):129–134.
520. Leach JD. Evolutionary perspective on dietary intake of fibre and colorectal cancer. *Eur J Clin Nutr.* 2007;61(1):140–142.
521. Martlett JA, McBurney MI, Slavin JL. Position of the American Dietetic Association: health implications of dietary fiber. *J Am Diet Assoc.* 2002;102:993–1000.
522. Ludwig DS, Pereira MA, Kroenke CJ, et al. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. *JAMA.* 1999;282:1539–1546.
523. Gross LS, Li S, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *Am J Clin Nutr.* 2004;79:774–779.
524. Ludwig DS, Peterson KE, Gortmaker SL. Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective, observational analysis. *Lancet.* 2001;357:505–508.
525. Faith MS, Dennison BA, Edmunds LS, Stratton HH. Fruit juice intake predicts increased adiposity gain in children from low-income families: weight status-by-environment interaction. *Pediatrics.* 2006;118:2066–2075.
526. Jurgens H, Haass W, Castaneda TR, et al. Consuming fructose-sweetened beverages increases body adiposity in mice. *Obes Res.* 2005;13(7):1146–1156.
527. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev.* 2005;63(5):133–157.
528. Le KA, Tappy L. Metabolic effects of fructose. *Curr Opin Nutr Metab Care.* 2006;9:469–475.
529. Nagai Y, Yonemitsu S, Erion DM, et al. The role of peroxisome proliferator-activated receptor gamma coactivator-1 beta in the pathogenesis of fructose-induced insulin resistance. *Cell Metab.* 2009;9(3):252–264.
530. Tuncman G, Hirosumi J, Solinas G, Chang L, Karin M, Hotamisligil GS. Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proc Natl Acad Sci USA.* 2006;103:10741–10746.
531. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose, not glucose-sweetened beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest.* 2009;119:1322–1334.
532. Teff KL, Grudziak J, Townsend RR, et al. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab.* 2009;94:1562–1569.
533. Lee O, Bruce WR, Dong Q, Bruce J, Mehta R, O'Brien PJ. Fructose and carbonyl metabolites and endogenous toxins. *Chem Biol Interact.* 2009;178:332–339.
534. Schwarz JM, Noworolski SM, Erkin-Cakmak A, et al. Effects of dietary fructose restriction on liver fat, de novo lipogenesis, and insulin kinetics in children with obesity. *Gastroenterology.* 2017;153(3):743–752.
535. Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr.* 2006;136(Suppl. 1):319S–323S.
536. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* 2009;9:311–326.
537. Higuchi N, Kato M, Miyakazi M, Tanaka M, Kohjima M, Ito T, et al. Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. *J Cell Biochem.* 2011;112(1):30–38.
538. Eaton S. Control of mitochondrial beta-oxidation flux. *Prog Lipid Res.* 2002;41(3):197–239.
539. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolic profiles and the risk of developing diabetes. *Nat Med.* 2011;17(4):448–453.
540. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia.* 2010;53(4):757–787.
541. Steinberg D, Pearson TA, Kuller LH. Alcohol and atherosclerosis. *Ann Intern Med.* 1991;114(11):967–976.
542. Suter PM, Schutz Y. The effect of exercise, alcohol or both combined on health and physical performance. *Int J Obes.* 2008;32(Suppl. 6):S48–S52.
543. Sozio M, Crabb DW. Alcohol and lipid metabolism. *Am J Physiol Endocrinol Metab.* 2008;295:E10–E16.
544. You M, Crabb DW. Molecular mechanisms of alcoholic fatty liver: role of sterol regulatory element-binding proteins. *Alcohol.* 2004;34(1):39–43.
545. Guzmán M, Castro J. Alterations in the regulatory properties of hepatic fatty acid oxidation and carnitine palmitoyltransferase I activity after ethanol feeding and withdrawal. *Alcohol Clin Exp Res.* 1990;14(3):472–477.
546. Yokoyama H, Hiroshi H, Ohgo H, Hibi T, Saito I. Effects of excessive ethanol consumption on the diagnosis of the metabolic syndrome using its clinical diagnostic criteria. *Intern Med.* 2007;46(17):1345–1352.
547. Pereira MA, Jacobs DR, van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults. *JAMA.* 2002;287:2081–2089.
548. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J.* 2000;14:1132–1138.
549. Shi H, Dirienzo D, Zemel MB. Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted ap2-agouti transgenic mice. *FASEB J.* 2001;15:291–293.
550. Lin YC, Lyle RM, McCabe LD, McCabe GP, Weaver CM, Teegarden D. Dietary calcium is related to changes in body composition during a two year exercise intervention in young women. *J Am Coll Nutr.* 2000;19:754–760.
551. Yanoff LB, Parikh SJ, Spitalnik A, et al. The prevalence of hypovitaminosis D and secondary hyperparathyroidism in obese Black Americans. *Clin Endocrinol.* 2006;64(5):523–529.
552. Heaney RP, Davies KM, Barger-Lux MJ. Calcium and weight: clinical studies. *J Am Coll Nutr.* 2002;21:152S–155S.
553. Walker WA. The importance of appropriate initial bacterial colonization of the intestine in newborn, child, and adult health. *Pediatr Res.* 2017;82(3):387–395.
554. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JL. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444:1027–1031.
555. Krajmalnik-Brown R, Ilhan ZE, Kang DW, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract.* 2012;27.
556. Rogers PC, Meacham LR, Oeffinger KC, Henry DW, Lange BJ. Obesity in pediatric oncology. *Pediatr Blood Cancer.* 2005;45:881–891.
557. Foster BJ, Shults J, Zemel BS, Leonard MB. Risk factors for glucocorticoid-induced obesity in children with steroid-sensitive nephrotic syndrome. *Pediatr Nephrol.* 2006;21(7):973–980.
558. Souverein PC, Berard A, Van Staa TP, et al. Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. *Heart.* 2004;90:859–865.

559. Bonny AE, Ziegler J, Harvey R, Debanne SM, Secic M, Cromer BA. Weight gain in obese and nonobese adolescent girls initiating depot medroxyprogesterone, oral contraceptive pills, or no hormonal contraceptive method. *Arch Pediatr Adol Med.* 2006; 160(1):40–45.
560. Quinn M, Ficociello LH, Rosner B. Change in glycemic control predicts change in weight in adolescent boys with type 1 diabetes. *Pediatr Diabetes.* 2003;4(4):162–167.
561. Vieweg WV, Sood AB, Pandurangi A, Silverman JJ. Newer antipsychotic drugs and obesity in children and adolescents. How should we assess drug-associated weight gain? *Acta Psychiatr Scand.* 2005;111(3):177–184.
562. Reekie J, Hosking SP, Prakash C, Kao KT, Juonala M, Sabin MA. The effect of antidepressants and antipsychotics on weight gain in children and adolescents. *Obes Rev.* 2015;16(7):566–580.
563. Styne DM, Arslanian SA, Connor EL, et al. Pediatric obesity—assessment, treatment, and prevention: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102(3):709–757.
564. Olney RC, Mougey EB. Expression of the components of the insulin-like growth factor axis across the growth-plate. *Mol Cell Endocrinol.* 1999;156(1-2):63–71.
565. Salerno M, Capalbo D, Cerbone M, De Luca F. Subclinical hypothyroidism in childhood - current knowledge and open issues. *Nat Rev Endocrinol.* 2016;12(12):734–746.
566. Wagenmakers M, Roerink S, Gil L, et al. Persistent centripetal fat distribution and metabolic abnormalities in patients in long-term remission of Cushing's syndrome. *Clin Endocrinol.* 2015;82(2):180–187.
567. Tirabassi G, Boscaro M, Arnaldi G. Harmful effects of functional hypercortisolism: a working hypothesis. *Endocrine.* 2014;46(3):370–386.
568. Masuzaki H, Paterson J, Shinyama H, et al. A transgenic model of visceral obesity and the metabolic syndrome. *Science (New York, NY).* 2001; 294(5549):2166–2170.
569. Mericq V, Medina P, Bouwman C, et al. Expression and activity of 11beta-hydroxysteroid dehydrogenase type 1 enzyme in subcutaneous and visceral adipose tissue of prepubertal children. *Horm Res.* 2009;71(2):89–93.
570. Draper N, Echwald SM, Lavery GG, et al. Association studies between microsatellite markers within the gene encoding human 11beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. *J Clin Endocrinol Metab.* 2002;87(11):4984–4990.
571. Tomlinson JW, Sinha B, Bujalska I, Hewison M, Stewart PM. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. *J Clin Endocrinol Metab.* 2002;87(12):5630–5635.
572. Li X, Wang J, Yang Q, Shao S. 11beta-hydroxysteroid dehydrogenase Type 1 in obese subjects with Type 2 diabetes mellitus. *Am J Med Sci.* 2017;354(4):408–414.
573. Barrett J, Maranda L, Nwosu BU. The relationship between subnormal peak-stimulated growth hormone levels and auxological characteristics in obese children. *Front Endocrinol (Lausanne).* 2014;5:35.
574. Cornford AS, Barkan AL, Horowitz JF. Rapid suppression of growth hormone concentration by overeating: potential mediation by hyperinsulinemia. *J Clin Endocrinol Metab.* 2011;96(3):824–830.
575. Chen M, Shrestha YB, Podyma B, et al. Galpha deficiency in the dorsomedial hypothalamus underlies obesity associated with Galpha mutations. *J Clin Invest.* 2017;127(2):500–510.
576. Levine MA. An update on the clinical and molecular characteristics of pseudohypoparathyroidism. *Curr Opin Endocrinol Diabetes Obes.* 2012;19(6):443–451.
577. Kaartinen JM, Kaar ML, Ohisalo JJ. Defective stimulation of adipocyte adenylate cyclase, blunted lipolysis, and obesity in pseudohypoparathyroidism 1a. *Pediatr Res.* 1994;35(5):594–597.
578. D'Angelo CS, Varela MC, de Castro CIE, et al. Chromosomal microarray analysis in the genetic evaluation of 279 patients with syndromic obesity. *Mol Cytogenet.* 2018;11:14.
579. Kaur Y, de Souza RJ, Gibson WT, Meyre D. A systematic review of genetic syndromes with obesity. *Obes Rev.* 2017;18(6):603–634.
580. Farooqi IS. Monogenic human obesity. *Front Horm Res.* 2008;36:1–11.
581. Farooqi S, O'Rahilly S. Genetics of obesity in humans. *Endocr Rev.* 2006;27(7):710–718.
582. Butler MG. Single gene and syndromic causes of obesity: illustrative examples. *Prog Mol Biol Transl Sci.* 2016;140:1–45.
583. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature.* 1997;387(6636):903–908.
584. Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest.* 2002;110(8):1093–1103.
585. Wabitsch M, Funcke JB, von Schnurbein J, et al. Severe early-onset obesity due to bioinactive leptin caused by a p.N103K mutation in the leptin gene. *J Clin Endocrinol Metab.* 2015;100(9):3227–3230.
586. Wabitsch M, Funcke JB, Lennerz B, et al. Biologically inactive leptin and early-onset extreme obesity. *N Engl J Med.* 2015;372(1):48–54.
587. Farooqi IS, Keogh JM, Kamath S, et al. Partial leptin deficiency and human adiposity. *Nature.* 2001;414(6859):34–35.
588. Farooqi IS, Wangenstein T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med.* 2007;356(3):237–247.
589. Clement K, Vaisse C, Lahlou N, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature.* 1998;392(6674):398–401.
590. He S, Tao YX. Defect in MAPK signaling as a cause for monogenic obesity caused by inactivating mutations in the melanocortin-4 receptor gene. *Int J Biol Sci.* 2014;10(10):1128–1137.
591. Li YQ, Shrestha Y, Pandey M, et al. G(q/11)alpha and G(s)alpha mediate distinct physiological responses to central melanocortins. *J Clin Invest.* 2016;126(1):40–49.
592. Yang LK, Tao YX. Biased signaling at neural melanocortin receptors in regulation of energy homeostasis. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863(10 Pt A):2486–2495.
593. Kuhnen P, Krude H, Biebermann H. Melanocortin-4 receptor signalling: importance for weight regulation and obesity treatment. *Trends Mol Med.* 2019;25(2):136–148.
594. Clement K, Biebermann H, Farooqi IS, et al. MC4R agonism promotes durable weight loss in patients with leptin receptor deficiency. *Nat Med.* 2018;24(5):551–555.
595. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet.* 1998;19(2):155–157.
596. Farooqi IS, Drop S, Clements A, et al. Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes.* 2006;55(9):2549–2553.
597. Kuhnen P, Handke D, Waterland RA, et al. Interindividual variation in DNA methylation at a putative POMC metastable epiallele is associated with obesity. *Cell Metab.* 2016;24(3):502–509.
598. Kuhnen P, Clement K, Wiegand S, et al. Proopiomelanocortin deficiency treated with a melanocortin-4 receptor agonist. *N Engl J Med.* 2016;375(3):240–246.
599. O'Rahilly S, Gray H, Humphreys PJ, et al. Brief report: impaired processing of prohormones associated with abnormalities of glucose homeostasis and adrenal function. *N Engl J Med.* 1995;333(21):1386–1390.
600. Jackson RS, Creemers JW, Ohagi S, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet.* 1997;16(3):303–306.
601. Frank GR, Fox J, Candela N, et al. Severe obesity and diabetes insipidus in a patient with PCSK1 deficiency. *Mol Genet Metab.* 2013;110(1-2):191–194.
602. Alsters SI, Goldstone AP, Buxton JL, et al. Truncating homozygous mutation of carboxypeptidase E (CPE) in a morbidly obese female with Type 2 diabetes mellitus, intellectual disability and hypogonadotropic hypogonadism. *PLoS One.* 2015;10(6):e0131417.
603. Lee YS, Poh LK, Loke KY. A novel melanocortin 3 receptor gene (MC3R) mutation associated with severe obesity. *J Clin Endocrinol Metab.* 2002;87(3):1423–1426.
604. Demidowich AP, Jun JY, Yanovski JA. Polymorphisms and mutations in the melanocortin-3 receptor and their relation to human obesity. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863(10 Pt A):2468–2476.

605. Vaisse C, Clement K, Durand E, Hercberg S, Guy-Grand B, Froguel P. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest*. 2000;106(2):253–262.
606. Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med*. 2003;348(12):1085–1095.
607. Reinehr T, Hinney A, de Sousa G, Austrup F, Hebebrand J, Andler W. Definable somatic disorders in overweight children and adolescents. *J Pediatr*. 2007;150(6):618–622. e611–615.
608. Hinney A, Schmidt A, Nottebom K, et al. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *J Clin Endocrinol Metab*. 1999;84(4):1483–1486.
609. Farooqi IS, Yeo GS, Keogh JM, et al. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. *J Clin Invest*. 2000;106(2):271–279.
610. Gu W, Tu Z, Kleyin PW, et al. Identification and functional analysis of novel human melanocortin-4 receptor variants. *Diabetes*. 1999;48(3):635–639.
611. Yeo GS, Lank EJ, Farooqi IS, Keogh J, Challis BG, O'Rahilly S. Mutations in the human melanocortin-4 receptor gene associated with severe familial obesity disrupts receptor function through multiple molecular mechanisms. *Hum Mol Genet*. 2003;12(5):561–574.
612. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr Rev*. 2010;31(4):506–543.
613. Tao YX. Constitutive activity in melanocortin-4 receptor: biased signaling of inverse agonists. *Adv Pharmacol*. 2014;70:135–154.
614. Greenfield JR, Miller JW, Keogh JM, et al. Modulation of blood pressure by central melanocortinergic pathways. *N Engl J Med*. 2009;360(1):44–52.
615. Agranat-Meged A, Ghanadri Y, Eisenberg I, Ben Neriah Z, Kieselstein-Gross E, Mitrani-Rosenbaum S. Attention deficit hyperactivity disorder in obese melanocortin-4-receptor (MC4R) deficient subjects: a newly described expression of MC4R deficiency. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(8):1547–1553.
616. Matharu N, Rattanasopha S, Tamura S, et al. CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science* (New York, NY), 2019;363(6424).
617. Li MM, Madara JC, Steger JS, et al. The paraventricular hypothalamus regulates satiety and prevents obesity via two genetically distinct circuits. *Neuron*. 2019;102(3):653–663.
618. Kublaoui BM, Holder Jr JL, Gemelli T, Zinn AR. Sim1 haploinsufficiency impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. *Mol Endocrinol* (Baltimore, Md), 2006;20(10):2483–2492.
619. Karkhanis A, Holleran KM, Jones SR. Dynorphin/kappa opioid receptor signaling in preclinical models of alcohol, drug, and food addiction. *Int Rev Neurobiol*. 2017;136:53–88.
620. Ramachandrapa S, Raimondo A, Cali AM, et al. Rare variants in single-minded 1 (SIM1) are associated with severe obesity. *J Clin Invest*. 2013;123(7):3042–3050.
621. Holder Jr JL, Butte NF, Zinn AR. Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet*. 2000;9(1):101–108.
622. Montagne L, Raimondo A, Delobel B, et al. Identification of two novel loss-of-function SIM1 mutations in two overweight children with developmental delay. *Obesity* (Silver Spring, Md), 2014;22(12):2621–2624.
623. Asai M, Ramachandrapa S, Joachim M, et al. Loss of function of the melanocortin 2 receptor accessory protein 2 is associated with mammalian obesity. *Science* (New York, NY), 2013;341(6143):275–278.
624. Hung CC, Luan J, Sims M, et al. Studies of the SIM1 gene in relation to human obesity and obesity-related traits. *Int J Obes* (2005). 2005;31(3):429–434.
625. Rouault AAJ, Srinivasan DK, Yin TC, Lee AA, Sebag JA. Melanocortin receptor accessory proteins (MRAPs): functions in the melanocortin system and beyond. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(10 Pt A):2462–2467.
626. Dykens EM, Maxwell MA, Pantino E, Kossler R, Roof E. Assessment of hyperphagia in Prader-Willi syndrome. *Obesity* (Silver Spring, Md), 2007; 15(7):1816–1826.
627. Han JC, Thurm A, Golden Williams C, et al. Association of brain-derived neurotrophic factor (BDNF) haploinsufficiency with lower adaptive behaviour and reduced cognitive functioning in WAGR/11p13 deletion syndrome. *Cortex*. 2013;49(10):2700–2710.
628. Sapio MR, Iadarola MJ, LaPaglia DM, et al. Haploinsufficiency of the brain-derived neurotrophic factor (BDNF) gene is associated with reduced pain sensitivity. *Pain*. 2019;160(5):1070–1081.
629. Shinawi M, Sahoo T, Maranda B, et al. 11p14.1 microdeletions associated with ADHD, autism, developmental delay, and obesity. *Am J Med Genet Part A*. 2011;155A(6):1272–1280.
630. Harcourt BE, Bullen DVR, Kao KT, et al. Maternal inheritance of BDNF deletion, with phenotype of obesity and developmental delay in mother and child. *Am J Med Genet Part A*. 2018;176(1):194–200.
631. Serra-Juhe C, Martos-Moreno GA, Bou de Pieri F, et al. Heterozygous rare genetic variants in non-syndromic early-onset obesity. *Int J Obes*. 2020;44(4):830–841.
632. Gunstad J, Schofield P, Paul RH, et al. BDNF Val66Met polymorphism is associated with body mass index in healthy adults. *Neuropsychobiology*. 2006;53(3):153–156.
633. Thorleifsson G, Walters GB, Gudbjartsson DF, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet*. 2009;41(1):18–24.
634. Zhao J, Bradfield JP, Li M, et al. The role of obesity-associated loci identified in genome-wide association studies in the determination of pediatric BMI. *Obesity* (Silver Spring, Md), 2009;17(12):2254–2257.
635. Wen W, Cho YS, Zheng W, et al. Meta-analysis identifies common variants associated with body mass index in east Asians. *Nat Genet*. 2012;44(3):307–311.
636. Gong J, Schumacher F, Lim U, et al. Fine mapping and identification of BMI loci in African Americans. *Am J Hum Genet*. 2013; 93(4):661–671.
637. Leon-Mimila P, Villamil-Ramirez H, Villalobos-Comparan M, et al. Contribution of common genetic variants to obesity and obesity-related traits in mexican children and adults. *PLoS One*. 2013;8(8). e70640.
638. Wu L, Xi B, Zhang M, et al. Associations of six single nucleotide polymorphisms in obesity-related genes with BMI and risk of obesity in Chinese children. *Diabetes*. 2010;59(12):3085–3089.
639. Mitchell JA, Hakonarson H, Rebeck TR, Grant SF. Obesity-susceptibility loci and the tails of the pediatric BMI distribution. *Obesity* (Silver Spring, Md), 2013;21(6):1256–1260.
640. Xi B, Cheng H, Shen Y, et al. Study of 11 BMI-associated loci identified in GWAS for associations with central obesity in the Chinese children. *PLoS One*. 2013;8(2). e56472.
641. Shugart YY, Chen L, Day IN, et al. Two British women studies replicated the association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) and BMI. *Eur J Hum Genet*. 2009;17(8):1050–1055.
642. Yeo GS, Connie Hung CC, Rochford J, et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat Neurosci*. 2004;7(11):1187–1189.
643. Gray J, Yeo G, Hung C, et al. Functional characterization of human NTRK2 mutations identified in patients with severe early-onset obesity. *Int J Obes* (2005). 2007;31(2):359–364.
644. Walters RG, Jacquemont S, Valsesia A, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature*. 2010;463(7281):671–675.
645. Selvanayagam T, Walker S, Gazzellone MJ, et al. Genome-wide copy number variation analysis identifies novel candidate loci associated with pediatric obesity. *Eur J Hum Genet*. 2018;26(11):1588–1596.
646. Shih CH, Chen CJ, Chen L. New function of the adaptor protein SH2B1 in brain-derived neurotrophic factor-induced neurite outgrowth. *PLoS One*. 2013;8(11). e79619.
647. Doche ME, Bochukova EG, Su HW, et al. Human SH2B1 mutations are associated with maladaptive behaviors and obesity. *J Clin Invest*. 2012;122(12):4732–4736.
648. Cassidy SB, Driscoll DJ. Prader-Willi syndrome. *Eur J Hum Genet*. 2009;17(1):3–13.
649. Holm VA, Cassidy SB, Butler MG, et al. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics*. 1993;91(2):398–402.
650. Schaaf CP, Gonzalez-Garay ML, Xia F, et al. Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nat Genet*. 2013;45(11):1405–1408.

651. Fountain MD, Schaaf CP. Prader-Willi syndrome and Schaaf-Yang syndrome: neurodevelopmental diseases intersecting at the MAGEL2 gene. *Diseases*. 2016;4(1).
652. Fountain MD, Aten E, Cho MT, et al. The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families. *Genet Med*. 2017;19(1):45–52.
653. Bischof JM, Stewart CL, Wevrick R. Inactivation of the mouse Magel2 gene results in growth abnormalities similar to Prader-Willi syndrome. *Hum Mol Genet*. 2007;16(22):2713–2719.
654. Cummings DE, Clement K, Purnell JQ, et al. Elevated plasma ghrelin levels in Prader-Willi syndrome. *Nat Med*. 2002;8(7):643–644.
655. Haqq AM, Farooqi IS, O'Rahilly S, et al. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. *J Clin Endocrinol Metab*. 2003;88(1):174–178.
656. Kweh FA, Miller JL, Sulsona CR, et al. Hyperghrelinemia in Prader-Willi syndrome begins in early infancy long before the onset of hyperphagia. *Am J Med Genet Part A*. 2015;167A(1):69–79.
657. De Waele K, Ishkhanian SL, Bogarin R, et al. Long-acting octreotide treatment causes a sustained decrease in ghrelin concentrations but does not affect weight, behaviour and appetite in subjects with Prader-Willi syndrome. *Eur J Endocrinol*. 2008;159(4):381–388.
658. Haqq AM, Stadler DD, Rosenfeld RG, et al. Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi syndrome. *J Clin Endocrinol Metab*. 2003;88(8):3573–3576.
659. Knani I, Earley BJ, Udi S, et al. Targeting the endocannabinoid/CB1 receptor system for treating obesity in Prader-Willi syndrome. *Mol Metab*. 2016;5(12):1187–1199.
660. Lindgren AC, Marcus C, Skwirut C, et al. Increased leptin messenger RNA and serum leptin levels in children with Prader-Willi syndrome and nonsyndromal obesity. *Pediatr Res*. 1997;42(5):593–596.
661. Goldstone AP, Unmehopa UA, Bloom SR, Swaab DF. Hypothalamic NPY and agouti-related protein are increased in human illness but not in Prader-Willi syndrome and other obese subjects. *J Clin Endocrinol Metab*. 2002;87(2):927–937.
662. Mercer RE, Michaelson SD, Chee MJ, Atallah TA, Wevrick R, Colmers WF. Magel2 is required for leptin-mediated depolarization of POMC neurons in the hypothalamic arcuate nucleus in mice. *PLoS Genet*. 2013;9(1). e1003207.
663. Merighi A, Salio C, Ghirri A, et al. BDNF as a pain modulator. *Prog Neurobiol*. 2008;85(3):297–317.
664. Groth R, Aanonson L. Spinal brain-derived neurotrophic factor (BDNF) produces hyperalgesia in normal mice while antisense directed against either BDNF or trkB, prevent inflammation-induced hyperalgesia. *Pain*. 2002;100(1-2):171–181.
665. Burnett LC, LeDuc CA, Sulsona CR, et al. Deficiency in prohormone convertase PC1 impairs prohormone processing in Prader-Willi syndrome. *J Clin Invest*. 2017;127(1):293–305.
666. Allen SJ, Dawbarn D. Clinical relevance of the neurotrophins and their receptors. *Clin Sci*. 2006;110(2):175–191.
667. Bekx MT, Carrel AL, Shriver TC, Li Z, Allen DB. Decreased energy expenditure is caused by abnormal body composition in infants with Prader-Willi syndrome. *J Pediatr*. 2003;143(3):372–376.
668. Carrel AL, Myers SE, Whitman BY, Allen DB. Benefits of long-term GH therapy in Prader-Willi syndrome: a 4-year study. *J Clin Endocrinol Metab*. 2002;87(4):1581–1585.
669. Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet*. 2013;21(1):8–13.
670. Sherafat-Kazemzadeh R, Ivey L, Kahn SR, et al. Hyperphagia among patients with Bardet-Biedl syndrome. *Pediatr Obes*. 2013;8(5):e64–e67.
671. Mariman EC, Vink RG, Roumans NJ, et al. The cilium: a cellular antenna with an influence on obesity risk. *Br J Nutr*. 2016;116(4):576–592.
672. Davis RE, Swiderski RE, Rahmouni K, et al. A knockin mouse model of the Bardet-Biedl syndrome 1 M390R mutation has cilia defects, ventriculomegaly, retinopathy, and obesity. *Proc Natl Acad Sci USA*. 2007;104(49):19422–19427.
673. Rahmouni K, Fath MA, Seo S, et al. Leptin resistance contributes to obesity and hypertension in mouse models of Bardet-Biedl syndrome. *J Clin Invest*. 2008;118(4):1458–1467.
674. Seo S, Guo DF, Bugge K, Morgan DA, Rahmouni K, Sheffield VC. Requirement of Bardet-Biedl syndrome proteins for leptin receptor signaling. *Hum Mol Genet*. 2009;18(7):1323–1331.
675. Guo DF, Cui H, Zhang Q, et al. The BBSome Controls Energy Homeostasis by Mediating the Transport of the Leptin Receptor to the Plasma Membrane. *PLoS Genet*. 2016;12(2). e1005890.
676. Wei Q, Gu YF, Zhang QJ, et al. Lztf1/BBS17 controls energy homeostasis by regulating the leptin signaling in the hypothalamic neurons. *J Mol Cell Biol*. 2018;10(5):402–410.
677. Barbari NF, Pasek RC, Malarkey EB, et al. Leptin resistance is a secondary consequence of the obesity in ciliopathy mutant mice. *Proc Natl Acad Sci USA*. 2013;110(19):7796–7801.
678. Feuillan PP, Ng D, Han JC, et al. Patients with Bardet-Biedl syndrome have hyperleptinemia suggestive of leptin resistance. *J Clin Endocrinol Metab*. 2011;96(3):E528–E535.
679. Haws RM, Fletty KL, McIntee TJ, et al. Effect of the melanocortin-4 receptor agonist setmelanotide on obesity and hyperphagia in individuals affected by Bardet-Biedl syndrome. *Eur Soc Paediatr Endocrinol*. 2018;89:1–105.
680. Collin GB, Marshall JD, Ikeda A, et al. Mutations in ALMS1 cause obesity, type 2 diabetes and neurosensory degeneration in Alstrom syndrome. *Nat Genet*. 2002;31(1):74–78.
681. Marshall JD, Muller J, Collin GB, et al. Alstrom syndrome: mutation spectrum of ALMS1. *Hum Mutat*. 2015;36(7):660–668.
682. Han JC, Reyes-Capo DP, Liu CY, et al. Comprehensive endocrine-metabolic evaluation of patients with Alstrom syndrome compared with BMI-matched controls. *J Clin Endocrinol Metab*. 2018;103(7):2707–2719.
683. Heydet D, Chen LX, Larter CZ, et al. A truncating mutation of Alms1 reduces the number of hypothalamic neuronal cilia in obese mice. *Dev Neurobiol*. 2013;73(1):1–13.
684. Collin GB, Cyr E, Bronson R, et al. Alms1-disrupted mice recapitulate human Alstrom syndrome. *Hum Mol Genet*. 2005;14(16):2323–2333.
685. Davenport JR, Watts AJ, Roper VC, et al. Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr Biol*. 2007;17(18):1586–1594.
686. Han JC, Smith WA, Burton ET, et al. Effect of setmelanotide (MC4R Agonist) on obesity and hunger in individuals with Alström syndrome. *Obes Wk*. 2018;89:1–105.
687. Burns B, Schmidt K, Williams SR, Kim S, Girirajan S, Elsea SH. Rai1 haploinsufficiency causes reduced Bdnf expression resulting in hyperphagia, obesity and altered fat distribution in mice and humans with no evidence of metabolic syndrome. *Hum Mol Genet*. 2010;19(20):4026–4042.
688. Tahir R, Kennedy A, Elsea SH, Dickinson AJ. Retinoic acid induced-1 (Rai1) regulates craniofacial and brain development in *Xenopus*. *Mech Dev*. 2014;133:91–104.
689. Alaimo JT, Hahn NH, Mullegama SV, Elsea SH. Dietary regimens modify early onset of obesity in mice haploinsufficient for Rai1. *PLoS One*. 2014;9(8). e105077.
690. Edelman EA, Girirajan S, Finucane B, et al. Gender, genotype, and phenotype differences in Smith-Magenis syndrome: a meta-analysis of 105 cases. *Clin Genet*. 2007;71(6):540–550.
691. Carmona-Mora P, Encina CA, Canales CP, et al. Functional and cellular characterization of human Retinoic Acid Induced 1 (RAI1) mutations associated with Smith-Magenis syndrome. *BMC Mol Biol*. 2010;11:63.
692. Duplomb L, Duvet S, Picot D, et al. Cohen syndrome is associated with major glycosylation defects. *Hum Mol Genet*. 2014;23(9):2391–2399.
693. Kolehmainen J, Black GC, Saarinen A, et al. Cohen syndrome is caused by mutations in a novel gene, COH1, encoding a transmembrane protein with a presumed role in vesicle-mediated sorting and intracellular protein transport. *Am J Hum Genet*. 2003;72(6):1359–1369.
694. Limoge F, Faivre L, Gautier T, et al. Insulin response dysregulation explains abnormal fat storage and increased risk of diabetes mellitus type 2 in Cohen Syndrome. *Hum Mol Genet*. 2015;24(23):6603–6613.
695. Seifert W, Holder-Espinasse M, Spranger S, et al. Mutational spectrum of COH1 and clinical heterogeneity in Cohen syndrome. *J Med Genet*. 2006;43(5). e22.
696. Seifert W, Kuhnisch J, Maritzen T, Horn D, Haucke V, Hennies HC. Cohen syndrome-associated protein, COH1, is a

- novel, giant Golgi matrix protein required for Golgi integrity. *J Biol Chem.* 2011;286(43):37665–37675.
697. Wang H, Falk MJ, Wensel C, Traboulsi EI. Cohen Syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews* ((R)); 1993. Seattle (WA).
 698. Alessandri JL, Dagoneau N, Laville JM, Baruteau J, Hebert JC, Cormier-Daire V. RAB23 mutation in a large family from Comoros Islands with Carpenter syndrome. *Am J Med Genet Part A.* 2010;152A(4):982–986.
 699. Hidestrand P, Vasconez H, Cottrill C. Carpenter syndrome. *J Craniofac Surg.* 2009;20(1):254–256.
 700. Jenkins D, Baynam G, De Catte L, et al. Carpenter syndrome: extended RAB23 mutation spectrum and analysis of nonsense-mediated mRNA decay. *Hum Mutat.* 2011;32(4):E2069–E2078.
 701. Jenkins D, Seelow D, Jehee FS, et al. RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. *Am J Hum Genet.* 2007;80(6):1162–1170.
 702. Perlyn CA, Marsh JL. Craniofacial dysmorphism of Carpenter syndrome: lessons from three affected siblings. *Plast Reconstr Surg.* 2008;121(3):971–981.
 703. Ramos JM, Davis GJ, Hunsaker 3rd JC, Balko MG. Sudden death in a child with Carpenter Syndrome. Case report and literature review. *Forensic Sci Med Pathol.* 2009;5(4):313–317.
 704. Twigg SR, Lloyd D, Jenkins D, et al. Mutations in multidomain protein MEGF8 identify a Carpenter syndrome subtype associated with defective lateralization. *Am J Hum Genet.* 2012;91(5):897–905.
 705. Jeanrenaud B. An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. *Diabetologia.* 1985;28(8):502–513.
 706. Satoh N, Ogawa Y, Katsuura G, et al. Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology.* 1997;138(3):947–954.
 707. Bray GA, Inoue S, Nishizawa Y. Hypothalamic obesity: the autonomic hypothesis and the lateral hypothalamus. *Diabetologia.* 1981;20(Suppl. 1):366–377.
 708. Bray GA, Nishizawa Y. Ventromedial hypothalamus modulates fat mobilisation during fasting. *Nature.* 1978;274(5674):900–902.
 709. Sklar CA. Craniopharyngioma: endocrine sequelae of treatment. *Pediatr Neurosurg.* 1994;21(Suppl. 1):120–123.
 710. Bray GA. Syndromes of hypothalamic obesity in man. *Pediatr Ann.* 1984;13(7):525–536.
 711. Daousi C, Dunn AJ, Foy PM, MacFarlane IA, Pinkney JH. Endocrine and neuroanatomic features associated with weight gain and obesity in adult patients with hypothalamic damage. *Am J Med.* 2005;118(1):45–50.
 712. Schofl C, Schleh A, Berger D, Terkamp C, von zur Muhlen A, Brabant G. Sympathoadrenal counterregulation in patients with hypothalamic craniopharyngioma. *J Clin Endocrinol Metab.* 2002;87(2):624–629.
 713. Coutant R, Maurey H, Rouleau S, et al. Defect in epinephrine production in children with craniopharyngioma: functional or organic origin? *J Clin Endocrinol Metab.* 2003;88(12):5969–5975.
 714. Shaikh MG, Grundy RG, Kirk JM. Reductions in basal metabolic rate and physical activity contribute to hypothalamic obesity. *J Clin Endocrinol Metab.* 2008;93(7):2588–2593.
 715. Lee HC, Curry DL, Stern JS. Direct effect of CNS on insulin hypersecretion in obese Zucker rats: involvement of vagus nerve. *Am J Physiol.* 1989;256(3 Pt 1):E439–E444.
 716. Preeyasombat C, Bacchetti P, Lazar AA, Lustig RH. Racial and etiopathologic dichotomies in insulin hypersecretion and resistance in obese children. *J Pediatr.* 2005;146(4):474–481.
 717. Tokunaga K, Fukushima M, Kemnitz JW, Bray GA. Effect of vagotomy on serum insulin in rats with paraventricular or ventromedial hypothalamic lesions. *Endocrinology.* 1978;103(4):1708–1711.
 718. Inoue S, Bray GA. The effects of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic obesity. *Endocrinology.* 1977;100(1):108.
 719. Smith DK, Sarfeh J, Howard L. Truncal vagotomy in hypothalamic obesity. *Lancet* (London, England), 1983;1(8337):1330–1331.
 720. van Iersel L, Brokke KE, Adan RAH, Bulthuis LCM, van den Akker ELT, van Santen HM. Pathophysiology and individualized treatment of hypothalamic obesity following craniopharyngioma and other suprasellar tumors: a systematic review. *Endocr Rev.* 2019;40(1):193–235.
 721. Lee JM, Shin J, Kim S, et al. Rapid-onset obesity with hypoventilation, hypothalamic, autonomic dysregulation, and neuroendocrine tumors (ROHHADNET) syndrome: a systematic review. *Biomed Res Int.* 2018;2018:1250721.
 722. Ize-Ludlow D, Gray JA, Sperling MA, et al. Rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation presenting in childhood. *Pediatrics.* 2007;120(1):e179–e188.
 723. Weese-Mayer DE, Bolk S, Silvestri JM, Chakravarti A. Idiopathic congenital central hypoventilation syndrome: evaluation of brain-derived neurotrophic factor genomic DNA sequence variation. *Am J Med Genet.* 2002;107(4):306–310.
 724. Thaker VV, Esteves KM, Towne MC, et al. Whole exome sequencing identifies RAI1 mutation in a morbidly obese child diagnosed with ROHHAD syndrome. *J Clin Endocrinol Metab.* 2015;100(5):1723–1730.
 725. Ibanez-Mico S, Marcos Oltra AM, de Murcia Lemauviel S, Ruiz Prunedra R, Martinez Ferrandez C, Domingo Jimenez R. Rapid-onset obesity with hypothalamic dysregulation, hypoventilation, and autonomic dysregulation (ROHHAD syndrome): A case report and literature review. *Neurologia.* 2017;32(9):616–622.
 726. Schwartz B, Jacobs DR, Moran A, Steinberger J, Hong CP, Sinaiko AR. Measurement of insulin sensitivity in children: comparison between the euglycemic-hyperinsulinemic clamp and surrogate measures. *Diabetes Care.* 2008;31(4):783–788.
 727. Bremer AA, Mietus-Snyder ML, Lustig RH. Toward a unifying hypothesis of metabolic syndrome. *Pediatrics.* 2012;129(3):557–570.
 728. Epstein LH, Valoski S, Wing R, McCurley J. Ten-year follow-up of behavioral, family-based treatment for obese children. *JAMA.* 1990;264:2519–2523.
 729. Oude Luttikhuis H, Baur L, Jansen H, et al. Interventions for treating obesity in children. *Cochrane Database Syst Rev.* 2009. CD001872.
 730. Flodmark CE, Marcus C, Britton M. Interventions to prevent obesity in children and adolescents: a systematic literature review. *Int J Obes.* 2006;30:579–589.
 731. Sumithran P, Prendergast LA, Delbridge E, et al. Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med.* 2011;365(17):1597–1604.
 732. Waters E, de Silva-Sanigorski A, Hall BJ, et al. Interventions for preventing obesity in children. *Cochrane Database Syst Rev.* 2011. CD001871.
 733. International Obesity Task Force. Assessment of childhood and adolescent obesity. Results from an international obesity task force workshop, Dublin, June 16, 1997. *Am J Clin Nutr.* 1999;70:117S–175S.
 734. Barlow SE, Dietz WH. Obesity evaluation and treatment: expert committee recommendations. The Maternal and Child Health Bureau, Health Resources and Services Administration and the Department of Health and Human Services. *Pediatrics.* 1998;102. e29.
 735. Baranowski T, Mendelein J, Resnicow K, Frank E, Cullen K, Baranowski J. Physical activity and nutrition in youth, an overview of obesity prevention. *Prevent Med.* 2000;31:S1–S10.
 736. Sothorn MS, Schumacher H, von Almen TK, Carlisle LK, Udall JN. Committed to kids: an integrated, 4-level team approach to weight management in adolescents. *J Am Diet Assoc.* 2002;102: S81–S85.
 737. Epstein LH, Roemmich JN, Raynor HA. Behavioral therapy in the treatment of pediatric obesity. *Pediatr Clin North Am.* 2001;48: 981–993.
 738. Resnicow K, Davis R, Rollnick S. Motivational interviewing for pediatric obesity: conceptual issues and evidence review. *J Am Diet Assoc.* 2006;106:2024–2033.
 739. Schwartz RP. Motivational interviewing (patient-centered counseling) to address childhood obesity. *Pediatr Ann.* 2010;39(3):154–158.
 740. James J, Thomas P, Cavan D, Kerr D. Preventing childhood obesity by reducing consumption of carbonated drinks: cluster randomised controlled trial. *BMJ (Clinical research ed).* 2004;328(7450):1237.

741. Ebbeling CB, Feldman HA, Osganian SK, Chomitz VR, Ellenbogen SJ, Ludwig DS. Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. *Pediatrics*. 2006;117:673–680.
742. Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA*. 2007;297(19):2092–2102.
743. Ebbeling CB, Swain JF, Feldman HA, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA*. 2012;307:2627–2634.
744. Nuutinen O. Long-term effects of dietary counseling on nutrient intake and weight loss in obese children. *Eur J Clin Nutr*. 1991;45:287–297.
745. Stice E, Cameron R, Killen J, Hayward C, Taylor CB. Naturalistic weight reduction efforts prospectively predict growth in relative weight and onset of obesity among female adolescents. *J Consult Clin Psychol*. 1999;67:967–974.
746. Klem ML, Wing RR, McGuire MT, Seagle HM, Hill JO. A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *Am J Clin Nutr*. 1997;66(2):239–246.
747. Ravussin E, Danforth E. Beyond sloth-physical activity and weight gain. *Science* (New York, NY). 1999;283:184–185.
748. Sothorn M. Exercise as a modality in the treatment of childhood obesity. *Pediatr Clin North Am*. 2001;48:931–945.
749. Gutin B, Barbeau P, Owens S, et al. Effects of exercise intensity on cardiovascular fitness, total body composition, and visceral adiposity of obese adolescents. *Am J Clin Nutr*. 2002;75:818–826.
750. McMurray RG, Bauman MJ, Harrell JS, Brown S, Gangdiwala SI. Effects of improvement in aerobic power on resting insulin and glucose concentrations in children. *Eur J Appl Physiol*. 2000;81:132–139.
751. McKechnie R, Mosca L. Physical activity and coronary heart disease: prevention and effect on risk factors. *Cardiol Rev*. 2003;11:21–25.
752. Ferguson MA, Gutin B, Le NA, et al. Effects of exercise training and its cessation on components of the insulin resistance syndrome in obese children. *Int J Obes and Relat Metab Disord*. 1999;22:889–895.
753. Zeitler P, Hirst K, Pyle L, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. *N Engl J Med*. 2012;366(24):2247–2256.
754. Nemet D, Barkan S, Epstein Y, Friedland O, Kowen G, Eliakim A. Short- and long-term beneficial effects of a combined dietary-behavioral-physical activity intervention for the treatment of childhood obesity. *Pediatrics*. 2005;115:e443–e449.
755. McGovern L, Johnson JN, Paulo R, et al. Clinical review: treatment of pediatric obesity: a systematic review and meta-analysis of randomized trials. *J Clin Endocrinol Metab*. 2007;93(12):4600–4605.
756. Yanovski SZ, Yanovski JA. Drug therapy: obesity. *N Engl J Med*. 2002;346:591–602.
757. Bray GA, Greenway FL. Current and potential drugs for treatment of obesity. *Endocr Rev*. 1999;20:805–875.
758. Abenhaim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and risk of primary pulmonary hypertension. International pulmonary hypertension study group. *N Engl J Med*. 1996;335:609–616.
759. Jick H, Vasilakis C, Weinrauch LA, Meier CR, Jick SS, Derby LE. A population-based study of appetite-suppressant drugs and the risk of cardiac valve regurgitation. *N Engl J Med*. 1998;339:719–724.
760. Weintraub M. Phenylpropanolamine as an anorexiatic agent in weight control: a review of published and unpublished studies. In: Morgan JP, Kagan DV, Bordy JS, eds. *Phenylpropanolamine: Risks, Benefits, and Controversies*. New York: Praeger; 1985:53–79.
761. Morgan JP, Funderburk FR. Phenylpropanolamine and blood pressure: a review of prospective studies. *Am J Clin Nutr*. 1992;55:206S–210S.
762. Mittendorf B, Ostlund RJ, Patterson B, Klein SZ. Orlistat inhibits dietary cholesterol absorption. *Obes Res*. 2001;9:599–604.
763. Guerciolini R, Radu-Radulescu L, Boldrin M, Dallas J, Moore R. Comparative evaluation of fecal fat excretion induced by orlistat and chitosan. *Obes Res*. 2001;9:364–367.
764. Zhi J, Melia AT, Guerciolini R, et al. Retrospective population-based analysis of the dose-response (fecal fat excretion) relationship of orlistat in normal and obese volunteers. *Clin Pharmacol Therap*. 1994;56:82–85.
765. Reitman JB, Castro-Cabezas M, de Bruin TW, Erkelens DW. Relationship between improved postprandial lipemia and low-density lipoprotein metabolism during treatment with tetrahydrolipstatin, a pancreatic lipase inhibitor. *Metab Clin Exp*. 1994;43:293–298.
766. Maahs D, de Serna DG, Kolotkin RL, et al. Randomized, double-blind, placebo-controlled trial of orlistat for weight loss in adolescents. *Endocr Pract*. 2006;12:18–28.
767. Chanoine JP, Hampf S, Jensen C, Boldrin M, Hauptman J. Effects of orlistat on weight and body composition in obese adolescents: a randomized controlled trial. *JAMA*. 2005;293(23):2873–2883.
768. Davidson MB, Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. *Am J Med*. 1997;102:99–110.
769. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995;333:550–554.
770. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995;333:541–549.
771. Jones KL, Arslanian S, Peterokova VA, Park JS, Tomlinson MJ. Effect of metformin in pediatric patients with Type 2 diabetes. *Diabetes Care*. 2002;25:89–94.
772. Group DPPR. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;346:393–403.
773. Mogul HR, Peterson SJ, Weinstein BI, Zhang S, Southren AL. Metformin and carbohydrate-modified diet: a novel obesity treatment protocol: preliminary findings from a case series of nondiabetic women with midlife weight gain and hyperinsulinemia. *Heart Dis*. 2001;3:285–292.
774. Lee A, Morley JE. Metformin decreases food-consumption and induces weight-loss in subjects with obesity with type-II non-insulin-dependent diabetes. *Obes Res*. 1998;6:47–53.
775. Paolisso G, Amato L, Eccellente R, et al. Effect of metformin on food intake in obese subjects. *Eur J Clin Invest*. 1998;28:441–446.
776. Bailey CJ, Turner RC. Metformin. *N Engl J Med*. 1996;334:574–579.
777. Lenhard JM, Kliever SA, Paulik MA, Plunket KD, Lehmann JM, Weiel JE. Effects of troglitazone and metformin on glucose and lipid metabolism: alterations of two distinct molecular pathways. *Biochem Pharmacol*. 1997;54:801–808.
778. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*. 2001;108:1167–1174.
779. Kumar N, Dey CS. Metformin enhances insulin signalling in insulin-dependent and -independent pathways in insulin resistant muscle cells. *Br J Pharmacol*. 2002;137(3):329–336.
780. Kiefer TJ, Habener JF. The glucagon-like peptides. *Endocr Rev*. 2001;20:876–913.
781. Mannucci E, Ognibene A, Cemasco F, et al. Effect of metformin on glucagon-like peptide-1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care*. 2001;24:489–494.
782. Drucker DJ. Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat Clin Pract Endo Metab*. 2005;1:22–31.
783. Freemark M, Bursley D. The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of Type 2 diabetes. *Pediatrics*. 2001;107. e55.
784. Wilson DM, Abrams SH, Aye T, et al. Metformin extended release treatment of adolescent obesity: a 48-week randomized, double-blind, placebo-controlled trial with 48-week follow-up. *Arch Pediatr Adol Med*. 2010;164(2):116–123.
785. Lustig RH, Mietus-Snyder ML, Bacchetti P, Lazar AA, Velasquez-Mieyer PA, Christensen ML. Insulin dynamics predict BMI and z-score response to insulin suppression or sensitization pharmacotherapy in obese children. *J Pediatr*. 2006;148(1):23–29.
786. Velasquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovarian syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metab Clin Exp*. 1994;43:647–654.
787. Pasquali R, Gambineri A, Biscotti D, et al. Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2000;85:2767–2774.

788. Jamieson MA. The use of metformin in adolescents with polycystic ovary syndrome: for and against. *J Pediatr Adolesc Gynecol*. 2002;15:109–114.
789. Ibanez L, Valls C, Potau N, Marcos MV, de Zegher F. Sensitization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia, and hyperinsulinism after precocious adrenarche. *J Clin Endocrinol Metab*. 2000;85:3526–3530.
790. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet*. 2001;358:893–894.
791. Schwimmer JB, Middleton MS, Deutsch R, Lavine JE. A phase 2 trial of metformin as a treatment for non-diabetic paediatric non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2005;21:871–879.
792. Morrison JA, Cottingham EM, Barton BA. Metformin for weight loss in pediatric patients taking psychotropic drugs. *Am J Psychiatry*. 2002;159:655–657.
793. Silverman B, Franklin GM, Bolin R. Lactic acidosis traced to thiamine deficiency related to nationwide shortage of multivitamins for total parenteral nutrition. *MMWR*. 1997;46(23):523–528.
794. Berthoud HR, Jeanrenaud B. Acute hyperinsulinemia and its reversal by vagotomy following lesions of the ventromedial hypothalamus in anesthetized rats. *Endocrinology*. 1979;105:146–151.
795. Rohner-Jeanrenaud F, Jeanrenaud B. Consequences of ventromedial hypothalamic lesions upon insulin and glucagon secretion by subsequently isolated perfused pancreases in the rat. *J Clin Invest*. 1980;65:902–910.
796. Jeanrenaud B. An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. *Diabetologia*. 1985;28:502–513.
797. Satoh N, Ogawa Y, Katsura G, et al. Pathophysiological significance of the obese gene product, leptin in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology*. 1997;138:947–954.
798. Bray GA, Inoue S, Nishizawa Y. Hypothalamic obesity. *Diabetologia*. 1978;20:366–377.
799. Bray GA, Nishizawa Y. Ventromedial hypothalamus modulates fat mobilization during fasting. *Nature*. 1978;274:900–902.
800. Bray GA. Syndromes of hypothalamic obesity in man. *Pediatr Ann*. 1984;13:525–536.
801. Daousi C, Dunn AJ, Foy PM, MacFarlane IA, Pinkney JH. Endocrine and neuroanatomic predictors of weight gain and obesity in adult patients with hypothalamic damage. *Am J Med*. 2005;118(1):45–50.
802. Thornton JE, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. *Endocrinology*. 1997;138:5063–5066.
803. Harz KJ, Muller HL, Waldeck E, Pudiel V, Roth C. Obesity in patients with craniopharyngioma: assessment of food intake and movement counts indicating physical activity. *J Clin Endocrinol Metab*. 2003;88(11):5227–5231.
804. Bray GA, Gallagher TF. Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a review of the literature. *Medicine*. 1975;54:301–333.
805. Lustig RH. Autonomic dysfunction of the β -cell and the pathogenesis of obesity. *Rev Endocr Metab Dis*. 2003;4:23–32.
806. Lustig RH. Hypothalamic obesity: the sixth cranial endocrinopathy. *Endocrinologist*. 2002;12:210–217.
807. Preeyasombat C, Bacchetti P, Lazar AA, Lustig RH. Racial and etiopathologic dichotomies in insulin secretion and resistance in obese children. *J Pediatr*. 2005;146(4):474–481.
808. Velasquez-Mieyer PA, Cowan PA, Buffington CK, et al. Suppression of insulin secretion promotes weight loss and alters macronutrient preference in a subset of obese adults. *Int J Obes*. 2003;27:219–226.
809. Hsu WH, Xiang HD, Rajan AS, Kunze DL, Boyd AE. Somatostatin inhibits insulin secretion by a G-protein-mediated decrease in Ca^{2+} entry through voltage-dependent Ca^{2+} channels in the beta-cell. *J Biol Chem*. 1991;266:837–843.
810. Mitra SW, Mezey E, Hunyady B, et al. Colocalization of somatostatin receptor sst5 and insulin in rat pancreatic β -cells. *Endocrinology*. 1999;140:3790–3796.
811. Bertoli A, Magnatera R, Borboni P, et al. Dose-dependent effect of octreotide on insulin secretion after OGTT in obesity. *Horm Res*. 1998;49:17–21.
812. Lustig RH, Greenway F, Velasquez-Mieyer P, et al. A multicenter, randomized, double-blind, placebo-controlled, dose-finding trial of a long-acting formulation of octreotide in promoting weight loss in obese adults with insulin hypersecretion. *Int J Obes (London, UK)*. 2006;30(2):331–341.
813. Krentz AJ, MacDonald LM, Schade DS. Octreotide: a long-acting inhibitor of endogenous hormone secretion for human metabolic investigations. *Metab Clin Exp*. 1994;43:24–31.
814. Lamberts SWJ, Van Der Lely AJ, De Herder WW, Hofland LJ. Drug therapy: octreotide. *N Engl J Med*. 1996;334:246–254.
815. Tauber MT, Harris AG, Rochiccioli P. Clinical use of the long-acting somatostatin analog octreotide in pediatrics. *Eur J Pediatr*. 1994;153:304–310.
816. Montague CT, Farooqi IS, Whitehead JP, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*. 1997;387:903–908.
817. Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T-cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest*. 2002;110:1093–1103.
818. Farooqi IS, Jebb SA, Langmack G, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*. 1999;341:913–915.
819. Gibson WT, Farooqi IS, Moreau M, et al. Congenital leptin deficiency due to homozygosity for the d1333G mutation: report of another case and evaluation of response to four years of leptin therapy. *J Clin Endocrinol Metab*. 2004;89:4821–4826.
820. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA*. 1999;282:1568–1575.
821. Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL. Low dose leptin administration reverses effects of sustained weight reduction on energy expenditure and circulating concentrations of thyroid hormones. *J Clin Endocrinol Metab*. 2002;87:2391–2394.
822. Shi ZQ, Chinookoswong N, Wang JL, et al. Additive effects of leptin and oral anti-obesity drugs in treating diet-induced obesity in rats. *Diabetes*. 2002;51(suppl. 2):1707.
823. Torekov SS, Madsbad S, Holst JJ. Obesity - an indication for GLP-1 treatment? Obesity pathophysiology and GLP-1 treatment potential. *Obes Rev*. 2011;12(8):593–601.
824. Danne T, Biester T, Kapitzke K, et al. Liraglutide in an adolescent population with obesity: a randomized, double-blind, placebo-controlled 5-week trial to assess safety, tolerability, and pharmacokinetics of liraglutide in adolescents aged 12–17 years. *J Pediatr*. 2017;181:146–153. e143.
825. Teter CJ, Early JJ, Gibbs CM. Treatment of affective disorder and obesity with topiramate. *Ann Pharmacother*. 2000;34:1262–1265.
826. Wilkes JJ, Nelson E, Osborne M, Demarest KT, Olefsky JM. Topiramate is an insulin-sensitizing compound in vivo with direct effects on adipocytes in female ZDF rats. *Am J Physiol Endocrinol Metab*. 2005;288:E617–E624.
827. Wilding J, Van Gaal L, Rissanen A, Vercauteren F, Fitchet M. A randomized double-blind placebo-controlled study of the long-term efficacy and safety of topiramate in the treatment of obese subjects. *Int J Obes*. 2004;28:1399–1410.
828. Wynne K, Park AJ, Small CJ, et al. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*. 2005;54:2390–2395.
829. Michalsky M, Kramer RE, Fullmer MA, Polfuss M, Porter R, Ward-Begnoche W, et al. Developing criteria for pediatric/adolescent bariatric surgery programs. *Pediatrics*. 2011;128(Suppl. 2):S65–S70.
830. Fried M, Yumuk V, Oppert JM, et al. Interdisciplinary European guidelines on metabolic and bariatric surgery. *Obes Surg*. 2014;24(1):42–55.
831. Kelly AS, Barlow SE, Rao G, et al. Severe obesity in children and adolescents: identification, associated health risks, and treatment approaches: a scientific statement from the American Heart Association. *Circulation*. 2013;128(15):1689–1712.
832. Pratt JSA, Browne A, Browne NT, et al. ASMBS pediatric metabolic and bariatric surgery guidelines, 2018. *Surg Obes Relat Dis*. 2018;14(7):882–901.

833. Olbers T, Beamish AJ, Gronowitz E, et al. Laparoscopic Roux-en-Y gastric bypass in adolescents with severe obesity (AMOS): a prospective, 5-year, Swedish nationwide study. *Lancet Diabetes Endocrinol.* 2017;5(3):174–183.
834. Inge TH, Courcoulas AP, Jenkins TM, et al. Weight loss and health status 3 years after bariatric surgery in adolescents. *N Engl J Med.* 2016;374(2):113–123.
835. Järholm K, Karlsson J, Olbers T, et al. Two-year trends in psychological outcomes after gastric bypass in adolescents with severe obesity. *Obesity* (Silver Spring, Md), 2015;23(10):1966–1972.
- 835a. Griggs CL, Perez NP, Goldstone RN, et al. National trends in the use of metabolic and bariatric surgery among pediatric patients with severe obesity. *JAMA Pediatr.* 2018;172(12):1191–1192.
836. Apovian CM, Baker C, Ludwig DS, et al. Best practice guidelines in pediatric/adolescent weight loss surgery. *Obes Res.* 2005;13:274–282.
837. Mun EC, Blackburn GL, Matthews JB. Current status of medical and surgical therapy for obesity. *Gastroenterology.* 2001;120:669–681.
838. Albaugh VL, Flynn CR, Tamboli RA, Abumrad NN. Recent advances in metabolic and bariatric surgery. *F1000Res.* 2016;5.
839. O'Brien PE, Sawyer SM, Laurie C, Brown WA, Skinner S, Veit F, et al. Laparoscopic adjustable gastric banding in severely obese adolescents: a randomized trial. *JAMA.* 2010;303(6):519–526.
840. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med.* 2002;346:1623–1630.
841. Boza C, Muñoz R, Salinas J, Gamboa C, Klaassen J, Escalona A, et al. Safety and efficacy of Roux-en-Y gastric bypass to treat type 2 diabetes mellitus in non-severely obese patients. *Obes Surg.* 2011;21:1330–1336.
842. Sjöström CD, Peltonen M, Sjöström L. Effects of 2 and 10 years weight loss retention on cardiovascular risk factors. *Int J Obes.* 2002;26(S218):865.
843. Shah M, Simha V, Garg A. Review: Long-term impact of bariatric surgery on body weight, co-morbidities, and nutritional status. *J Clin Endocrinol Metab.* 2006;91(11):4223–4231.
844. Inge TH, Jenkins TM, Zeller M, Dolan L, Daniels SR, Garcia VF, et al. Baseline BMI is a strong predictor of nadir BMI after adolescent gastric bypass. *J Pediatr.* 2010;156(1):103–108.
845. Towbin S, Inge TH, Garcia VF, et al. Beriberi after gastric bypass surgery in adolescence. *J Pediatr.* 2004;145:263–267.
846. Golomb I, Ben David M, Glass A, Kolitz T, Keidar A. Long-term metabolic effects of laparoscopic sleeve gastrectomy. *JAMA Surg.* 2015;150(11):1051–1057.
847. Mason EE, Scott DH, Doherty C, et al. Vertical banded gastroplasty in the severely obese under age twenty-one. *Obes Surg.* 1995;5:23–33.
848. Greenstein RJ, Rabner JG. Is adolescent gastric-restrictive anti-obesity surgery warranted? *Obes Surg.* 1995;5:138–144.
849. Breaux CW. Obesity surgery in children. *Obes Surg.* 1995;5:279–284.
850. Nguyen NT, Paya M, Stevens M, Mavandadi S, Zainabadi K, Wilson SE. The relationship between hospital volume and outcome in bariatric surgery at academic medical centers. *Ann Surg.* 2004;240(4):586–594.
851. Zingmond DS, McGory ML, Ko CY. Hospitalization before and after gastric bypass surgery. *JAMA.* 2005;294(15):1918–1924.
852. Inge TH, Krebs NF, Garcia VF, et al. Bariatric surgery for overweight adolescents: concerns and recommendations. *Pediatrics.* 2004;114(1):217–223.
853. Ray RM, Senders CW. Airway management in the obese child. *Pediatr Clin North Am.* 2001;48:1055–1063.
854. Strauss R. Perspectives on childhood obesity. *Curr Gastroenterol Rep.* 2002;4:244–250.
855. DeBoer MD, Marks DL. Therapy insight: use of melanocortin antagonists in the treatment of cachexia in chronic disease. *Nat Clin Pract Endo Metab.* 2006;2:459–466.
856. Walsh D, Nelson KA. Autonomic nervous system dysfunction in advanced cancer. *Support Care Cancer.* 2002;10(7):523–528.
857. Barber MD, Ross JA, Fearon KC. Disordered metabolic response with cancer and its management. *World J Surg.* 2000;24(6):681–689.
858. Gahagan S, Holmes R. A stepwise approach to evaluation of undernutrition and failure to thrive. *Ped Clin North Am.* 1998;45:169–187.
859. Krugman SD, Dubowitz H. Failure to thrive. *Am Fam Phys.* 2003;68(5):879–884.
860. Schmitt BD, Mauro RD. Non-organic failure to thrive: an outpatient approach. *Child Abuse Negl.* 1989;13:235–248.
861. Kuhnle U, Lewicka S, Fuller PJ. Endocrine disorders of sodium regulation. Role of adrenal steroids in genetic defects causing sodium loss or sodium retention. *Horm Res.* 2004;61(2):68–83.
862. Sills RH. Failure to thrive. The role of clinical and laboratory evaluation. *Am J Dis Child.* 1978;132:967–969.
863. Berwick DM, Levy JC, Kleinerman R. Failure to thrive: diagnostic yield of hospitalization. *Arch Dis Child.* 1982;57:347–351.
864. Gahagan S. Failure to thrive: a consequence of undernutrition. *Pediatr Rev.* 2006;27(1):e1–e11.
865. Gabay MP. Galactogogues: medications that induce lactation. *J Hum Lact.* 2002;18(3):274–279.
866. Maggioni A, Lifshitz F. Nutritional management of failure to thrive. *Ped Clin North Am.* 1995;42:791–810.
867. Hershkovitz E, Printzman L, Segev Y, Levy J, Phillip M. Zinc supplementation increases the level of serum insulin-like growth factor-I but does not promote growth in infants with nonorganic failure to thrive. *Horm Res.* 1999;52(4):200–204.
868. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. *N Engl J Med.* 1991; 325(10):687–694.
869. Shyh-Chang N. Metabolic changes during cancer cachexia pathogenesis. *Adv Exp Med Biol.* 2017;1026:233–249.
870. Davis MP. Anorexia and cachexia in cancer. In: Yeung S, Escalante C, Gagel RF, eds. *Internal Medicine Care of Cancer Patients.* New York: Decker; 2007.
871. Kelly JF, Elias CF, Lee CE, et al. Ciliary neurotrophic factor and leptin induce distinct patterns of immediate early gene expression in the brain. *Diabetes.* 2004;53(4):911–920.
872. Argiles JM, Busquets S, Lopez-Soriano FJ. The role of uncoupling proteins in pathophysiological states. *Biochem Biophys Res Comm.* 2002;293(4):1145–1152.
873. Patel HJ, Patel BM. TNF- α and cancer cachexia: Molecular insights and clinical implications. *Life Sci.* 2017;170:56–63.
874. Makino T, Noguchi Y, Yoshikawa T, Doi C, Nomura K. Circulating interleukin 6 concentrations and insulin resistance in patients with cancer. *Br J Surg.* 1998;85(12):1658–1662.
875. Yoshikawa T, Noguchi Y, Doi C, Makino T, Okamoto T, Matsumoto A. Insulin resistance is connected with the alterations of substrate utilization in patients with cancer. *Cancer Lett.* 1999;141:93–98.
876. Yoshikawa T, Noguchi Y, Doi C, Makino T, Nomura K. Insulin resistance in patients with cancer: relationships with tumor site, tumor stage, body-weight loss, acute-phase response, and energy expenditure. *Nutrition.* 2001;17:590–593.
877. Tayek JA, Katz J. Glucose production, recycling, Cori cycle, and gluconeogenesis in humans: relationship to serum cortisol. *Am J Physiol.* 1997;272:E476–E484.
878. Russell A. A diencephalic syndrome of emaciation in infancy and childhood. *Arch Dis Child.* 1951;26:338–343.
879. Fleischman A, Brue C, Poussaint TY, et al. Diencephalic syndrome: a cause of failure to thrive and a model of partial growth hormone resistance. *Pediatrics.* 2005;115:e742–e748.
880. Brauner R, Trivin C, Zerah M, et al. Diencephalic syndrome due to hypothalamic tumor: a model of the relationship between weight and puberty onset. *J Clin Endocrinol Metab.* 2006;91(7):2467–2473.
881. Vlachopapadopoulou E, Tracey KJ, Capella M, Gilker C, Matthews DE. Increased energy expenditure in a patient with diencephalic syndrome. *J Pediatr.* 1993;122(6):922–924.
882. Lindvall Dahlgren C, Wisting L, Ro O. Feeding and eating disorders in the DSM-5 era: a systematic review of prevalence rates in non-clinical male and female samples. *J Eat Disord.* 2017;5:56.
883. Johnson JG, Cohen P, Kasen S, Brook JS. Eating disorders during adolescence and the risk for physical and mental disorders during early adulthood. *Arch Gen Psychiatry.* 2002;59:545–552.
884. Rigotti NA, Neer RM, Skates SJ, Herzog DB, Nussbaum SR. The clinical course of osteoporosis in anorexia nervosa. A longitudinal study of cortical bone mass. *JAMA.* 1991;265(9):1133–1138.
885. Bulik CM, Sullivan PF, Fear JL, Pickering A, Dawn A, McCullin M. Fertility and reproduction in women with anorexia nervosa: a controlled study. *J Clin Psychiatry.* 1999;60(2):130–135.

886. Lambe EK, Katzman DK, Mikulis DJ, Kennedy SH, Zipursky RB. Cerebral gray matter volume deficits after weight recovery from anorexia nervosa. *Arch Gen Psychiatry*. 1997;54(6):537–542.
887. Strober M, Freeman R, Morrell W. The long-term course of severe anorexia nervosa in adolescents: survival analysis of recovery, relapse, and outcome predictors over 10–15 years in a prospective study. *Int J Eating Dis*. 1997;22(4):339–360.
888. Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: mechanisms and management. *Nat Rev Endocrinol*. 2017;13(3):174–186.
889. Tamai H, Mori K, Matsubayashi S, et al. Hypothalamic-pituitary-thyroidal dysfunctions in anorexia nervosa. *Psychother Psychosom*. 1986;46(3):127–131.
890. Gianotti L, Arvat E, Valetto MR, et al. Effects of beta-adrenergic agonists and antagonists on the growth hormone response to growth hormone-releasing hormone in anorexia nervosa. *Biol Psychiatry*. 1998;43(3):181–187.
891. Licinio J, Wong ML, Gold PW. The hypothalamic pituitary adrenal axis in anorexia nervosa. *Horm Res*. 1996;62:75–83.
892. Misra M, Klibanski A. Anorexia nervosa and osteoporosis. *Rev Endo Metab Dis*. 2006;7:91–99.
893. Misra M, Miller KK, Tsai P, et al. Elevated peptide YY levels in adolescent girls with anorexia nervosa. *J Clin Endocrinol Metab*. 2006;91(3):1027–1033.
894. Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med*. 2004;351(10):987–997.
895. Balligand JL, Brichard SM, Brichard V, Desager JP, Lambert M. Hypoleptinemia in patients with anorexia nervosa: loss of circadian rhythm and unresponsiveness to short-term refeeding. *Eur J Endocrinol*. 1998;138:415–420.
896. Monteleone P, Fabrazzo M, Tortorella A, Fuschino A, Maj M. Opposite modifications in circulating leptin and soluble leptin receptor across the eating disorder spectrum. *Mol Psychiatry*. 2002;7:641–646.
897. Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet*. 2005;366:74–85.
898. Brichard SM, Delporte ML, Lambert M. Adipocytokines in anorexia nervosa: a review focusing on leptin and adiponectin. *Horm Metab Res*. 2003;35(6):337–342.
899. Tagami T, Satoh N, Usui T, Yamada K, Shimatsu A, Kuzuya H. Adiponectin in anorexia nervosa and bulimia nervosa. *J Clin Endocrinol Metab*. 2004;89(4):1833–1837.
900. Bosity-Westphal A, Brabant G, Haas V, et al. Determinants of plasma adiponectin levels in patients with anorexia nervosa examined before and after weight gain. *Eur J Nutr*. 2005;44(6):355–359.
901. Association AP. Treatment of patients with eating disorders, third edition. *Am J Psychiatr*. 2006;163:4–54.
902. Klibanski A, Biller BM, Schoenfeld DA, Herzog DB, Saxe VC. The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. *J Clin Endocrinol Metab*. 1995;80(3):898–904.
903. Robinson E, Bachrach LK, Katzman DK. Use of hormone replacement therapy to reduce the risk of osteopenia in adolescent girls with anorexia nervosa. *J Adolesc Health*. 2000;26(5):343–348.
904. Lustig RH. Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the first law of thermodynamics. *Nat Clin Pract Endo Metab*. 2006;2:447–458.
905. Mietus-Snyder ML, Lustig RH. Childhood obesity: adrift in the “limbic triangle” *Ann Rev Med*. 2008;59:147–162.
906. Ross MG, Huber I, Desai M. Intrauterine growth restriction, small for gestational age, and experimental obesity. In: Lustig RH, ed. *Obesity Before Birth: Maternal and Prenatal Effects on the Offspring*. New York: Springer; 2010:215–239.
907. Lustig RH. Fructose: metabolic, hedonic, and societal parallels with ethanol. *J Am Diet Assoc*. 2010;110(9):1307–1321.

CHAPTER OUTLINE

INTRODUCTION	1004
METABOLISM	1004
Primary Dyslipidemias	1007
Disorders of Cholesterol Metabolism	1007
Disorders of Overproduction of Very High-Density Lipoprotein	1008
Disorders of Marked Hypertriglyceridemia	1008
HYPOLIPEMIAS	1008
Low High-Density Lipoprotein Cholesterol	1008
Abetalipoproteinemia	1008
Hypobetalipoproteinemia	1009
Loss-of-Function Mutations for PCSK9	1009
Disorders With Lipoprotein Clearance via ApoE Pathways	1009
SECONDARY CAUSES	1009
VASCULAR CHANGES AND DYSLIPEMIA	1010
SCREENING FOR LIPID DISORDERS	1011
Routine Screening	1011
Genetic Testing	1012
DIET THERAPY IN MANAGING DYSLIPEMIA	1012
PHARMACOLOGIC MANAGEMENT	1014
Bile Acid–Binding Agents	1014
HMG-CoA Reductase Inhibitors	1015
Inhibitors of Cholesterol Absorption	1015
Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors	1015
Fibric Acid Derivatives	1016
Niacin	1016
Dietary Additives and Supplements	1016
Low-Density Lipoprotein Apheresis	1016
CONCLUSIONS AND FUTURE DIRECTIONS	1016
References	1017

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is a major cause of morbidity and mortality among adults in industrialized countries. Dyslipidemia (specifically elevated low-density lipoprotein [LDL] cholesterol, low high-density lipoprotein [HDL] cholesterol, and high non-HDL cholesterol and triglycerides [TGs]) has been identified as an independent risk factor in the development of ASCVD. There is strong evidence that lipoprotein levels track from childhood into adulthood and that abnormal levels of LDL cholesterol and perhaps other lipoproteins are associated with atherosclerosis, and therefore with related adverse outcomes.

This chapter reviews the evidence for the role of lipid abnormalities in the early natural history of atherosclerosis. In addition, a general overview of lipoprotein metabolism is

provided—followed by a review of genetic disorders in the metabolism of lipoproteins. Secondary causes of high cholesterol are explained, including the increasing prevalence of obesity and metabolic syndrome, as a cause of lipid abnormalities in the pediatric population. Standards and approaches to screening for hyperlipidemia in children are reviewed, as well as current approaches to the dietary and pharmacologic management of pediatric lipid disorders.

METABOLISM

Lipid disorders in children and adolescents can result from defects in the production, transport, or degradation of lipoproteins. To understand the diverse causes of lipoprotein abnormalities, a brief review of lipoprotein structure, function, and metabolism is provided. Table 25.1 summarizes the lipoprotein subclasses, the source of each one, and the constituent lipids and apolipoproteins associated with each particle.

TGs, cholesterol esters, phospholipids, and plant sterols within food post-ingestion are digested to fatty acids, 2-monoglycerides, lysophospholipids, unesterified cholesterol, and plant sterols. Absorption of these digestive end products occurs through two mechanisms: passive diffusion and carrier-mediated transport. In passive diffusion, nonpolar lipids are solubilized with the aid of bile acids and lysophospholipids into mixed micelles that can diffuse through the apical surface of the enteric membrane. Carrier-mediated transport involves several different transport proteins for fatty acids and sterols. CD 36/scavenger receptor B2 (SR-B2), a fatty acid translocase, promotes long-chain fatty acid and cholesterol absorption in the proximal small intestine.¹ At least two additional transporters, Niemann-Pick C1-like 1 protein (NPC1L1) and SR-B1, play a role in sterol uptake.^{2,3} As such, NPC1L1 and SR-B1 are targets for the cholesterol-lowering medication ezetimibe, a potent inhibitor of cholesterol and plant sterol absorption.

Most of the plant sterols ingested and about half of the absorbed cholesterol are excreted from the intestinal cell back into the lumen by two adenosine triphosphate (ATP)-binding cassette (ABC) half-transporters, G5 and G8, thus limiting the amount of sterols that are absorbed.^{4,5} A rare mutation of either ABCG5 or ABCG8, known as *sitosterolemia*, results in abnormally high plant sterol levels in plasma and tissues and deposition of sterols in the skin and arteries. Individuals with this disorder are at an increased risk of premature atherosclerosis.⁶ Sterols that remain in the enterocyte are converted to sterol esters by acyl-CoA cholesterol acyl transferase (ACAT), which attaches a fatty acid to the sterol for storage within the cytoplasm of the cell. Within the enterocyte, lipids are aggregated into lipoproteins through the action of a chaperone protein, microsomal triglyceride transfer protein (MTTP), and perhaps several additional proteins.⁷ MTTP conjugates TGs, phospholipids, cholesterol, and cholesterol ester with apolipoprotein B-48 (apoB-48) on the luminal side of the endoplasmic reticulum (ER) membrane to create a mature chylomicron.⁷ A similar process is used to aggregate TG, phospholipids, cholesterol, and cholesterol ester with apoB-100 in the liver to form very low-density lipoprotein (VLDL) particles. In the genetic disorder abetalipoproteinemia, mutations in the gene encoding MTTP result in an inability to produce chylomicrons and VLDL,

TABLE 25.1 Lipoprotein Subclasses and Associated Apolipoproteins and Lipid Constituents

Lipoprotein	Apolipoprotein	Source	Lipid Constituents
Chylomicrons	ApoB-48, apoC-II, ^a apoC-III, apoE ^a	Intestine	Dietary triglycerides
VLDL	ApoB-100, C-II, ^a C-III, ^a apoE ^a	Liver	Endogenous cholesterol and triglyceride
IDL	ApoB-100, apoE	VLDL metabolism	Cholesterol and triglyceride
LDL	ApoB-100	VLDL metabolism	Cholesterol
HDL	ApoA-I, apoA-II, apoC-II, apoE	Liver and intestine	Cholesterol and phospholipid

^aTransferred from HDL.

HDL, High-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

suggesting the essential nature of MTP in chylomicron and VLDL biogenesis.⁷ The recently approved drug lomitapide inhibits MTP, reducing lipoprotein assembly and secretion, and lowers plasma cholesterol by around 50% in patients with homozygous familial hypercholesterolemia.⁸

Chylomicrons once formed are too large to penetrate the capillary membrane. Consequently, they are secreted into the lymphatic system and enter the venous plasma compartment through the thoracic lymph duct. As the nascent particles are released into the plasma, several apolipoproteins (including apoC-II, C-III, and apoE) are preferentially transferred to the chylomicrons from circulating HDLs.⁹ Fig. 25.1 depicts chylomicron metabolism.

Chylomicrons transport dietary TG and cholesterol to sites of storage or metabolism.¹⁰ The size of the particles varies depending on the amount of fat ingested. They are rapidly cleared from the circulation through the action of lipoprotein lipase (LPL). LPL is a TG hydrolase found on the capillary endothelium of various tissues, with its highest concentration in muscle and adipose tissues.¹¹ Glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1) anchors LPL to the capillary endothelium.¹² LPL is activated by apoC-II and inhibited by apoC-III on the chylomicron. Loss-of-function mutations in LPL, apoC-II, and GPIHBP1 can result in marked

hypertriglyceridemia. Loss-of-function mutations in apoC-III are associated with increased LPL activity and decreased TG levels.^{12,13} As the TG contained within the chylomicron is hydrolyzed, free fatty acids are liberated for oxidation via a variety of cell types and the particle decreases in size. When approximately 80% of the initial TG has been removed, apoC-II dissociates from its surface.¹¹ The transfer of apoC-II from chylomicrons to HDL decreases the ability of LPL to further breakdown TGs. The TG-depleted chylomicrons, now considered chylomicron remnants, are taken up by the liver through the LDL receptor (LDLR), a receptor that recognizes apoE on the chylomicron surface and apoB100 on the surface of liver-derived lipoproteins.⁹ A smaller fraction of remnants may also be internalized via an LDLR-related protein-1 (LRP1)-mediated endocytosis.^{14,15}

VLDLs originate from the liver, and like chylomicrons they are TG-rich particles (Fig. 25.2). In contrast to the intestinally derived chylomicrons, the fatty acids contained within the VLDL TG come from de novo synthesis from dietary carbohydrate, lipoprotein remnants, or circulating fatty acids, internalized by the liver from plasma.⁹ Similar to chylomicrons, the size of the VLDL particles can vary depending on the quantity of the TG carried in the particle. When TG production in the liver is increased, the secreted VLDL particles are large. Within the hepatocyte, TG and cholesterol ester are assembled by an MTP and surrounded with a phospholipid membrane associated with apoB-100.⁸ The mature VLDL particles are released into the lymph and ultimately into the vascular space, where other apolipoproteins (including apoC-II, apoC-III, and apoE) adsorb to the VLDL surface. The metabolism of the VLDL particle follows a route similar to that of the chylomicron: apoC-II on its surface activates LPL, LPL hydrolyzes the VLDL TG, free fatty acids are liberated, the particle decreases in size (after an 80% loss of TG), and ultimately apoC-II dissociates—resulting in the formation of VLDL remnants (also known as *intermediate-density lipoproteins* [IDLs]). Approximately half of the IDL is then removed from plasma through the interaction of apoE with the LDLR and LRP1 on the surface of liver cells.⁹ The rest of the IDL is converted to LDL through further hydrolysis of TGs and phospholipids by hepatic TG lipase (HL).¹⁶ ApoE is transferred from IDL to HDL during the transition of the remnant to LDL.¹²

LDL, the major carrier of cholesterol in plasma, is taken up into peripheral tissues and liver cells by the LDLR assisted by an adaptor protein (AP). The AP binds to the LDLR and clathrin, suggesting a role for AP in the recruitment and retention of LDLR in clathrin-coated pits.¹⁷ Upon receptor binding, the LDL particle

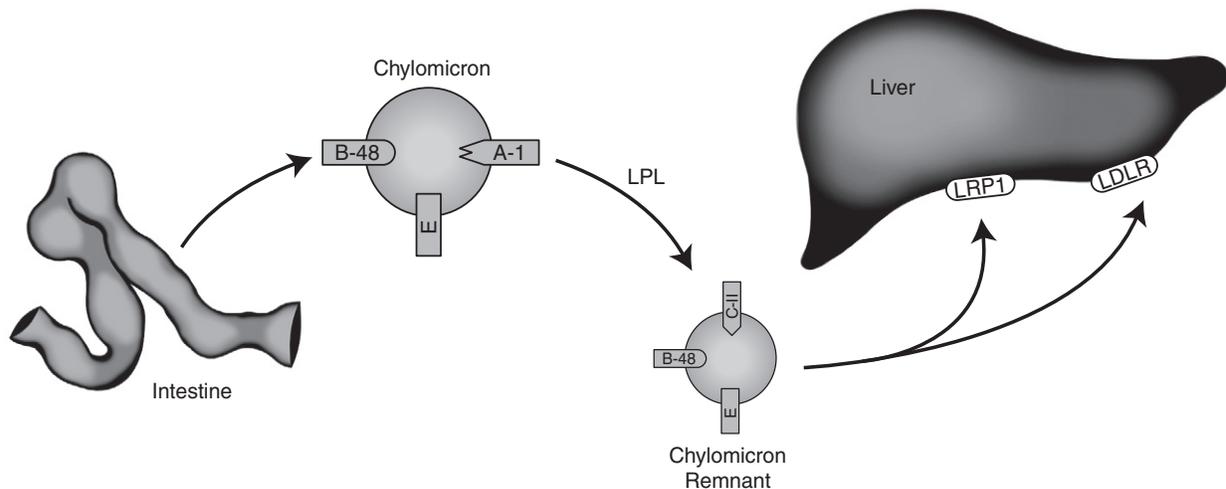


Fig. 25.1 Endogenous lipoprotein metabolism. See text for details. A-1, Apolipoprotein A1; B-48, apolipoprotein B-48; C-II, apolipoprotein C2; E, apolipoprotein E; LDLR, low-density lipoprotein; LPL, lipoprotein lipase; LRP1, low-density lipoprotein receptor-related protein 1. (Courtesy Emilie Graham, University of Cincinnati.)

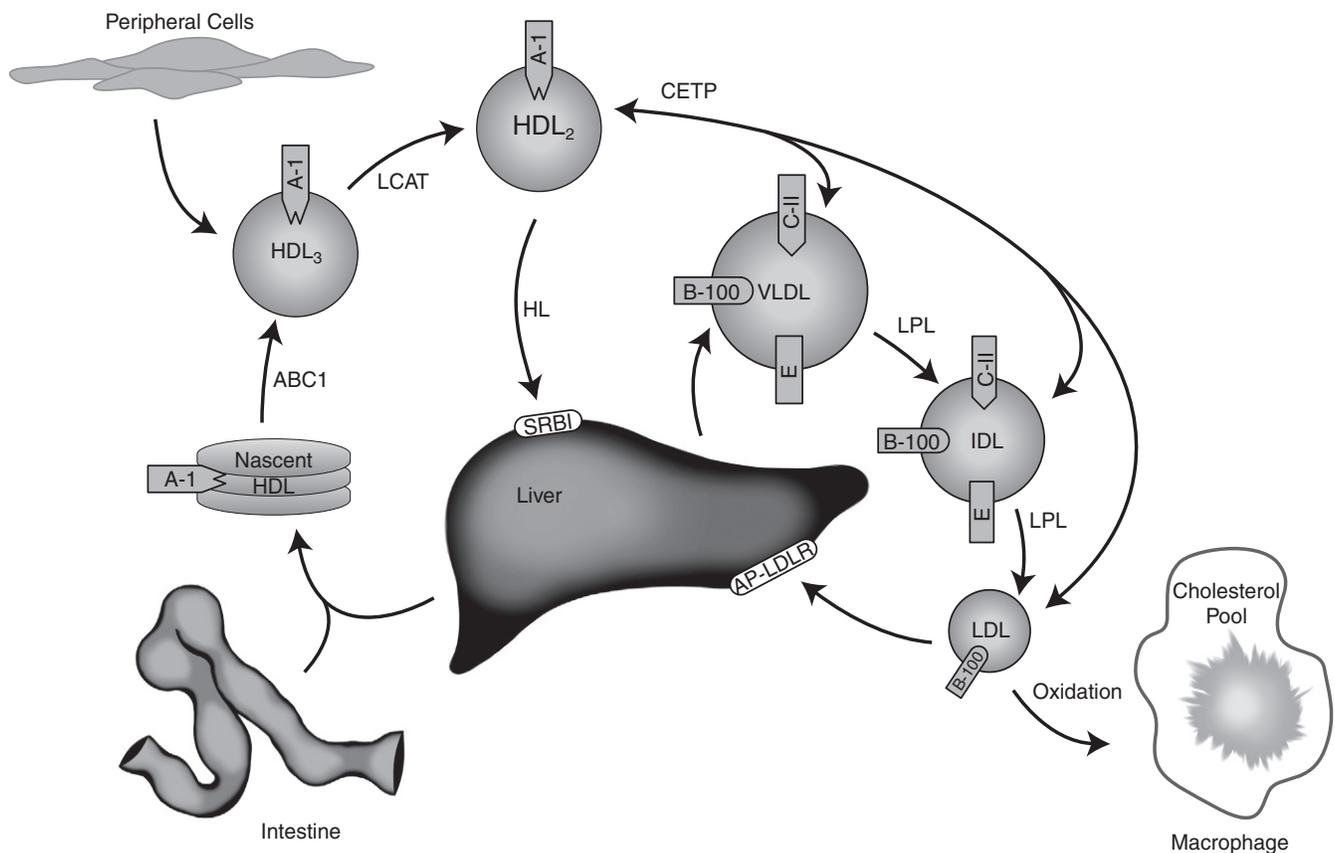


Fig. 25.2 Exogenous lipoprotein metabolism. See text for details. A-1, Apolipoprotein A-1; ABC1, ATP binding cassette transporter A1; AP-LDLR, adaptor protein-low-density lipoprotein receptor; B-100, apolipoprotein B100; C-II, apolipoprotein C2; CETP, cholesterol ester transfer protein; E, apolipoprotein E; HDL, high-density lipoprotein; HL, hepatic triglyceride lipase; LCAT, lecithin cholesteryl acyl transferase; LPL, lipoprotein lipase; SRBI, scavenger receptor beta 1. (Courtesy Emilie Graham, University of Cincinnati.)

bound to LDLR/AP is rapidly internalized into clathrin-coated pits by endocytosis. Within the cell, the newly formed endosome becomes acidified through the action of an ATP-dependent proton pump.¹⁸ Acidification causes degradation of the clathrin coat, dissociation of the LDLR from LDL, and subdivision of the endosomal membranes. The endosome containing the LDLR recirculates back to the cell membrane for additional LDL uptake. Alternatively, proprotein convertase subtilisin/kexin type 9 (PCSK9) binds LDLR, and short-circuits recycling of LDLR from the endosome, leading to its degradation.¹⁹ The remaining LDL-containing endosome fuses with a lysosome, where hydrolytic enzymes digest the lipoprotein into its component parts: unesterified cholesterol, fatty acids, and free amino acids.¹⁸

The amount of cholesterol released from endosomal uptake regulates hepatic synthesis of LDLR and cholesterol. When cellular concentration of cholesterol is low, sterol receptor binding proteins (SREBPs) move from the ER to the Golgi, where proteases cleave SREBPs into active transcription factors. SREBPs translocate to the nucleus, where they stimulate the transcription of LDLR and hydroxymethylglutaryl (HMG) CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis.²⁰ If cholesterol levels in the cell are high, SREBPs remains in the ER in an inactive form and do not stimulate LDLR synthesis. In this way, intracellular hepatic cholesterol concentration regulates the amount of cholesterol internalized and synthesized by the cell.

When excess LDL and other small apoB-containing lipoproteins (chylomicron remnants and IDL) are present in the plasma, the capacity of the LDLR to remove them is exceeded and these particles become more susceptible to oxidation. Oxidized apoB-containing lipoproteins can be taken up by scavenger receptors

on macrophages in the subendothelium of arteries and may contribute to the formation of atherosclerotic lesions.²¹

HDL transfers cholesterol and other lipids from peripheral tissues (including arterial atheroma) back to the liver. The particles are synthesized predominantly in the liver (and to a lesser extent in the intestine) as lipid-poor precursor particles (pre-beta HDL) containing apoA-I (see Fig. 25.2).⁹ Nascent HDL interacts with the plasma membrane of cells, collecting lipid through an ABCA1 mechanism.^{5,7} The cholesterol and phospholipids transferred through this process adsorb to the HDL, forming a disk-shaped particle referred to as HDL₃. Dysfunction of ABCA1 will significantly decrease HDL levels and thereby dramatically impair cholesterol and lipid transport functions.⁹ A rare autosomal recessive disorder called *Tangier disease* is caused by lack of functional ABCA1 protein and is characterized by an absence of HDL along with hypertriglyceridemia and low LDL levels. Within the plasma, HDL₃ interacts with the enzyme lecithin cholesterol acyl transferase (LCAT)—which catalyzes the esterification of particle-associated cholesterol. ApoA-I on the HDL surface activates LCAT. Once formed, the cholesterol ester is more hydrophobic and moves to the interior of the particle—creating a sphere-shaped HDL particle known as HDL₂.²²

As HDL₂ increases in size, the particle becomes substrate for cholesterol ester transfer protein (CETP). This enzyme promotes the exchange of esterified cholesterol within HDL₂ for TG contained within apoB-100-associated lipoproteins.²³ This lipid exchange is the primary mechanism whereby HDL participates in reverse cholesterol transport from tissues back to the liver. The rest of the cholesterol ester is selectively taken up from HDL by hepatocytes via a SR-B1, without concomitant uptake

TABLE 25.2 Pediatric Lipoprotein Disorders

Lipoprotein Disorder	Lipoprotein Analysis	Blood Lipids	Genetic Defect
Familial hypercholesterolemia Autosomal recessive	↑↑LDL	↑↑Cholesterol	LDL receptor (<i>LDLR</i>)
hypercholesterolemia Autosomal dominant	↑↑LDL	↑↑ Cholesterol	<i>LDLRAP</i>
hypercholesterolemia	↑↑LDL (with increase in function mutations)	↑↑ Cholesterol	<i>PCSK9</i>
Familial ligand-defective apoB-100	↑↑ LDL	↑↑Cholesterol	ApoB-100
Sitosterolemia	↑ LDL	↑ Cholesterol	<i>ABCG5</i> or <i>ABCG8</i>
Familial combined hyperlipidemia	↑ VLDL, ↑ LDL, ↓ HDL	↑ Cholesterol, ↑ triglycerides	Unknown
Familial hypertriglyceridemia	↑↑ VLDL, ↓ HDL	↑ Triglycerides	Unknown
Familial chylomicronemia syndrome	↑↑ Chylomicrons ↑ VLDL	↑↑Triglycerides	Lipoprotein lipase (<i>LPL</i>), <i>ApoC-II</i> , <i>Apo A-V</i> , <i>GP1HBP1</i>
Hypoalphalipoproteinemia	↓ HDL	Normal	<i>ApoA-1</i>
Dysbetalipoproteinemia	↑↑ Chylomicron remnants, ↑↑ IDL	↑↑ Cholesterol, ↑↑ triglycerides	ApoE

↑↑ Very high; ↑ moderately elevated; and ↓ decreased.

HDL, High-density lipoprotein; *IDL*, intermediate-density lipoprotein; *LDL*, low-density lipoprotein; *VLDL*, very low-density lipoprotein. See text for details.

of the entire HDL particle. This latter process may require the action of HL.²⁴ The lipid-poor pre-beta HDL resulting from this process is released for recycling.¹⁷

Primary Dyslipidemias

Lipoprotein synthesis, transport, and metabolism occur in many steps and involve many specialized proteins. A number of genetic defects have been identified in these processes and are referred to as *primary dyslipidemias*. Most of these genetic defects present in childhood. Table 25.2 summarizes pediatric lipoprotein disorders with reference to the characteristic lipoprotein profile of each one. The genetic and metabolic etiologies of these disorders are detailed in the following material.

Disorders of Cholesterol Metabolism

Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is the most common single gene disorder of lipoprotein metabolism. FH is inherited as an autosomal-dominant trait with relatively low prevalence in Western countries. The prevalence has been reported to be 10 times higher in certain populations with a presumed founder effect, such as the Lebanese, the French Canadians, and the South Afrikaners.²⁵ The heterozygous form is found in one in 250 persons, and the homozygous form is found in one in 1 million persons.²⁶ The disorder is caused by a mutation in the *LDLR* gene.²⁷ More than 1200 mutations in this gene have been identified, including those that affect receptor synthesis, intracellular transport, ligand binding, internalization, and recycling.²⁸ In the heterozygous form, inheritance of one defective *LDLR* gene results in plasma LDL cholesterol levels 2 to 3 times higher than normal.²⁷ TG and HDL cholesterol levels are usually unaffected by FH-causing gene mutations, but may be altered by obesity and insulin resistance.²⁸

Individuals with heterozygous FH are at an increased risk of developing early-onset ASCVD, usually between the ages of 30 and 60 years.²⁵ In the homozygous form, individuals inherit a mutant allele for FH from both parents, resulting in plasma LDL cholesterol concentrations that are 4 to 6 times higher than normal.²⁹ A more severe phenotype is found in individuals with receptor-negative mutations (those with 5% residual LDL receptor activity) compared with those with receptor-defective mutations (5%–30% of normal LDL receptor activity).³⁰ Because of the excessively high plasma cholesterol levels in individuals with homozygous FH, cholesterol deposits

are common in the tendons (xanthomas) and eyelids (xanthelasmas)—generally by the age of 5 years.³¹ In the heterozygous form, xanthomas occur less frequently and generally not until one reaches older adulthood. Children with homozygous FH have early-onset atherosclerosis and often have myocardial infarction in the first decade of life, and death from ASCVD in the second decade.³¹

Autosomal-Dominant and Autosomal-Recessive Hypercholesterolemia

Autosomal-dominant hypercholesterolemia (ADH) is another inherited disorder resulting in a phenotype that is expressed as marked elevations or low levels of LDL cholesterol. ADH is caused by mutations in a serine protease, *PCSK9*.³² This protein binds and favors degradation of the *LDLR* and thereby modulates the plasma levels of LDL cholesterol. Some of the naturally occurring *PCSK9* mutations result in an increase in the function of the protein and cause hypercholesterolemia by increasing the degradation of *LDLR*, whereas other mutations result in a loss of function, and hence increase in *LDLR* abundance, and are associated with low LDL cholesterol. The latter mutations appear to confer protection from developing ASCVD.

Autosomal recessive hypercholesterolemia (ARH) is caused by mutations in the *ARH* gene, which encodes the adaptor protein required for normal *LDLR*-mediated endocytosis in hepatocytes.³³ Several different mutations in this protein have been identified, all leading to a lack of or suboptimal internalization of the *LDLR*.³⁴ Cholesterol levels in individuals with ARH are 5 to 6 times higher than normal. Children with this disorder are clinically similar to those with homozygous FH. However, their parents usually have normal lipoprotein profiles.³³

Familial Ligand-Defective ApoB-100

Familial ligand-defective apoB-100 (FDB) is a monogenic disorder that clinically resembles heterozygous FH. The disease is characterized by moderate to markedly high plasma LDL cholesterol levels, normal TGs, and tendon xanthomas. The disorder is caused by poor binding of the LDL particle to the *LDLR*, because of a mutation in apoB-100.³⁵ Specifically two mutations, R3500Q and R3500W remain the most frequently identified mutations that cause FDB.³⁶ Deficient *LDLR* binding results in a decreased clearance of LDL from plasma. The disorder is most common in individuals of European descent (one per 1000).⁹ Patients with FDB are at moderate to high risk of developing ASCVD.³⁷

Sitosterolemia

Sitosterolemia is a rare autosomal-recessive disease caused by a mutation in either of two genes (*ABCG5* or *ABCG8*) encoding the ABC half-transporters.³⁸ These genes are expressed in enterocytes and hepatocytes. The ABC half-transporters limit the absorption of cholesterol and plant sterols (and possibly shellfish sterols) in the gut. They also promote biliary and fecal excretion of cholesterol and phytosterols.^{39,40} Defective proteins result in an abnormally high absorption of plant sterols (and, to a lesser extent, cholesterol) into the enterocyte, and decreased excretion of these sterols from the liver into the bile. Plasma cholesterol can be mildly, moderately, or markedly elevated, whereas plant sterol concentrations in the plasma are markedly increased. Patients with sitosterolemia develop premature ASCVD and xanthomas in childhood, and may develop aortic stenosis.³⁸

Disorders of Overproduction of Very Low-Density Lipoprotein

Familial combined hyperlipidemia (FCHL) is an autosomal-dominant disorder with a prevalence of 1% to 2% in Western populations.⁴¹ There is overlap in the lipid phenotype between FCHL and combined dyslipidemia (CD) of obesity, which likely has genetic underpinnings but is primarily influenced by lifestyle factors. CD is highly prevalent in youth, occurring in 30% to 60% of obese children and adolescents.⁴² Individuals with FCHL and CD generally share the same metabolic defect, which is overproduction of hepatic VLDL. Families with FCHL have multiple patterns of hyperlipidemia, including hypercholesterolemia, hypertriglyceridemia, and elevated apoB levels. A diagnosis of FCHL is based on the presence of increased levels of cholesterol, TG, or apoB in patients and their first-degree relatives.⁴¹ Veerkamp and colleagues have developed a nomogram to calculate the probability that a person is likely to be affected by FCHL.⁴³ FCHL can manifest in childhood, but is usually not fully expressed until adulthood.⁴¹ Patients with FCHL and CD often have concurrent problems with insulin resistance, central obesity, and hypertension and are at an increased risk of premature ASCVD.⁴⁴

Syndromes with a similar phenotype to FCHL and CD are hyperapobeta-lipoproteinemia, LDL subclass pattern B, and the clustering of ASCVD risk factors known as *metabolic syndrome in adults*.⁴¹ Of the three, the latter syndrome is much more prevalent in children. Rates of metabolic syndrome are continuing to rise with the prevalence of obesity in the pediatric population.⁴⁵ There appears to be a mechanistic link between central obesity, insulin resistance, and dyslipidemia—with central obesity generally preceding both glucose and lipid abnormalities. Currently, there is no agreed upon definition for metabolic syndrome in childhood.⁴⁶

Disorders of Marked Hypertriglyceridemia

Familial Hypertriglyceridemia

Familial hypertriglyceridemia (FHTG) follows an autosomal-dominant inheritance pattern expressed predominantly in adulthood, with a population prevalence of around 5% to 10%.⁴¹ The prevalence in children is increasing. Obesity is an important factor that can expedite the expression of FHTG, and patients often have concurrent glucose intolerance. The phenotype for FHTG is moderate to markedly high serum TGs (200–500 mg/dL range) and low to normal LDL and HDL cholesterol levels. The metabolic cause of the disorder is hepatic secretion of large TG-rich VLDL particles that are catabolized slowly.⁴⁷ The fundamental genetic defect for FHTG has not been identified.

Familial Chylomicronemia Syndrome

Chylomicronemia syndrome is a compilation of rare monogenetic disorders that cause marked impairment of LPL activity. These disorders are phenotypically expressed as hypertriglyceridemia (usually TGs >1000 mg/dL), because of diminished or absent hydrolysis of chylomicron and VLDL-associated TGs by LPL.⁴³ The estimated prevalence is one in 500,000 to 1,000,000.⁴⁸ Impairment of LPL activity may be related to LPL deficiency, apoC-II (cofactor for LPL) deficiency, or the more recently described apoA5 and GPIHBP1 loss-of-function mutations that result in poor hydrolysis of chylomicron and VLDL-associated TGs.^{49,50}

In homozygous chylomicronemia, fasting plasma has a viscous, creamy appearance because of the presence of large numbers of chylomicron particles. Risks for pancreatitis and hepatosplenomegaly are increased because of the markedly elevated serum TGs.⁴⁹ In addition, eruptive xanthomas and neurologic symptoms may be apparent. Individuals heterozygous for the syndrome may have a mild to moderate elevation in plasma TGs that can range from 200 to 750 mg/dL. Environmental factors, such as weight gain, may exacerbate hypertriglyceridemia. Premature cardiovascular disease (CVD) is generally not a feature of chylomicronemia, but cases have been reported.⁵⁰

HYPOLIPIDEMIAS

Low High-Density Lipoprotein Cholesterol

In clinical practice, patients with low HDL cholesterol levels commonly have concurrent high TGs, with or without elevations in small dense LDL cholesterol.⁴¹ These patients are usually obese, and the mechanistic explanation for this dyslipidemic triad is VLDL overproduction. Less common are familial disorders of HDL, including familial hypoalphalipoproteinemia, mutations of the apoA-1 protein, Tangier disease, and LCAT deficiency.⁵¹ These disorders are characterized by a low HDL cholesterol level, with no other lipid abnormality. Familial hypoalphalipoproteinemia follows an autosomal-dominant inheritance pattern.⁵² ApoA-1 levels are also often low because of decreased production of HDL.

A number of mutations have been described in the *apoA-1* gene and are associated with low HDL cholesterol and low apoA-1.^{51,53} Tangier disease is caused by mutations in the *ABCA1* gene.⁵³ Patients affected by this disease are not able to actively withdraw cholesterol from cells onto nascent HDL particles, causing rapid degradation of the nascent HDL. ApoA-1 is rapidly cleared before it is able to acquire cholesterol. In Tangier disease, HDL cholesterol levels are close to zero and the apoA-1 levels are less than 5 mg/dL. The risk of premature ASCVD in these patients is mild to moderate.^{52,53} LCAT deficiency is a very rare autosomal recessive disorder caused by mutations in LCAT, an enzyme synthesized by the liver and secreted into the plasma, where it associates with lipoproteins.⁵⁴ LCAT esterifies free cholesterol on the surface of HDL and enables the accumulation of cholesteryl esters in the core of HDL. In LCAT deficiency, lack of normal cholesterol esterification impairs formation of mature HDL particles, which are readily catabolized along with apoA-1. Remarkably, despite the extremely low levels of plasma HDL cholesterol (usually <10 mg/dL) and apoA-1, premature CVD is not a consistent feature of this disorder.

Abetalipoproteinemia

Abetalipoproteinemia is associated with low serum cholesterol (<50 mg/dL) and TGs (~2–45 mg/dL). Patients with this disorder present with steatorrhea and fatty liver. Without

treatment, ataxia follows (with acanthocytosis and retinitis pigmentosa). Abetalipoproteinemia is caused by a defect in MTP.⁵⁵ Without MTP, no chylomicrons, VLDL, or LDL appear in the plasma. In these patients, HDL takes over as the primary cholesterol carrier. Thus the defect is not fatal because of significant fat malabsorption, fat-soluble vitamin status is impaired.

In particular, because vitamin E absorption and cellular uptake require chylomicron and LDL transport, high doses of vitamin E are required to prevent retinal and sensory neuron degeneration. Additional dietary considerations include restricting long-chain dietary TGs to less than 15 g/day to alleviate the steatorrhea. Medium-chain triglycerides (MCT oils) can be used as an alternative source of energy.⁵⁰

Hypobetalipoproteinemia

Hypobetalipoproteinemia is an autosomal-dominant disorder resulting from a defect in the *apoB* gene that produces a truncated apolipoprotein B.⁵⁶ Cholesterol levels in patients with heterozygous hypobetalipoproteinemia are usually 50% of those of an unaffected family member. The heterozygous form of this condition is benign. However, homozygous hypobetalipoproteinemia is associated with severe hypocholesterolemia, significant steatorrhea, fatty liver, acanthocytosis retinopathy, and peripheral neuropathy.⁵⁷

Loss-of-Function Mutations for PCSK9

PCSK9 loss-of-function mutations are associated with very low LDL-cholesterol levels and reduced ASCVD risk (see section on Autosomal-Dominant and Autosomal-Recessive Hypercholesterolemia for mechanism for low LDL-cholesterol level). These mutations are found in approximately 2% of the population.⁵⁸ Compared with noncarriers, heterozygous carriers have a 28% reduction in LDL-C levels and an 88% reduction in ASCVD risk,⁵⁹ and may also protect carriers from myocardial infarction. Preventing PCSK9-mediated LDLR degradation with monoclonal antibodies is a novel strategy for LDL-cholesterol lowering in patients with severe forms of hypercholesterolemia.⁵⁹

Disorders With Lipoprotein Clearance via ApoE Pathways

Dysbetalipoproteinemia is characterized by elevated cholesterol and TG levels.⁴¹ The disorder results from the presence of a polymorphism of the *apoE* allele (*apoE2*, rather than the more common *apoE3* or less common *apoE4*).⁶⁰ Metabolically, this defect results in a poor uptake of remnant particles and abnormal remnant catabolism because of the abnormal *apoE*. Increased remnants, VLDL, chylomicrons, and apoE are all present. Xanthomas may occur, and premature ASCVD has been reported. This lipoprotein disorder is rare in children and often presents in young adulthood.⁴¹

SECONDARY CAUSES

Secondary dyslipidemias can result from a variety of diseases and conditions (Box 25.1). In the United States, the most prevalent cause of secondary dyslipidemia is overweight and obesity.⁶¹ The dyslipidemic triad (namely, elevated TGs and small dense LDL and low HDL cholesterol) is commonly associated with overweight (in particular, with central adiposity).^{61,62} In addition to dyslipidemia, insulin resistance and elevated blood pressure may be present. This cluster of abnormalities is known in adults as the *metabolic syndrome*. Empirical evidence in children also indicates that obesity during

BOX 25.1 Selected Secondary Causes of Pediatric Hyperlipoproteinemia

ENDOCRINE

- Hypothyroidism
- Diabetes
- Pregnancy

EXOGENOUS

- Drugs
- Obesity
- Alcohol

RENAL

- Nephrotic syndrome
- Chronic renal failure

HEPATIC

- Cholestasis
- Biliary atresia
- Hepatitis
- Biliary cirrhosis

IMMUNOLOGIC

- Human immunodeficiency virus infection/acquired immunodeficiency syndrome

childhood is associated with the same risk factor clustering seen in adults, that it continues into adult life, and that it is associated with an increased risk for accelerated early atherosclerosis.⁶³ The primary approach to treating this disorder in both adults and children is weight management. Improvement in weight status and a decrease in body fatness have been shown to be associated with improvements in the dyslipidemia and other comorbidities associated with obesity.⁶⁴

Metabolic lipid perturbations in adult patients with types 1 and 2 diabetes mellitus are similar to those found in patients with the metabolic syndrome, but often are more severe.⁶⁵ In general, in adults with diabetes, TGs are elevated and HDL cholesterol is low—and LDL cholesterol can be normal, mildly, or moderately elevated. Diabetes in adults is considered an ASCVD risk equivalent according to the National Cholesterol Education Program (NCEP). This means that the risk for developing ASCVD in patients with poorly controlled diabetes is equivalent to those with established ASCVD.⁶⁶ For this reason, the NCEP recommends aggressive treatment of dyslipidemia in adult patients with diabetes.

Although type 1 diabetes is currently the main form of diabetes seen in children, in the United States a growing number of patients with type 2 diabetes are under the age of 18 years.⁶⁷ Change in the prevalence of type 2 diabetes in youth is likely related to the growing obesity epidemic occurring in the pediatric population.^{67,68} Data on lipid concentrations in children and adolescents with diabetes are few, particularly in those with type 2 diabetes.

The Search for Diabetes in Youth Study assessed the prevalence of serum lipid abnormalities among a representative sample of US children and adolescents with type 1 and type 2 diabetes.⁶⁹ Findings from this study showed a substantial number of diabetic children over the age of 10 years with abnormal serum lipids: nearly 50% had an LDL cholesterol level above the optimal level of 100 mg/dL. For children with type 2 diabetes, 37% had elevated TG levels and 44% had low HDL cholesterol. These data highlight the importance of serum lipid screening in children with diabetes. A growing body of literature also shows early vascular dysfunction in children with diabetes, regardless of type.⁷⁰ This is thought to be caused by

glycemic and lipid abnormalities associated with poorly managed diabetes. For this reason, new treatment guidelines recommend intensive glucose and lipid management for children with diabetes.⁷¹ These guidelines are discussed later in the chapter.

Other causes of secondary dyslipidemia include hypothyroidism, nephrotic syndrome, other renal diseases, liver diseases, and infection.¹⁰ The risk of development of atherosclerosis with these conditions is unknown but is likely proportionate to the length of exposure and extent of elevation in serum LDL cholesterol levels. CVD is common in patients with chronic renal insufficiency.⁷² Dyslipidemias can also result from the ingestion of a variety of medications. These medications include progestins, estrogens, androgens, anabolic steroids, corticosteroids, cyclosporine, and retinoids. Secondary causes of dyslipidemias should be identified by patient historical data and a careful physical examination.¹⁰ Laboratory tests (including thyroid, renal, and liver function panels) can confirm the diagnosis.

The treatment of dyslipidemia in patients with secondary causes is focused on managing the underlying disease. Diet and physical activity changes may also be recommended to reduce elevated LDL cholesterol and TG levels.

VASCULAR CHANGES AND DYSLIPIDEMIA

It is well established that elevated concentrations of total cholesterol and LDL cholesterol in adult life are strong and reversible risk factors for ASCVD.⁶⁶ Whether dyslipidemia during childhood contributes to atherosclerotic lesions in coronary and other arteries has been a subject of debate, but accumulating evidence from pathology and in vivo imaging studies favors a relationship. Atherosclerotic lesions result from deposits of lipid and cholesterol in the intima of the arterial wall.⁷³ Early lesions, called *fatty streaks*, are formed from the accumulation of macrophages filled with lipid droplets (foam cells).

Fatty streaks do not disorganize the normal structure of the intima, do not deform or obstruct the artery, and are in and of themselves not considered harmful.⁷⁴ However, some continue to accumulate macrophage foam cells and extracellular lipid and smooth muscle cells—forming raised plaques. From these, more advanced lesions may develop—with further deposition of extracellular lipid, cholesterol crystals, collagen, and potentially calcium.⁷⁵ It is these raised lesions that result in a myocardial infarction because of their increasing size and obstruction of the arterial lumen or because of rupture of the fibrous plaque, which results in the release of thrombogenic substances from the necrotic core.⁷⁵

Pathobiological studies of the coronary arteries of young individuals who died from causes unrelated to heart disease have been useful in documenting the progression of atherosclerosis by age and risk factor determinants. Stary and colleagues studied more than 500 postmortem samples of coronary arteries from persons younger than 30 years of age and found the presence of fatty streaks in the majority of children younger than 9 years of age, raised lesions in about half of adolescents, and more advanced lesions in about one-third of the young adults studied.⁷⁶ In 93 autopsies of young adults for whom childhood risk factor data were available, Berenson and colleagues found that the extent of the surface of arteries covered with fatty streaks and fibrous plaques was positively associated with LDL cholesterol, TGs, blood pressure, and body mass index (BMI), and negatively associated with HDL cholesterol levels in childhood.⁷⁷

The Pathobiological Determinants of Atherosclerosis in Youth study reached similar conclusions from examination of more than 3000 postmortem samples of coronary arteries of young adults who died from noncardiovascular events and

BOX 25.2 Pediatric Risk Factors for Atherosclerotic Cardiovascular Disease

POSITIVE RISK FACTORS

- Elevated LDL cholesterol (≥ 130 mg/dL)
- Family history of premature (aged >55 years) coronary heart disease, CVD, or peripheral vascular disease
- Smoking
- Hypertension
- Obesity (≥ 95 th percentile weight for height on National Center for Health Statistics [NCHS] growth chart)
- Physical inactivity
- Diabetes

NEGATIVE RISK FACTORS

- High HDL cholesterol (60 mg/dL)

CVD, Cardiovascular disease, HDL, high-density lipoprotein, LDL, low-density lipoprotein.

who likewise had a variety of surrogates for antimortem risk factor measures available.⁷⁸ In general, pathology studies have made important contributions to the identification of risk factors for early aspects of the atherosclerotic process. In conjunction with findings from longitudinal studies, such as the Framingham Heart Study (in which risk factor assessments of participants preceded the development of CVD),⁷⁹ a group of risk factors, often referred to as the *traditional risk factors* for ASCVD has been established. A complete list of pediatric risk factors for ASCVD is found in Box 25.2.

Advances in vascular imaging technology have provided a means of measuring early pathologic changes and functional abnormalities against coronary and other arteries in response to adverse changes in CVD risk factors. The advantage in using this technology is that walls of superficial arteries can be imaged noninvasively in real time at high resolution, and changes to the arterial wall can be measured as a continuous variable from childhood to adulthood in patients with and without the presence of risk factors for ASCVD.⁸⁰ Computed tomography (CT) scanning is considered one of the most sensitive noninvasive tools for imaging the extent and location of coronary artery calcium present in atheroma.⁸¹

The presence of coronary artery calcium has been associated with adverse CVD outcomes in adults.⁸² In adolescents, several prospective studies have shown associations between risk factors for ASCVD in youth and coronary artery calcium in young adulthood. In the Muscatine Study, in which participants were assessed for CVD risk factors during their school-age years, and later assessed for cardiovascular changes by CT scan, 31% of men and 10% of women aged 29 to 37 years had significant coronary artery calcification.⁸³ In this study, childhood risk factors associated with calcification were obesity, increased blood pressure, and low HDL cholesterol. In the Cardiovascular Risk in Young Finns Study, elevated total and LDL cholesterol, Apo-B levels and systolic blood pressure measured in adolescence were associated with coronary artery calcium in middle-age, highlighting the role of lifelong risk factor exposure to the pathophysiology of CVD.⁸⁴ Gidding and colleagues showed significant coronary calcium by electron beam CT in 7 of 29 young adults with heterozygous familial hypercholesterolemia.⁸⁵ Overweight was found to increase the likelihood of calcium being present in individuals already at high risk.

Vascular ultrasound imaging has been used to assess alterations in brachial artery flow-mediated dilation, which is a measure of endothelial function, and carotid intima-media thickness (IMT).⁸¹ In adults, both measures have been

associated with adverse changes in traditional ASCVD risk factors,⁸⁶ respond to normalization of risk factors,⁸⁷ and are considered important early markers for the progression of atherosclerotic disease.^{86–89} Although fewer studies have used ultrasound technology to evaluate coronary arteries in the young, children with hypercholesterolemia have been assessed using measures of the carotid and brachial arteries and have been found to have abnormalities of carotid IMT^{90,91} and brachial artery vasodilation.^{92–94} Early initiation and longer duration of statin therapy in youth with FH was related to better carotid IMT at 10 years of follow-up, as demonstrated by no difference in rate of increased carotid IMT between youths with FH compared with their nonaffected siblings.⁹⁵ Regression of carotid IMT has also been demonstrated in youth with treatment of dyslipidemia,⁹⁶ whereas a study of statin therapy in low-risk adults demonstrated lack of progression of carotid IMT but did not result in regression of atherosclerosis.⁹⁷ In a systematic review of 51 studies evaluating over 4000 FH patients and 700 FCHL patients, impaired endothelial function, as measured by reduced brachial artery flow-mediated dilation (FMD), was significantly improved with statins. Consistent with studies of carotid IMT, improvement was proportional to duration and intensity of statin therapy.⁹⁸

In summary, these studies confirm the utility of vascular imaging for detecting early pathologic and functional changes to coronary vessels, and associations with modifiable CVD risk factors in the young. Clinically, vascular imaging by ultrasound may be a valuable means of estimating the benefit of treating multiple CVD risk factors in children and adolescents. However, the collection of more normative data across age, race, and gender groups, and longitudinal studies to determine age and puberty-related changes in these measures is needed before these methods could be adopted in clinical evaluation.^{99,100} In general, CT scans may be less useful in younger patients because calcium depositions are uncommon before young adulthood.

SCREENING FOR LIPID DISORDERS

Routine Screening

The approach to pediatric screening for dyslipidemia has been controversial. Since the 1990s, pediatric guidelines established by the NCEP have provided the standard of care, with respect to lipid screening and treatment of dyslipidemia in children.¹⁰¹ These guidelines recommend selective blood cholesterol screening in children, based on a positive family history of premature CVD (before age 55 years), presence of dyslipidemia in a parent (total cholesterol >240 mg/dL), or presence of additional CVD risk factors in the child, such as hypertension, diabetes, and obesity. If family history is unknown, recommendations suggest that lipid screening of a child be done at the discretion of the primary care provider. Selective screening is endorsed for young children between 2 to <9 years of age by the National Heart, Lung and Blood Institutes (NHLBI) Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents.⁶⁴

For older children ages 9 to 11 years and again after puberty (ages 17–21 years), the NHLBI Expert Panel recommends universal screening for dyslipidemia. This is based on evidence that targeted screening for dyslipidemia reportedly missed many children with moderate dyslipidemia (as many as 30%–60%) and failed to detect a substantial number who likely had genetic dyslipidemia who might require more intensive therapy.¹⁰² FH is a relatively common problem, with the heterozygous form occurring in one in 250 individuals.²⁶ FH has been clearly associated with an increased lifetime risk of ASCVD, and earlier treatment is associated with reduced

subclinical evidence of atherosclerosis.³⁰ To increase the likelihood of detecting young patients with FH and other genetic dyslipidemias, the American Heart Association (AHA)¹⁰³ and the National Lipid Association¹⁰⁴ also endorse lipid screening during childhood and adolescence. The US Preventive Services Task Force 2016 guidelines on pediatric lipid screening do not specifically advocate for or against pediatric lipid screening, but cite a lack of knowledge regarding the relationship between childhood lipid levels and hard cardiovascular outcomes, and the need for further evidence in this regard.¹⁰⁵

The NHLBI Expert Panel recommendations recognized that there is considerable variation in LDL cholesterol with age during growth and development, especially during puberty.^{106,107} Total and LDL cholesterol levels tend to decline during puberty, meaning that some adolescents will appear normal, when in fact they will have elevated levels after puberty.¹⁰⁸ For this reason, 9 to 11 years of age was selected as a good age to screen, before the effect of puberty lowers LDL cholesterol levels, but closer to an age when drug therapy may be appropriate.¹⁰⁹

The Expert Panel⁶⁴ also included non-HDL cholesterol as a screening tool for the identification of dyslipidemia in children. Non-HDL cholesterol is calculated by subtracting the HDL cholesterol from the total cholesterol. This measure reflects the amount of cholesterol carried by atherogenic apolipoprotein B-containing lipoproteins (VLDL, IDL, and LDL). In both adults and children, non-HDL cholesterol has been determined to be more predictive of persistent dyslipidemia and therefore atherosclerosis and future CVD events than total cholesterol, LDL cholesterol, or HDL cholesterol alone.¹⁰⁸ A major advantage of non-HDL cholesterol is that it can be accurately calculated in a nonfasting state and is therefore practical to obtain in a primary care setting. Percentiles for non-HDL cholesterol have been established (Table 25.3) and are based on data from the Bogalusa Heart Study,¹⁰⁹ where non-HDL cholesterol greater than or equal to the 95th percentile is considered “abnormal/high” and between the 75th to 95th percentile is considered “borderline.”

If a nonfasting non-HDL cholesterol is found to be abnormal, a fasting (12-hour) lipoprotein analysis is recommended⁶⁴ to allow quantification of total cholesterol, HDL cholesterol, TGs, and calculation of LDL cholesterol. The Friedewald equation—LDL cholesterol = total cholesterol – (HDL cholesterol + TGs/5)—can be used to calculate LDL cholesterol, as long as the serum TG level is less than 400 mg/dL.¹¹⁰ Direct measurement of LDL cholesterol concentration is available through some commercial laboratories and is indicated for individuals whose fasting TG level is 400 mg/dL or more.

TABLE 25.3 Normal Plasma Lipid and Lipoprotein Concentrations (mg/dL) for Children and Adolescents

Category	Low	Acceptable	Borderline	High
Total cholesterol	—	<170	170–199	≥200
LDL cholesterol	—	<110	110–129	≥130
Non-HDL cholesterol	—	<120	120–144	≥145
Apolipoprotein B	—	<90	90–109	≥110
Triglycerides	—			
0–9 y		<75	75–99	≥100
10–19 y		<90	90–129	≥130
HDL cholesterol	<40	> 45	40–45	—
Apolipoprotein A-1	<115	<120	115–120	—

Values for plasma lipids and lipoproteins are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. Pediatrics, 128, S1–S44.

HDL, High-density lipoprotein; LDL, low-density lipoprotein.

The lipid/lipoprotein cut points to be used in screening are presented in Table 25.3. Cut points for total cholesterol and LDL cholesterol in children are from the Lipid Research Clinics Prevalence Study.¹⁰¹ The 75th and 95th percentiles for lipid values from this study were used to define “borderline” and “high” risk categories, respectively. If a lipoprotein analysis reveals LDL cholesterol to be borderline or high, there is consensus among guidelines that the test should be repeated and the average value of the two tests be considered for clinical decision making.⁶⁴

Although the Lipid Research Clinics Prevalence Study provided population percentiles for TG and HDL-C concentrations, recommended cut points for these variables were not established back in 1992.¹⁰¹ Measurement of these variables has become more important because they are part of the clustering of risk factors associated with the growing epidemic of pediatric obesity in the United States.⁶² Data from pathology and imaging studies show adverse changes in vascular structure and function in adulthood related to low HDL cholesterol and high TG levels in childhood.¹⁰⁰ Therefore the latest guideline⁶⁴ recommends cut points for TGs and HDL cholesterol based on percentiles provided by the NCEP.¹¹¹ The 75th and 95th percentiles for TG values were used to define borderline and high risk categories, respectively, and the 25th and the 10th percentiles were used to define borderline and high risk groups for HDL cholesterol (see Table 25.3).

Although well-standardized immunochemical methods are available for the determination of apoB and apoA-1 measurements, guidelines suggest that measurements of apolipoproteins for universal screening provide no additional advantage over measuring non-HDL cholesterol, LDL cholesterol, and HDL cholesterol levels, except in youth with premature CVD in parents.^{102,112} Cut points for apoB and apoA-1 from the National Health and Nutrition Examination Survey should be used in these cases¹¹¹ (see Table 25.3). Measurement of lipoprotein subclasses and their sizes in children and adolescents, by advanced lipoprotein testing, has been found to have no sufficient clinical utility to warrant routine use.⁶⁴

Clinical evaluation of children and adolescents at high risk for ASCVD, based on abnormal lipid levels, should include a careful review of the patient’s medical and family history, and physical examination to identify additional risk factors and secondary causes of dyslipidemia. Assessment should include the following: review of past medical or family history for hypertension, diabetes mellitus, medication use, obesity, poor dietary habits (including excessive intake of saturated fat), sedentary behavior, and tobacco use; measurement of height, weight, and calculation of BMI; Tanner staging to assess pubertal growth; blood pressure measurement; physical inspection of skin, eyes, and tendons for lipid deposition and palpitation of the thyroid gland and liver for signs of enlargement; and laboratory tests (including thyroid, renal, and liver function panels). Glucose and insulin levels should be measured to assess for the presence of metabolic syndrome or diabetes.

Genetic Testing

Increasingly, deoxyribonucleic acid–based tests are being used to confirm the diagnosis of FH in patients with a family member who has a mutation or in a young patient with high LDL cholesterol, with tendon xanthomas or atherosclerotic disease.¹¹³ Currently, three genes (*LDLR*, *apoB*, and *PCSK9*) have been identified in association with mutations that cause this disorder.¹¹⁴ In addition to *LDLR*, most laboratories test for the *apolipoprotein B* gene r500Q mutation. Rapid and relatively inexpensive methods have been developed to test a selected subset of the *LDLR* mutations. However, more expensive “complete gene scans” are needed for mutation-negative samples.¹¹⁴

Once a mutation is identified, relatives can be tested rapidly and cheaply.

It is expected that genetic testing will facilitate the diagnosis of FH and the initiation and intensity of recommended lipid-lowering therapy.¹¹⁵ Studies have shown that the degree of cholesterol lowering achieved by statins is influenced by the type of mutation (e.g., individuals with the *APOB* r3500Q mutation showed a strong positive response to statin therapy).^{116,117} In addition, the diagnosis of FH based on genetic testing improved uptake and adherence to treatment in several studies.^{118,119} Data from the CASCADE FH Registry (Cascade Screening for Awareness and Detection of FH) indicate that FH genetic testing is underused for patients in the United States, with genetic testing reported in 3.9% of individuals in the registry with a clinical diagnosis of FH.¹²⁰ Concerns remain about the long-term benefits and potential side effects from lifelong treatment with statins for identified children. As progress in this area continues, current treatment algorithms may need modification to describe the role of genetic testing in clinical practice.

DIET THERAPY IN MANAGING DYSLIPIDEMIA

New pediatric nutrition recommendations from the Expert Panel⁶⁴ support a two-pronged dietary approach to managing pediatric hypercholesterolemia: one geared to the population in general and the second focused on an individualized treatment of dyslipidemia, including an approach to elevated LDL-C and an approach to elevated TGs. On the population level for healthy infants, the Expert Panel⁶⁴ has recommended breastfeeding as optimal to 12 months of age if possible with supplementation of complementary foods when appropriate; the introduction of iron-fortified formula is recommended if breastfeeding is stopped or reduced before 1 year of age. Previous nutrition recommendations from the NCEP¹⁰¹ did not include recommendations for infants; however, evidence in favor of the sustained cardiovascular benefits of breastfeeding, including lower total cholesterol, BMI, and carotid IMT into adulthood, is now strong.^{121–123} These guidelines are in accordance with the Surgeon General’s Office, the World Health Organization, the American Academy of Pediatrics, and the American Academy of Family Physicians.

The 2015 Dietary Guidelines for Americans (DGA)¹²⁴ provide optimal nutrition guidance for primary care providers to use in promoting nutrient adequacy and CVD risk reduction in children over 2 years of age. In keeping with the goal of reducing chronic disease risk among all Americans, the DGA supports a dietary fat composition of 25% to 35% of calories, with less than 10% of calories from saturated fat and no or minimal trans fats included. Notably, the 2015 DGA no longer designates dietary cholesterol as a nutrient of concern for overconsumption. This is based on available evidence showing no appreciable relationship between dietary cholesterol intake and serum cholesterol. The DGA also emphasizes setting an appropriate daily calorie goal for healthy weight stability. This dietary pattern closely matches the former NCEP Step 1 diet,¹⁰¹ and is viewed as a primary preventive measure to optimize serum lipids, thereby reducing the risk of ASCVD in the pediatric population at large. Similar food-based dietary recommendations are available through the World Health Organization (<http://www.who.int/nutrition/topics/nutrecomm/en/>).

Within appropriate gender- and age-specific requirements for growth and development in normal children and children with dyslipidemia, the Expert Panel⁶⁴ has recommended an early transition (between 1 and 2 years of age) to reduced fat, unflavored milk (from whole to 2%), and a dietary composition that matches that recommended by the DGA.¹²⁴ Advice to transition early to a low-fat diet (minimum of 30% of total calories to age 2 years) is supported by data from the ongoing

TABLE 25.4 Estimated Calorie Needs per Day by Age, Gender, and Physical Activity Level

Gender	Age (Years)	Calorie Requirements (kcal) by Activity Level			
		Sedentary	Moderately Active	Active	
Child	2–3	1000–1200	1000–1400	1000–1400	
	Female	4–8	1200–1400	1400–1600	1400–1800
		9–13	1400–1600	1600–2000	1800–2200
Males	14–18	1800	2000	2400	
	4–8	1200–1400	1400–1600	1600–2000	
	9–13	1600–2000	1800–2200	2000–2600	
	14–18	2000–2400	2400–2800	2800–3200	

Estimated amounts of calories needed to maintain caloric balance for various age and gender groups at three different levels of physical activity are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. *Pediatrics*, 128, S1–S44.

Special Turku Risk Intervention Program study,^{125–127} which showed that limiting total fat and saturated fat to DGA levels could be instituted safely after 6 months of age, under medical supervision and lowered total cholesterol, LDL cholesterol, and BMI more than a usual higher-fat diet. The Expert Panel⁶⁴ has cautioned that any dietary changes initiated during childhood should be tailored to the child to ensure optimal growth and development and administered under the guidance of the child's primary care physician. Estimated calorie requirements based on age and gender at three levels of physical activity from the Institute of Medicine¹²⁸ are found in Table 25.4.

The Expert Panel's⁶⁴ population approach for youth ages 4 years and older encourages consumption of a plant-based diet comparable to the Dietary Approaches to Stop Hypertension (DASH)¹²⁹ dietary pattern. This dietary pattern recommends 7 to 10 servings/day of fruits and vegetables and 6 to 10 servings of breads, cereals, and grains. Whole fruits and vegetables rather than juice, and whole grains rather than processed grains are encouraged. Key sources of saturated fat and cholesterol are moderated on the DASH dietary plan. For example, red meats are limited to modest amounts of lean cuts (e.g., 5 to 6 oz per day), and dairy products are limited to skim or low-fat varieties (16–24 oz/day). Plant-based foods should constitute the largest proportion of energy in children's diets. Most choices within these food groups are low in fat, cholesterol free, and high in fiber—and will help displace energy sources containing saturated fat.^{130,131} The major health benefits of a DASH style of eating are lowered blood pressure, improved lipids, and improved weight status.^{132,133} Box 25.3 highlights some practical dietary strategies for lowering saturated fat and cholesterol in the diets of the young.

In a recent systematic review of randomized controlled trials, aimed to reduce dietary saturated fat to modify CVD risk factors in children, the greatest lowering of LDL-C was achieved by replacing saturated fat with polyunsaturated fats (PUFA) or a mixture of PUFA and monounsaturated fats.¹³⁴ Similar to DASH, a Mediterranean-style diet, if appropriately planned, also emphasizes whole, unprocessed foods, a mixture of PUFA and monounsaturated fats, and plant sources of protein. This food pattern has been recognized as effective in modifying cardiovascular risk factors, including lowering blood pressure, improving blood lipids, and promoting weight loss.¹³⁵ Greater improvements in arterial distensibility measures were reported in obese adolescents with hypercholesterolemia on a 12-week Mediterranean-type diet intervention compared with a control group.¹³⁶

BOX 25.3 Practical Dietary Strategies for Lowering Saturated Fat and Cholesterol

- Eat 7 to 10 servings of fresh, frozen, or canned fruits and vegetables daily.
- Use vegetable oils and soft margarines low in saturated fat and trans fatty acids, instead of butter or most other animal fats in the diet.
- Eat whole-grain breads and cereals rather than processed grain products.
- Use nonfat (skim) or low-fat milk and dairy products daily.
- Eat more fish, especially oily fish (broiled or baked).
- Eat lean cuts of meat, and trim any obvious fat from red meat before cooking.
- Take the skin off chicken or turkey and eat only the white meat.
- Avoid processed meats, such as hotdogs, sausage, and bologna.
- Avoid creams or sauces made with butter or whole-milk dairy products.
- Choose low-fat snacks, such as ginger snaps, graham crackers, pretzels, plain popcorn, animal crackers, and vanilla wafers.
- Use recommended portion sizes on food labels when preparing and serving food.

For the individual child with identified dyslipidemia, a positive family history of premature CVD, obesity, or hypertension, the Expert Panel⁶⁴ has recommended initiating the same dietary approach recommended for the general population (as described previously). The Expert Panel has deemed this plan the Cardiovascular Health Integrated Lifestyle Diet (CHILD-1) and considers this the first line of therapy to remediate CVD risk factors, with a primary target of lowering LDL cholesterol levels. Meal plans for different calorie levels have been published for DASH and Mediterranean-style diet patterns¹²⁴ that conform to the CHILD-1 diet and can be modified for progressive saturated fat lowering and calorie and nutrient adequacy of children.¹³⁷ If LDL cholesterol levels remain abnormal (LDL cholesterol ≥ 130 mg/dL) after 3 to 6 months of dietary adherence to the CHILD-1, further restriction of dietary saturated fat (<7% of total calories) and cholesterol (<200 mg/day), referred to as the CHILD-2 (LDL), is recommended. Repeated studies have shown that a dietary saturated fat reduction to 7% of total calories and cholesterol to 200 mg/day is safe and efficacious in lowering LDL cholesterol in children with hypercholesterolemia.^{138–140}

Low-fat diets should be designed to be nutritionally adequate and consultation with a registered dietitian is particularly helpful in that regard. In some studies, lower intakes of calcium, zinc, vitamin E, and phosphorus on low-fat diets were reported for diets initiated without medical supervision.¹⁴⁰ To prevent overzealous implementation of a very low fat diet for children with dyslipidemia, which could lead to failure to thrive,¹⁴¹ the Expert Panel⁶⁴ has recommended 25% as the lower end of the range for total fat calories, consistent with the acceptable macronutrient distribution range stipulated by the DGA¹²⁴ for children 2 years of age and older. In most cases, dietary compliance to the CHILD-2 diet should normalize borderline-high LDL cholesterol levels. The reported response to diets with this composition of total fat, saturated fat, and cholesterol in children with LDL cholesterol levels over 130 mg/dL has ranged from 3% to 12%.^{141–144}

For children older than 2 years of age, with persistently high LDL cholesterol levels, additional dietary adjuncts can be used. These may include margarines containing plant stanol/sterol esters or water-soluble fibers, such as psyllium. About three servings of stanol containing margarine daily (equivalent to

approximately 2 g/day), consumed as part of a low-fat diet, can reduce LDL cholesterol by 5% to 15%.^{145–147} Psyllium at a dose of 6 g/day added to a low-fat diet may provide an additional 5% to 10% reduction in LDL cholesterol.^{147,148} The extent to which LDL cholesterol is lowered may depend on previous dietary intake and baseline LDL cholesterol levels. Dietary supplements are covered in more detail later in the chapter (see the section Dietary Additives and Supplements.).

For children with elevated TGs or low HDL cholesterol, the Expert Panel⁶⁴ has recommended following the CHILD-2 (TG) dietary pattern, which includes reducing one's intake of simple sugars from processed desserts, snacks, and sugared-sweetened beverages. CHILD-2 (TG) guidelines to minimize added sugars are consistent with a recent scientific statement from the AHA for children to consume no more than 25 g (100 calories or 6 teaspoons) of added sugars per day to lower cardiovascular risk factors.¹⁴⁹ Foods that are high in simple sugar generally add excess calories to the diet, which may contribute to weight gain and elevate serum TGs.¹⁵⁰ Weight management should be a goal of diet therapy for children with a BMI greater than or equal to the 85th percentile. Weight-reduction approaches should focus on decreasing the child's weight-for-height percentile, while maintaining linear growth.¹⁵¹ Although weight loss may temporarily lower HDL cholesterol, weight stabilization at a new lower level will lead to a gradual increase in HDL cholesterol over time.¹⁵² Fish oils from fatty fish, such as salmon and tuna, contain omega-3 fatty acids, which may also help to lower serum TGs.¹⁵³ Increased physical activity should be encouraged with a goal of 1 hour per day of moderate to vigorous activity, and sedentary activities (such as watching television and playing computer and video games) should be limited to no more than 2 hours per day.⁶⁴

For children with very high TG, such as those with hypercholesterolemia, a CHILD-2 (TG) diet and fat restriction as low as 15% of daily calories may be helpful in lowering TG and avoiding pancreatitis. MCT may also be considered as a source of calories in these cases. MCT are comprised of fatty acids, with a chain length of 10 to 12 carbons. MCTs are absorbed efficiently, without the need for emulsification, and do not require repackaging into chylomicrons for transport; therefore MCTs are particularly beneficial for disorders that have defects in bile secretion or chylomicron aggregation or clearance processes.¹⁵⁴

PHARMACOLOGIC MANAGEMENT

For many children with moderately or severely elevated LDL cholesterol, which usually results from a genetic dyslipidemia, diet alone will not lower their cholesterol levels to the acceptable or even borderline range. In these cases, lipid-lowering drug therapy will be required to achieve LDL cholesterol treatment goals in children age 10 years and older. Long-term drug therapy is associated with a decreased incidence of heart disease and overall mortality in adults.⁶⁶ Although no studies directly demonstrate the efficacy of administering lipid-lowering drug therapy in children to prevent CVD, there is evidence from vascular imaging studies that statins may delay the atherosclerotic disease process.^{155,156}

The Expert Panel⁶⁴ has recommended using medication in patients who are at least 10 years of age and whose postdietary (e.g., CHILD-1 → CHILD-2 after 6 months) LDL cholesterol level is 190 mg/dL (or higher) or whose LDL cholesterol level is 160 mg/dL (or higher), and there is a family history of early CVD, with at least one high-level or at least two moderate-level risk factors for CVD. In children with FH who have substantially elevated LDL cholesterol, medication management may need to be started earlier than age 10 years.¹⁵⁶ A lipid specialist should be consulted in these cases. A follow-up LDL cholesterol

TABLE 25.5 Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents Recommendations for Lipid Management of Children and Adolescents With Diabetes

Type 1 and Type 2 Diabetes Mellitus	
Screening	After glycemic control >2 years at diagnosis if other CVD risk factors; otherwise at 10 years; if normal, rescreen every 5 years At diagnosis regardless of age; if normal, rescreen every 2 years
Lipid goals	LDL-C ≤100 mg/dL Non-HDL-C <120 mg/dL Triglycerides <90 mg/dL
Treatment strategies	Glycemic control Diet (CHILD-1 → CHILD-2) Physical activity Weight reduction if appropriate Medication indications if initial management fails: <ul style="list-style-type: none"> • Age ≥10 years • If LDL >160 mg/dL • If LDL 130–159 mg/dL consider based on CVD risk profile • Statins with or without resins • Fibrates if TG >1000 mg/dL • Manage other CVD risk factors (see Box 25.2) if appropriate
Recommendations are from the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (2011). Summary report. <i>Pediatrics</i> , 128, S1–S44. CVD, Cardiovascular disease; CHILD, Cardiovascular Health Integrated Lifestyle Diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride.	

assessment is recommended 6 weeks after starting drug therapy and every 3 months thereafter, until LDL cholesterol goals are met. Thereafter, follow-up can be less frequent.

For children in general, the goal for LDL cholesterol is less than 130 mg/dL. For children with diabetes, the goal for LDL cholesterol is lower than 100 mg/dL.^{68,157} The lower LDL cholesterol goal for children with diabetes reflects a synthesis of pediatric guidelines and treatment recommendations for adults with diabetes that now consider the presence of diabetes a coronary heart disease risk equivalent.⁶⁶ Table 25.5 summarizes the recommendations of the Expert Panel⁶⁴ regarding lipid screening and management of children and youth with diabetes. Medications used in the treatment of specific lipid abnormalities are summarized in Table 25.6 and are reviewed in the following material.

Bile Acid–Binding Agents

Bile acid–binding agents lower serum cholesterol indirectly by binding with bile acids in the gastrointestinal tract. This action prevents their reabsorption into the enterohepatic circulation, resulting in their loss from the body and removal from the cholesterol pool.¹⁵⁸ To compensate for this loss, the liver increases endogenous cholesterol synthesis and upregulates LDL-receptor synthesis—thereby lowering LDL cholesterol levels in the blood. In bile-acid resin trials in children, a dose of 8 g/day, while on a cholesterol-lowering diet, resulted in a decrease in LDL cholesterol ranging from 10% to 20%.^{159,160}

Bile-acid resins come in powder and tablet form. The powder is usually taken twice daily (4-g scoop) mixed with water or juice. Resins in this form tend to be gritty in texture and children complain that they are unpleasant to drink. Tablets are more palatable but are large and difficult for some children

TABLE 25.6 Drugs Used in the Treatment of Pediatric Lipid Disorders

Class of Medication	Common Name	Mechanism of Action	Change in Lipid Profile	Adverse Effects
Bile acid-binding agents	Cholestyramine	Binds intestinal bile acids; more cholesterol converted into bile acids; decrease hepatic cholesterol pool	↓ LDL	Constipation Abdominal cramping
	Colestipol		↑ TG	
HMG Co-A reductase inhibitors	Colesevelam	Inhibits cholesterol synthesis in hepatic cells; decreases cholesterol pool resulting in upregulation of LDLR	↓ LDL	Dyspepsia ↑ Liver transaminases ↑ CK Myositis
	Atorvastatin		↓ TG	
	Simvastatin		↑ HDL	
	Pravastatin			
	Rosuvastatin			
	Lovastatin			
Fibrates ^a	Fluvastatin	Increases degradation of VLDL cholesterol and TG; hepatic synthesis of VLDL may be decreased	↓ TG	Constipation Myositis
	Fenofibrate		↑ HDL	
Niacin ^a	Niacin extended release	Inhibits release of free fatty acids from adipose tissue; decreases VLDL and LDL cholesterol production and HDL cholesterol degradation	Gemfibrozil	Anemia
			↓ LDL	Flushing
PCSK9 Inhibitors ^a	Alirocumab	Binds to the extracellular domain of the LDL receptor and targets the receptor for degradation	↓ TG	Headache
			↑ HDL	↑ Liver transaminases
Cholesterol absorption blocker	Ezetimibe	Inhibits intestinal absorption of cholesterol and plant sterols	↓ LDL	Not reported in children or adolescents

^aNot US Food and Drug Administration approved for youths (as of this writing).

CK, Creatine kinase; HDL, high-density lipoproteins; HMG CoA, hydroxymethylglutaryl CoA; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9; TG, triglycerides; VLDL, very low-density lipoprotein.

to swallow. Overall, studies report poor to fair compliance with the medication.^{158,161} Side effects are few and are mainly gastrointestinal in nature, including constipation and gas. These can be minimized with increased intake of water and fiber. Resins may increase TG levels and may interfere with the absorption of certain medications and fat-soluble vitamins.¹⁶² Supplementation with a multivitamin and folate (1 mg daily) is usually recommended.¹⁵⁸

HMG-CoA Reductase Inhibitors

HMG-CoA reductase inhibitors (also known as *statins*) lower LDL cholesterol in the blood by blocking hepatic HMG Co-A reductase, the rate-limiting enzyme in cholesterol biosynthesis.¹⁵⁶ This action depletes the intracellular cholesterol pool, leading to an upregulation of LDL receptors and a decrease in serum cholesterol. Doses ranging from 5 to 40 mg/day in children with FH have resulted in a 23% to 40% lowering of LDL cholesterol.^{154–156} Statins may be taken in combination with bile acid-binding agents for patients who fail to meet LDL cholesterol targets with statin monotherapy. No adverse effects of the combined therapies were noted in a pediatric trial of the medications, and the efficacy of the two medications taken together was additive.¹⁶³

In vivo imaging studies have demonstrated improvements in surrogate markers for atherosclerosis with statin therapy. Reversal of endothelial dysfunction and regression of carotid IMT have been reported in children treated with statins over a 2-year period.^{155,156} This latter finding suggests that the initiation of lipid-lowering statins in childhood may inhibit progression or might even lead to a regression of atherosclerosis.

Adverse effects of the medication are few but have included gastrointestinal complaints, elevated liver transaminase and creatine kinase (CK) levels, and myositis.^{158,162,164} For this reason, statins would not be recommended for patients with liver disease. Follow-up of patients treated with statins should include a laboratory assessment of liver transaminase and CK levels, and identification of adverse physical symptoms, such as muscle cramps.

Because statins are potentially teratogenic, it is essential that physicians determine that adolescent girls are not pregnant or likely to become pregnant before initiating therapy.¹⁵⁸ The longest statin trials have been 2 years in length. Thus whether statins adversely affect long-term growth and development and are safe for lifelong use has not been ascertained. Longer-term safety and efficacy studies are needed, particularly with a follow-up of vascular end points.

Inhibitors of Cholesterol Absorption

Ezetimibe is a cholesterol-lowering agent that prevents the absorption of cholesterol and plant sterols by inhibiting the passage of sterols across the intestinal wall.¹⁶⁵ The reduction in cholesterol absorption leads to a decrease in hepatic cholesterol uptake and availability. As a result, there is a compensatory increase in hepatic cholesterol biosynthesis, an upregulation of LDL receptor expression, and an overall decrease in blood LDL cholesterol levels. In children and adolescents, a daily intake of 10 mg/day reduced LDL cholesterol by approximately 20%.¹⁶³ Kusters et al. studied the safety and efficacy of ezetimibe in children aged 6 to 10 years of age.¹⁶⁶ They found that after 12 weeks of treatment LDL-C was reduced by 27% and non-HDL-C was reduced by 26%. Ezetimibe was well tolerated with few adverse effects. They concluded that ezetimibe could be used as monotherapy for lowering LDL-C in some children. Ezetimibe is US Food and Drug Administration (FDA) approved for use in individuals age 10 years and above.

Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors

In the early 2000s it was discovered that a gain of function of the gene for PCSK9 results in autosomal dominant hypercholesterolemia.¹⁶⁷ PCSK9 binds to the extracellular domain of the LDL receptor and targets the receptor for degradation. Loss-of-function mutations result in very low circulating LDL-C and also very low risk of CVD.¹⁶⁸ These findings have led to the development of agents that inhibit PCSK9 and lower

circulating LDL-C. These agents use monoclonal antibodies, which are fully human immunoglobulin G subtypes that bind with an approximate 1:1 stoichiometry to circulating PCSK9, which prohibits its binding to the LDL receptor. This results in an accumulation of LDL receptors on the membrane of hepatocytes. This increases clearance of LDL particles and reduces plasma levels of LDL-C.¹⁶⁹

PCSK9 inhibitors have been studied in adult populations, particularly individuals with prior CVD. They result in reduction of LDL-C by 55% to 72%, resulting in very low levels of plasma LDL-C.¹⁶⁹ These reductions in LDL-C have been associated with reduction of atherosclerotic CVD rates.¹⁷⁰

PCSK9 inhibitors have not been extensively studied in children and adolescents. They require injections, which may inhibit their use in pediatric patients. These agents are also costly and approval by insurance providers has proven difficult in adult patients. However, for patients with genetic dyslipidemia and marked elevation of LDL-C who may require more aggressive treatment using LDL apheresis, the PCSK9 inhibitors may prove to be valuable. Pediatric studies with PCSK9 inhibitors are underway, but long-term efficacy and safety data will take some time to acquire.

Fibric Acid Derivatives

Fibric acid derivatives (also known as *fibrates*) lower blood TG levels by increasing degradation and reducing the hepatic production of VLDL.^{158,165} The drug also increases the production of apoA-I, resulting in higher HDL cholesterol levels. Side effects are similar to those for statins and include gastrointestinal complaints, elevated liver transaminase activity, and myopathy.¹⁵⁸ For this reason, fibrates are not recommended for use with statins. These medications are not FDA approved for use with children and adolescents and should be used only in consultation with a lipid specialist for adolescents with genetic hypertriglyceridemia.⁶⁴

Niacin

Niacin decreases hepatic VLDL production, leading to decreased production of LDL cholesterol.^{158,165} Children with heterozygous FH treated with 1000 to 2250 mg of niacin daily, over an average of 8 months, showed a 23% to 30% reduction in LDL cholesterol.¹⁷¹ However, 76% of the children had adverse effects from therapy (e.g., flushing, headache, nausea, glucose intolerance, myopathy, abnormal liver function) and 38% discontinued the drug. Niacin is not recommended for children younger than 2 years of age and is generally not used to treat children with FH, unless LDL cholesterol is persistently elevated or unusual hypertriglyceridemia and low HDL cholesterol are present.⁶⁴ Niacin given in combination with statins has been used to treat homozygous FH. Niacin is available in immediate and slow-release forms (Niaspan, Slo-Niacin).

Dietary Additives and Supplements

Plant sterols and stanols consumed at levels of 2 g per day have been shown to reduce LDL cholesterol levels by 9% to 20% in adults.⁶⁶ Foods containing these additives (e.g., margarines and salad dressings) lower serum cholesterol by preventing dietary cholesterol absorption in the gastrointestinal tract.¹⁴⁵ In children with FH, use of 2 g/day of plant sterols decreased LDL cholesterol by 5% to 15%, but did not improve endothelial function.¹⁴⁶ This suggests that LDL cholesterol must be reduced to a certain threshold level before improvement of endothelial function can occur.

More studies examining the long-term effects of plant sterols on the vascular endothelium are warranted. Concern has been

raised about the potential for the malabsorption of fat and fat-soluble vitamins in children consuming plant sterols chronically.⁶⁶ The Expert Panel⁶⁴ recommends reserving the use of foods supplemented with plant sterols/stanols to children with moderate to severe elevation in cholesterol, and monitoring fat-soluble vitamin status.¹⁴⁰

Dietary supplements containing soluble fiber, garlic, and fish oils have been used to treat pediatric dyslipidemia with limited to moderate efficacy. In a randomized controlled trial, Davidson and colleagues studied the effect of psyllium fiber versus placebo on change in blood cholesterol levels of 6- to 18-year-old children with hypercholesterolemia.¹⁴⁸ Psyllium fiber (6 g/day) and the placebo were added to a ready-to-eat cereal. Compliance to treatment was excellent; consumption of the enriched cereal resulted in a modest 7% reduction in LDL cholesterol concentrations compared with the control cereal.

Garlic extract therapy was studied in a randomized controlled trial for effects on serum lipoproteins in 8- to 18-year-old children with FH.¹⁷² The extract was given in three daily doses of 300 mg versus a placebo for 8 weeks. No significant effects of garlic treatment versus placebo were noted on total, LDL, or HDL cholesterol.

Red yeast rice, a traditional Chinese culinary product, has been marketed to lower blood cholesterol. Although one pediatric trial showed significant LDL-C lowering with this product,¹⁷³ red yeast rice contains monacolin K, which is chemically identical to the cholesterol-lowering drug lovastatin. Therefore red yeast rice products may not be safe and are not recommended for children.

Omega-3 fatty acids, as found in fish oils, are known to reduce serum TG levels in adults.⁶⁶ In a randomized crossover study, children with FH and FCHL were supplemented with either 1.2 g/day of docosahexa-enoic acid (DHA) or a placebo for 6 weeks.¹⁵⁵ All children were given dietary counseling to reduce saturated fat intake to less than 7% of calories. Outcomes studied included TGs, LDL and HDL cholesterol, and endothelial function, as measured by FMD of the brachial artery. Findings showed that DHA supplementation was associated with increased levels of total, LDL, and HDL cholesterol, but no change in TGs compared with the placebo group. FMD improved significantly after DHA supplementation compared with baseline in both groups, and the change was greater in the DHA-treated group versus the controls. This finding suggests that in children, DHA may not be effective for positively modifying serum lipids. However, the endothelium may be a therapeutic target for DHA in hyperlipidemic children.

Low-Density Lipoprotein Apheresis

For children with homozygous FH and extremely elevated LDL cholesterol (>500 mg/dL), where LDL cholesterol cannot be reduced through the combination of diet therapy and multiple medication management, biweekly LDL apheresis has been used successfully to achieve significant lipid lowering, under the care of a lipid specialist. In this process, apoB-containing particles are selectively removed from the circulation through extracorporeal precipitation.¹⁷⁴ Apheresis reduced LDL concentrations up to 72% compared with maximal drug therapy in patients with homozygous FH. The procedure is FDA approved, and qualified medical sites that perform this procedure are listed on the National Lipid Association website (www.lipid.org).

CONCLUSIONS AND FUTURE DIRECTIONS

Increasing evidence indicates that extreme elevation in LDL cholesterol is associated with vascular pathology in youth. With new recommendations for universal screening of pediatric patients from the NHLBI Expert Panel, more patients with

significant hypercholesterolemia are likely to be identified. Fortunately, appropriate therapy is available to modify and potentially reverse vascular abnormalities. Pediatric clinical trials of lifestyle and drug therapy to treat dyslipidemia suggest similar effectiveness and safety to that observed in adults. Most studies, however, have been of short or medium term in design. Longer-term safety and efficacy studies are needed, particularly with follow-up of vascular end points. Also few clinical data are available to document at what age drug therapy should be appropriately and safely started. More work is needed in this regard to support clinical judgments on what age and dosage should be used in children at high risk for ASCVD.

REFERENCES

- Glatz JF, Luiken JJ. From fat to FAT (CD36/SR-B2): understanding the regulation of cellular fatty acid uptake. *Biochimie*. 2017;136(5):21–26.
- Bettors JL, Yu L. NPC1L1 and cholesterol transport. *FEBS Lett*. 2010;584(13):2740–2747.
- Yamanashi Y, Takada T, Kurauchi R, Tanaka Y, Komine T, Suzuki H. Transporters for the intestinal absorption of cholesterol, vitamin E and vitamin K. *J Atheroscler Thromb*. 2017;24(4):347–359.
- D'Aquila T, Hung YH, Carreiro A, Buhman KK. Recent discoveries on absorption of dietary fat: presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes. *Biochim Biophys Acta*. 2016;1861(8 pt A):730–747.
- Cavelier C, Lorenzi I, Rohrer L, von Eckardstein A. Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. *Biochim Biophys Acta*. 2006;1761(7):655–666.
- Yoo EG. Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis and management. *Ann Pediatr Endocrinol*. 2016;21:7–14.
- Sirwi A, Hussain MM. Lipid transfer proteins in the assembly of apo-B containing lipoproteins. *J Lip Res*. 2018;59:1094–1102.
- Walsh MT, Hussain MM. Targeting microsomal triglyceride transfer protein and lipoprotein assembly to treat homozygous familial hypercholesterolemia. *Crit Rev Clin Lab Sci*. 2017;54:26–48.
- Sook Sul H, Storch J. Cholesterol and lipoproteins: synthesis, transport and metabolism. In: Stipanuk MH, ed. *Biochemical, Physiological, and Molecular Aspects of Human Metabolism*. 4th ed. St. Louis: Saunders/Elsevier; 2018:393–415.
- Daniels SR. Lipid metabolism and secondary forms of dyslipoproteinemia in children. *Prog Ped Card*. 2003;17:135–140.
- He PP, Jiang T, OuYang XP, Liang YQ, Zou JQ, et al. Lipoprotein lipase: Biosynthesis, regulatory factors, and its role in atherosclerosis and other diseases. *Clin Chim Acta*. 2018;480:126–137.
- Dallinga-Thie GM, Franssen R, Mooij HL, Visser ME, Hassing HC, Peelman F, et al. The metabolism of triglyceride-rich lipoproteins revisited: new players, new insight. *Atherosclerosis*. 2010;211:1–8.
- Dijk W, Kersten S. Regulation of lipid metabolism by angiopoietin-like proteins. *Curr Opin Lipid*. 2016;27:249–256.
- Au DT, Strickland DK, Muratoglu SC. The LDL receptor-related protein 1: at the crossroads of lipoprotein metabolism and insulin signaling. *J Diabetes Res*. 2017;8356537.
- Actis DV, Chiabrando GA. The role of low density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. *Int J Mol Sci*. 2018;19: E1780.
- Olivecrona G, Olivecrona T. (2010). Triglyceride lipases and atherosclerosis. *Curr Opin Lipidol*. 2010;21(5):409–415.
- Hegele RA. Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet*. 2009;10(2):109–121.
- Beglova N, Blacklow SC. The LDL receptor: how acid pulls the trigger. *Trend Biochem Sci*. 2005;30(6):309–317.
- TKrysa JA, Ooi TC, Proctor SD, Vine DF. Nutritional and lipid modulation of PCSK9: Effects on cardiometabolic risk factors. *J Nutr*. 2017;147:473–481.
- Walker AK, Naar AM. SREBPs: regulators of cholesterol/lipids as therapeutic targets in metabolic disorders, cancers and viral diseases. *Clin Lipidol*. 2012;7:27–36.
- Rigotti A. Scavenger receptors and atherosclerosis. *Biol Res*. 2000;33(2):97–103.
- Krimbou L, Marcil M, Genest J. New insights into the biogenesis of human high-density lipoproteins. *Curr Opin Lipidol*. 2006;17(3):258–267.
- Schaefer EJ, Asztalos BF. Cholesteryl ester transfer protein inhibition, high-density lipoprotein metabolism and heart disease risk reduction. *Curr Opin Lipidol*. 2006;17(4):394–398.
- Ohashi R, Mu H, Wang X, Yao Q, Chen C. Reverse cholesterol transport and cholesterol efflux in atherosclerosis. *QJM*. 2005;98(12):845–856.
- Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol*. 2009;29(4):431–438.
- de Ferranti SD, Rodday AM, Mendelson MM, Wong JB, Leslie LK, Sheldrick RC. Prevalence of familial hypercholesterolemia in the 1999 to 2012 United States National Health and Nutrition Examination Surveys (NHANES). *Circulation*. 2016;113(11):1067–1072.
- Ose L. Familial hypercholesterolemia from children to adults. *Cardiovasc Drug Therapy*. 2002;16(4):289–293.
- Gidding SS, Champagne MA, de Ferranti SD, Defesche J, Ito MK, et al. The agenda for familial hypercholesterolemia: a scientific statement from the American Heart Association. *Circulation*. 2015;132:1–5.
- Usifo E, Leigh SE, Whittall RA, Lench N, Taylor A, Yeats C, et al. Low density lipoprotein receptor gene familial hypercholesterolemia variant database: update and pathophysiological assessment. *Ann Hum Genet*. 2012;76:387–401.
- Fahed AC, Nemer GM. Familial hypercholesterolemia: the lipids or the genes? *Nutr Metab*. 2011;8(1):23.
- Naoumova RP, Thompson GR, Soutar AK. Current management of severe homozygous hypercholesterolaemias. *Curr Opin Lipidol*. 2004;15(4):413–422.
- van der Graaf A, Avis HJ, Kusters DM, Vissers MN, Hutten BA, Defesche JC, et al. Molecular basis of autosomal dominant hypercholesterolemia: assessment in a large cohort of hypercholesterolemic children. *Circulation*. 2011;123(11):1167–1173.
- Arca M, Zuliani G, Wilund K, Campagna F, Fellini R, Bertolini S, et al. Autosomal recessive hypercholesterolaemia in Sardinia, Italy, and mutations in ARH: a clinical and molecular genetic analysis. *Lancet (London, England)*. 2002;359(9309):841–847.
- Lind S, Olsson AG, Eriksson M, Rudling M, Eggertsen G, Angelin B. Autosomal recessive hypercholesterolaemia: normalization of plasma LDL cholesterol by ezetimibe in combination with statin treatment. *J Intern Med*. 2004;256(5):406–412.
- Imes CC, Austin MA. Low density lipoprotein cholesterol, apolipoprotein B and risk of coronary heart disease: from familial hyperlipidemia to genomics. *Biol Res Nurs*. 2012;1:1–17.
- Patni N, Ahmad Z, Wilson DP. Genetics and dyslipidemia. In: DeGroot LJ, Chrousos K, et al., eds. *Diagnosis and Treatment of Disease of Lipids and Lipoprotein Metabolism in Adults and Children*; 2016. Endotext [Internet]. South Dartmouth (MA), MDText.com. Inc.; Last updated November 6, 2016.
- Liyanage KE, Burnett JR, Hooper AJ, van Bockxmeer FM. Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. *Crit Rev Clin Lab Sci*. 2011;48(1):1–18.
- Othman RA, Myrie SB, Jones PJ. Non-cholesterol sterols and cholesterol metabolism in sitosterolemia. *Atherosclerosis*. 2013;231:291–299.
- Yoo EG. Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis, and management. *Ann Pediatr Endocrinol Metab*. 2016;21:7–14.
- Bartnikowska E. Biological activities of phytosterols with particular attention to their effects on lipid metabolism. *Polish J Food Nutr Sci*. 2009;59:105–112.
- Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*. 2011;123(20):2292–2333.
- Kavey RE. Combined dyslipidemia in children and adolescents. In: De Groot LJ, Chrousos K, et al., eds. *Diagnosis and Treatment of Disease of Lipids and Lipoprotein Metabolism in Adults and Children*; 2016. Endotext [Internet]. South Dartmouth (MA), MDText.com. Inc.; Last updated August 10, 2016.
- Veerkamp MJ, de Graaf J, Hendriks JC, Demacker PN, Stalenhoef AF. Nomogram to diagnose familial combined

- hyperlipidemia on the basis of results of a 5-year follow-up study. *Circulation*. 2004;109(24):2980–2985.
44. Wiesbauer F, Blessberger H, Azar D, Goliasch G, Wagner O, Gerhold L, et al. Familial-combined hyperlipidaemia in very young myocardial infarction survivors (≤ 40 years of age). *Eur Heart J*. 2009;30(9):1073–1079.
 45. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet (London, England)*. 2005;365(9468):1415–1428.
 46. Steinberger J, Daniels SR, Eckel RH, et al. Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association. *Circulation*. 2009;119:628–647.
 47. Hopkins PN, Heiss G, Ellison RC, Province MA, Pankow JS, Eckfeldt JH, et al. Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case-control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. *Circulation*. 2003;108(5):519–523.
 48. Shah AS, Wilson DP. Genetic disorders causing hypertriglyceridemia in children and adolescents. In: DeGroot LJ, Chrousos K, et al., eds. *Diagnosis and Treatment of Disease of Lipids and Lipoprotein Metabolism in Adults and Children*; 2016. Endotext [Internet]. South Dartmouth (MA), MDText.com Inc.; Last updated June 23, 2016.
 49. Falko JM. Familial chylomicronemia syndrome: a clinical guide for endocrinologists. *Endocr Pract*. 2018;24:756–763.
 50. Goldberg IJ. (2009). Hypertriglyceridemia: impact and treatment. *Endocrinol Metab Clin North Am*. 2009;38(1):137–149.
 51. Hovingh GK, de Groot E, van der Steeg W, Boekholdt SM, Hutten BA, Kuivenhoven JA, et al. Inherited disorders of HDL metabolism and atherosclerosis. *Curr Opin Lipidol*. 2005;16(2):139–145.
 52. Clauss SB, Kwiterovich PO. Genetic disorders of lipoprotein transport in children. *Prog Ed Card*. 2003;17:123–133.
 53. Assmann G, Von Eckardstein A, Brewer Jr HB. Familial analphalipoproteinemia: Tangier disease. In: Scriver C, Beaudet A, Sly W, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill; 2001:2937–2960.
 54. Asztalos BF, Schaefer EJ, Horvath KV, Yamashita S, Miller M, Franceschini G, et al. Role of LCAT in HDL remodeling: investigation of LCAT deficiency states. *J Lipid Res*. 2007;48(3):592–599.
 55. Di Filippo M, Crehalet H, Samson-Bouma ME, Bonnet V, Aggerbeck LP, Rabes JP, et al. Molecular and functional analysis of two new MTP gene mutations in an atypical case of abetalipoproteinemia. *J Lipid Res*. 2012;53(3):548–555.
 56. Schonfeld G. Familial hypobetalipoproteinemia: a review. *J Lipid Res*. 2003;44(5):878–883.
 57. Tarugi P, Averna M. Hypobetalipoproteinemia: genetics, biochemistry, and clinical spectrum. *Adv Clin Chem*. 2011;54:81–107.
 58. Bergeron N, Phan BA, Ding Y, et al. Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. *Circulation*. 2015;132:1648–1666.
 59. Marais AD, Kim JB, Wasserman SM, Lambert G. PCSK9 Inhibition in LDL cholesterol reduction: genetics and therapeutic implications of very low plasma lipoprotein levels. *Pharmacol Ther*. 2015;145:58–66.
 60. Smelt AH, de Beer F. Apolipoprotein E and familial dysbetalipoproteinemia: clinical, biochemical, and genetic aspects. *Semin Vasc Med*. 2004;4(3):249–257.
 61. Rosenson RS. *Secondary causes of dyslipidemia*; 2012. Available from: www.uptodate.com/contents/secondary-causes-of-dyslipidemia.
 62. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. *Circulation*. 2005;111(15):1999–2012.
 63. Brambilla P, Lissau I, Flodmark CE, Moreno LA, Widhalm K, Wabitsch M, et al. Metabolic risk-factor clustering estimation in children: to draw a line across pediatric metabolic syndrome. *Int J Obes*. 2007;31(4):591–600.
 64. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. Summary report. *Pediatrics*. 2011;128:S1–S44.
 65. Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am*. 2006;35(3):491–510. vii–viii.
 66. Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001;285(19):2486–2497.
 67. Kaufman FR. Type 2 diabetes mellitus in children and youth: a new epidemic. *J Pediatr Endocrinol Metab*. 2002;15(Suppl 2):737–744.
 68. American Diabetes Association. Management of dyslipidemia in children and adolescents with diabetes. *Diabetes Care*. 2003;26(7):2194–2197.
 69. Kershner AK, Daniels SR, Imperatore G, Palla SL, Pettitt DB, Pettitt DJ, et al. Lipid abnormalities are prevalent in youth with type 1 and type 2 diabetes: the SEARCH for Diabetes in Youth Study. *J Pediatr*. 2006;149(3):314–319.
 70. Naylor LH, Green DJ, Jones TW, Kalic RJ, Suriano KL, Shah M, et al. Endothelial function and carotid intima-medial thickness in adolescents with type 2 diabetes mellitus. *J Pediatr*. 2011;159(6):971–974.
 71. American Diabetes Association. Children and adolescents: standards of medical care in diabetes - 2018. *Diabetes Care*. 2018;41:S126–S136.
 72. Galley R. Improving outcomes in renal disease. *JAAPA*. 2006;19(9):20–25.
 73. Badimon L, Martinez-Gonzalez J, Llorente-Cortes V, Rodriguez C, Pedro T. Cell biology and lipoproteins in atherosclerosis. *Curr Mol Med*. 2006;6(5):439–456.
 74. Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arterioscler Thromb Vasc Biol*. 2006;26(8):1702–1711.
 75. Kher N, Marsh JD. Pathobiology of atherosclerosis—a brief review. *Semin Thrombos Hemostasanti*. 2004;30(6):665–672.
 76. Stary HC, Stoll JD, Yin J, et al. The natural history of atherosclerosis in the aorta in the first forty years of life. In: Fuster V, ed. *Syndromes of Atherosclerosis: Correlations of Clinical Imaging and Pathology*. Armonk, NY: Futura Publishing; 1996:225–238.
 77. Berenson GS, Srinivasan SR, Bao W, Newman 3rd WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med*. 1998;338(23):1650–1656.
 78. McGill Jr HC, McMahan CA, Herderick EE, Zieske AW, Malcom GT, Tracy RE, et al. Obesity accelerates the progression of coronary atherosclerosis in young men. *Circulation*. 2002;105(23):2712–2718.
 79. Lloyd-Jones DM, Wilson PW, Larson MG, Beiser A, Leip EP, D'Agostino RB, et al. Framingham risk score and prediction of lifetime risk for coronary heart disease. *Am J Cardiol*. 2004;94(1):20–24.
 80. de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, et al. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation*. 2004;109(23 Suppl 1):Iii33–38.
 81. Sankatsing RR, de Groot E, Jukema JW, de Feyter PJ, Pennell DJ, Schoenhagen P, et al. Surrogate markers for atherosclerotic disease. *Curr Opin Lipidol*. 2005;16(4):434–441.
 82. Keelan PC, Bielak LF, Ashai K, Jamjoum LS, Denktas AE, Rumberger JA, et al. Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. *Circulation*. 2001;104(4):412–417.
 83. Mahoney LT, Burns TL, Stanford W, Thompson BH, Witt JD, Rost CA, et al. Coronary risk factors measured in childhood and young adult life are associated with coronary artery calcification in young adults: the Muscatine Study. *J Am Coll Cardiol*. 1996;27(2):277–284.
 84. Hartialam S, Kajander S, Knuuti J, Ukkonen H, Saraste A, et al. Life course risk factor level and coronary artery calcification. The Cardiovascular Risk in Young Finns Study. *Intern J Cardiol*. 2016;225:23–29.
 85. Gidding SS, Bookstein LC, Chomka EV. Usefulness of electron beam tomography in adolescents and young adults with

- heterozygous familial hypercholesterolemia. *Circulation*. 1998;98(23):2580–2583.
86. Kobayashi K, Akishita M, Yu W, Hashimoto M, Ohni M, Toba K. Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. *Atherosclerosis*. 2004;173(1):13–18.
 87. Watanabe K, Sugiyama S, Kugiyama K, Honda O, Fukushima H, Koga H, et al. Stabilization of carotid atheroma assessed by quantitative ultrasound analysis in nonhypercholesterolemic patients with coronary artery disease. *J Am Coll Cardiol*. 2005;46(11):2022–2030.
 88. Teragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H, Chayama K. Usefulness of flow-mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease. *Am J Cardiol*. 2001;88(10):1147–1151.
 89. Furumoto T, Fujii S, Saito N, Mikami T, Kitabatake A. Relationships between brachial artery flow mediated dilation and carotid artery intima-media thickness in patients with suspected coronary artery disease. *Japan Heart J*. 2002;43(2):117–125.
 90. Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA*. 2003;290(17):2277–2283.
 91. Sanchez A, Barth JD, Zhang L. The carotid artery wall thickness in teenagers is related to their diet and the typical risk factors of heart disease among adults. *Atherosclerosis*. 2000;152(1):265–266.
 92. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, et al. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Biol*. 1996;16(8):984–991.
 93. Davis PH, Dawson JD, Riley WA, Lauer RM. Carotid intimal-medial thickness is related to cardiovascular risk factors measured from childhood through middle age: The Muscatine Study. *Circulation*. 2001;104(23):2815–2819.
 94. Lavrencic A, Kosmina B, Keber I, et al. Carotid intima-media thickness in young patients with familial hypercholesterolemia. *Heart*. 1996;76:321–325.
 95. Kusters DM, Avis HJ, DeGroot E, et al. Ten-year follow-up after initiation of statin therapy in children with familial hypercholesterolemia. *JAMA*. 2014;312:1055–1057.
 96. Wiegman A, Hutten BA, DeGroot E, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA*. 2004;292:331–332.
 97. Crouse JR, Raichlen JS, Riley WA, et al. Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: The METEOR trial. *JAMA*. 2007;297(12):1344–1353.
 98. Masoura C, Pitsavos C, Aznaouridis K, Skoumas I, Vlachopoulos C, Stefanadis C. Arterial endothelial function and wall thickness in familial hypercholesterolemia and familial combined hyperlipidemia and the effect of statins. A systemic review and meta-analysis. *Atherosclerosis*. 2011;214:129–138.
 99. Leeson CP, Kattenhorn M, Morley R, Lucas A, Deanfield JE. Impact of low birth weight and cardiovascular risk factors on endothelial function in early adult life. *Circulation*. 2001;103(9):1264–1268.
 100. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, et al. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension (Dallas, Tex: 1979)*. 2009;54(5):919–950.
 101. National Cholesterol Education Program (NCEP). Highlights of the report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents. *Pediatrics*. 1992;89(3):495–501.
 102. Rifai N, Neufeld E, Ahlstrom P, Rimm E, D'Angelo L, Hicks JM. Failure of current guidelines for cholesterol screening in urban African-American adolescents. *Pediatrics*. 1996;98(3 Pt 1):383–388.
 103. Gidding SS, Champagne MA, de Ferranti SD, et al. American Heart Association Atherosclerosis, Hypertension, and Obesity in Young Committee of Council on Cardiovascular Disease in Young. Council on Cardiovascular and Stroke Nursing. Council on Functional Genomics and Translational Biology, and Council on Lifestyle and Cardiometabolic Health. The agenda for familial hypercholesterolemia: a scientific statement from the American Heart Association. *Circulation*. 2015;132(22):2167–2192.
 104. Jabobson TA, Maki KC, Orringer CE, et al. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 2. *J Clin Lipidol*. 2015;9(6). (suppl), S1, S122.
 105. Lozano P, Henrikson NB, Dunn J, Morrison CC, Nguyen M, et al. Lipid screening in childhood and adolescence for detection of familial hypercholesterolemia; evidence report and systematic review for the US Preventive Services Task Force. *JAMA*. 2016;316:645–655.
 106. Morrison JA. A longitudinal evaluation of the NCEP-Peds guidelines for evaluated total and LDL cholesterol in adolescent girls and boys. *Prog Pediatr Cardiol*. 2003;17:159–168.
 107. Labarthe DR, Dai S, Fulton JE. Cholesterol screening in children: insights from Project HeartBeat! and NHANES III. *Prog Pediatr Cardiol*. 2003;17:169–178.
 108. Srinivasan SR, Frontini MG, Xu J, Berenson GS. Utility of childhood non-high-density lipoprotein cholesterol levels in predicting adult dyslipidemia and other cardiovascular risks: the Bogalusa Heart Study. *Pediatrics*. 2006;118(1):201–206.
 109. Srinivasan SR, Myers L, Berenson GS. Distribution and correlates of non-high-density lipoprotein cholesterol in children: the Bogalusa Heart Study. *Pediatrics*. 2002;110(3). e29.
 110. Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem*. 1990;36(1):15–19.
 111. Bachorik PS, Lovejoy KL, Carroll MD, Johnson CL. Apolipoprotein B and AI distributions in the United States, 1988–1991: results of the National Health and Nutrition Examination Survey III (NHANES III). *Clin Chem*. 1997;43(12):2364–2378.
 112. Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS, et al. Beyond low-density lipoprotein cholesterol: defining the role of low-density lipoprotein heterogeneity in coronary artery disease. *J Am Coll Cardiol*. 2007;50(18):1735–1741.
 113. Vergopoulos A, Knoblauch H, Schuster H. DNA testing for familial hypercholesterolemia: improving disease recognition and patient care. *Am J Pharmacogenomic*. 2002;2(4):253–262.
 114. Hadfield SG, Humphries SE. Implementation of cascade testing for the detection of familial hypercholesterolaemia. *Curr Opin Lipidol*. 2005;16(4):428–433.
 115. Sturm AC, Knowles JW, Gidding SS, et al. Clinical genetic testing for familial hypercholesterolemia. *J Am Coll Cardiol*. 2018;72:662–680.
 116. Miltiados G, Xenophontos S, Bairaktari E, Ganotakis M, Cariolou M, Elisaf M. Genetic and environmental factors affecting the response to statin therapy in patients with molecularly defined familial hypercholesterolaemia. *Pharmacogenet Genomics*. 2005;15(4):219–225.
 117. Humphries SE, Whittall RA, Hubbart CS, Maplebeck S, Cooper JA, Soutar AK, et al. Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. *J Med Genet*. 2006;43(12):943–949.
 118. Umans-Eckenhausen MA, Defesche JC, Sijbrands EJ, Scheerder RL, Kastelein JJ. Review of first 5 years of screening for familial hypercholesterolaemia in the Netherlands. *Lancet (London, England)*. 2001;357(9251):165–168.
 119. Leren TP, Manshaus T, Skovholt U, Skodje T, Nossen IE, Teie C, et al. Application of molecular genetics for diagnosing familial hypercholesterolemia in Norway: results from a family-based screening program. *Semin Vasc Med*. 2004;4(1):75–85.
 120. Ahmad RL, Andersen LH, Andersen, et al. US Physician practices for diagnosing familial hypercholesterolemia: data from the CASCADE-FH registry. *J Clin Lipidol*. 2016;10:1223–1229.
 121. Demmers TA, Jones PJ, Wang Y, Krug S, Creutzinger V, Heubi JE. Effects of early cholesterol intake on cholesterol biosynthesis and plasma lipids among infants until 18 months of age. *Pediatrics*. 2005;115(6):1594–1601.

122. Bayley TM, Alasmi M, Thorkelson T, Jones PJ, Corcoran J, Krug-Wisppe S, et al. Longer term effects of early dietary cholesterol level on synthesis and circulating cholesterol concentrations in human infants. *Metab Clin Exp*. 2002;51(1):25–33.
123. Mize CE, Uauy R, Kramer R, Benser M, Allen S, Grundy SM. Lipoprotein-cholesterol responses in healthy infants fed defined diets from ages 1 to 12 months: comparison of diets predominant in oleic acid versus linoleic acid, with parallel observations in infants fed a human milk-based diet. *J Lipid Res*. 1995;36(6):1178–1187.
124. US. Department of Health and Human Services and US Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at <https://health.gov/dietaryguidelines/2015/guidelines/>.
125. Simell O, Niinikoski H, Ronnema T, Lapinleimu H, Routi T, Lagstrom H, et al. Special Turku Coronary Risk Factor Intervention Project for Babies (STRIP). *Am J Clin Nutr*. 2000;72(5 Suppl):1316s–1331s.
126. Gidding S. The STRIP Study: Long term impact of a low saturated fat/low cholesterol diet. *Curr Cardiovasc Risk Rep*. 2014;8:410–415.
127. Talvia S, Lagstrom H, Rasanen M, Salminen M, Rasanen L, Salo P, et al. A randomized intervention since infancy to reduce intake of saturated fat: calorie (energy) and nutrient intakes up to the age of 10 years in the Special Turku Coronary Risk Factor Intervention Project. *Arch Pediatr Adolesc Med*. 2004;158(1):41–47.
128. Medicine IO. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. In: *The National Academies Press*. Washington: DC; 2002.
129. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med*. 1997;336(16):1117–1124.
130. Kronsberg SS, Obarzanek E, Affenito SG, Crawford PB, Sabry ZI, Schmidt M, et al. Macronutrient intake of black and white adolescent girls over 10 years: the NHLBI Growth and Health Study. *J Am Diet Assoc*. 2003;103(7):852–860.
131. Ritchie LD, Spector P, Stevens MJ, Schmidt MM, Schreiber GB, Striegel-Moore RH, et al. Dietary patterns in adolescence are related to adiposity in young adulthood in black and white females. *J Nutr*. 2007;137(2):399–406.
132. Obarzanek E, Sacks FM, Vollmer WM, Bray GA, Miller 3rd ER, Lin PH, et al. Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. *Am J Clin Nutr*. 2001;74(1):80–89.
133. Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N Engl J Med*. 2001;344(1):3–10.
134. Morenga LT, Montez JM. Health effects of saturated and trans fatty acid intake in children and adolescents: systematic review and meta-analysis. *PLOS One*. 2017;12. e0186672.
135. Velazquez-Lopez L, Santiago-Diaz G, Nava-Hernandez J, et al. Mediterranean-style diet reduces metabolic syndrome and components in obese children and adolescents with obesity. *BMC Pediatr*. 2014;14:175–179.
136. Giannini C, Diessle L, D'Adams E, et al. Influence of Mediterranean die ton carotid-intima media thickness in hypercholesterolemic children: A 12-month intervention study. *Nutr Metab Cardiovasc Dis*. 2014;24:75–82.
137. Van Horn L, Vincent E. The CHILd-1 and DASH diets: rationale and translational applications. *Pediatr Ann*. 2013;42:372–374.
138. Lauer RM, Obarzanek E, Hunsberger SA, Van Horn L, Hartmuller VW, Barton BA, et al. Efficacy and safety of lowering dietary intake of total fat, saturated fat, and cholesterol in children with elevated LDL cholesterol: the Dietary Intervention Study in Children. *Am J Clin Nutr*. 2000;72(5 Suppl):1332s–1342s.
139. Obarzanek E, Kimm SY, Barton BA, Van Horn LL, Kwiterovich Jr PO, Simons-Morton DG, et al. Long-term safety and efficacy of a cholesterol-lowering diet in children with elevated low-density lipoprotein cholesterol: seven-year results of the Dietary Intervention Study in Children (DISC). *Pediatrics*. 2001;107(2):256–264.
140. Jacobson MS, Tomopoulos S, Williams CL, Arden MR, Deckelbaum RJ, Starc TJ. Normal growth in high-risk hyperlipidemic children and adolescents with dietary intervention. *Prevent Med*. 1998;27(6):775–780.
141. Williams LA, Wilson DP. Nutritional management of pediatric dyslipidemia. In: DeGroot LJ, Chrousos G, Guban K, et al., eds. *Endotext [Internet]. Diagnosis and Treatment of Disease of Lipids and Lipoprotein Metabolism in Adults and Children*; 2016. South Dartmouth, MA; last updated June 22, 2016.
142. Lifshitz F, Moses N. Growth failure. A complication of dietary treatment of hypercholesterolemia. *Am J Dis Child (1960)*. 1989;143(5):537–542.
143. Williams CL, Hayman LL, Daniels SR, Robinson TN, Steinberger J, Paridon S, et al. Cardiovascular health in childhood: a statement for health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation*. 2002;106(1):143–160.
144. Tershakovec AM, Shannon BM, Achterberg CL, McKenzie JM, Martel JK, Smicklas-Wright H, et al. One-year follow-up of nutrition education for hypercholesterolemic children. *Am J Pub Health*. 1998;88(2):258–261.
145. Gylling H, Siimes MA, Miettinen TA. Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. *J Lipid Res*. 1995;36(8):1807–1812.
146. Amundsen AL, Ose L, Nenseter MS, Ntanos FY. Plant sterol ester-enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia. *Am J Clin Nutr*. 2002;76(2):338–344.
147. Williams CL, Bollella M, Spark A, Puder D. Soluble fiber enhances the hypocholesterolemic effect of the step I diet in childhood. *J Am Coll Nutr*. 1995;14(3):251–257.
148. Davidson MH, Dugan LD, Burns JH, Sugimoto D, Story K, Drennan K. A psyllium-enriched cereal for the treatment of hypercholesterolemia in children: a controlled, double-blind, crossover study. *Am J Clin Nutr*. 1996;63(1):96–102.
149. Vos MB, Kaar JL, Welsh, et al. Added sugars and cardiovascular risk in children: A scientific statement from the American Heart Association. *Circulation*. 2017;135:e1017–e1034.
150. Starc TJ, Shea S, Cohn LC, Mosca L, Gersony WM, Deckelbaum RJ. Greater dietary intake of simple carbohydrate is associated with lower concentrations of high-density-lipoprotein cholesterol in hypercholesterolemic children. *Am J Clin Nutr*. 1998;67(6):1147–1154.
151. Gidding SS, Dennison BA, Birch LL, Daniels SR, Gillman MW, Lichtenstein AH, et al. Dietary recommendations for children and adolescents: a guide for practitioners. *Pediatrics*. 2006;117(2):544–559.
152. Reinehr T, de Sousa G, Toschke AM, Andler W. Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. *Am J Clin Nutr*. 2006;84(3):490–496.
153. Engler MM, Engler MB, Malloy M, Chiu E, Besio D, Paul S, et al. Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. *Int J Clin Pharmacol Ther*. 2004;42(12):672–679.
154. Shah AS, Wilson DP. Primary hypercholesterolemia in children and adolescents. *J Clin Lipidol*. 2015;9:S20–S28.
155. Wiegman A, Hutten BA, de Groot E, Rodenburg J, Bakker HD, Buller HR, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA*. 2004;292(3):331–337.
156. Rodenburg J, Vissers MN, Wiegman A, van Trotsenburg AS, van der Graaf A, de Groot E, et al. Statin treatment in children with familial hypercholesterolemia: the younger, the better. *Circulation*. 2007;116(6):664–668.
157. American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2004;28(Suppl 1):S4–S36.
158. McCrindle BW. Drug therapy of hyperlipidemia. *Prog Pediatr Cardiol*. 2003;17:141–150.
159. McCrindle BW, O'Neill MB, Cullen-Dean G, Helden E. Acceptability and compliance with two forms of cholestyramine in the treatment of hypercholesterolemia in children: a randomized, crossover trial. *J Pediatr*. 1997;130(2):266–273.
160. Tonstad S, Knudtzon J, Sivertsen M, Refsum H, Ose L. Efficacy and safety of cholestyramine therapy in peripubertal and prepubertal children with familial hypercholesterolemia. *J Pediatr*. 1996;129(1):42–49.

161. McCrindle BW. Hyperlipidemia in children. *Thrombos Res.* 2006;118(1):49–58.
162. McCrindle BW, Helden E, Cullen-Dean G, Conner WT. A randomized crossover trial of combination pharmacologic therapy in children with familial hyperlipidemia. *Pediatr Res.* 2002;51(6):715–721.
163. Daniels SR, Gidding SS, de Ferranti SD. Pediatric aspects of familial hypercholesterolemias: recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol.* 2011;5(3 Suppl):S30–S37.
164. Arambepola C, Farmer AJ, Perera R, Neil HA. Statin treatment for children and adolescents with heterozygous familial hypercholesterolemia: a systematic review and meta-analysis. *Atherosclerosis.* 2007;195(2):339–347.
165. Rodenburg J, Vissers MN, Daniels SR, et al. Lipid-lowering medications. *Pediatr Endocrinol Rev.* 2004;2:171–180.
166. Kusters DM, Caceres M, Coll M, Cuffie C, Gagné C, Jacobson MS, et al. Efficacy and safety of ezetimibe monotherapy in children with heterozygous familial or nonfamilial hypercholesterolemia. *J Pediatr.* 2015;166:1377–1384.
167. Marais DA, Blom DJ, Petrides F, Gouëffic Y, Lambert G. Proprotein convertase subtilisin/kexin type 9 inhibition. *Curr Opin Lipidol.* 2012;23:511–517.
168. Cohen JC, Boerwinkle E, Mosley Jr TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med.* 2006;354:1264–1272.
169. Rosenson RS, Hegele RA, Fazio S, Cannon CP. The Evolving Future of PCSK9 Inhibitors. *J Am Coll Cardiol.* 2018;72:314–329.
170. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, et al. Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med.* 2015;372:1500–1509.
171. Colletti RB, Neufeld EJ, Roff NK, McAuliffe TL, Baker AL, Newburger JW. Niacin treatment of hypercholesterolemia in children. *Pediatrics.* 1993;92(1):78–82.
172. McCrindle BW, Helden E, Conner WT. Garlic extract therapy in children with hypercholesterolemia. *Arch Pediatr Adolesc Med.* 1998;152(11):1089–1094.
173. Guardamagna O, Abello F, Baracco V, et al. The treatment of hypercholesterolemic children: efficacy and safety of a combination of red yeast rice extract and policosanols. *Nutr Metab Cardiovasc Dis.* 2011;21:424–429.
174. Lee WP, Datta BN, Ong BB, Rees A, Halcox J. Defining the role of lipoprotein apheresis in the management of familial hypercholesterolemia. *Am J Cardiovasc Drug.* 2011;11(6):363–370.

Index

Note: Page numbers followed by “f” indicate figures, “t” indicate tables, and “b” indicate boxes.

A

- ABCC8, 285–286
Abdominal ionizing radiation, 864
Abetalipoproteinemia, 1008–1009
Abnormal extracellular fluid, 327
Absorptiometry, 266. *See also* Dual-energy X-ray absorptiometry (DEXA)
ACA. *See* Adrenal cytoplasmic autoantibodies (ACA)
Acanthosis nigricans, type A insulin resistance with, 864
Accumbens, nucleus, 947
Accuracy/method comparison, 90
Acetoacetate, 830
Acetones, 830
ACH, 328
Achondroplasia, 796–798
 C-type natriuretic peptide agonists, 342
Acidic serine aspartate-rich MEPE-associated motif (ASARM), 221t, 231–232, 802t
Acid-labile subunit deficiency, 324
Acidophilic cells, 1–2
Acidosis, 836
Acne, 598–599
Acrodysostosis, 719
Acromegaly, 6
Acromesomelic dysplasia-Maroteaux type, 265, 328
ACTH. *See* Adrenocorticotropic hormone (ACTH)
Activating function (AF), 221t
Activation function-1 (AF-1), 242
Active insulin, 846
Activins, 549, 666
Acute thyroiditis, 407
Acyl-coenzyme A dehydrogenase deficiency, 194–195, 194f
Adaptor-related protein complex 2 (AP2S1), 223–226t
ADCY5, 117
Addison disease
 in APS, 885t, 889–890, 892
 autoantigens in, 887t
 isolated, autoantibodies in, 897
 stages of, 895t
Adenoma
 in Cushing syndrome, 468
 familial isolated pituitary adenomas, 504
Adenomatous polyposis coli (APC), 221t, 514
Adenosine monophosphate (AMP), 221t, 802t
Adenosine triphosphatase (ATPase), 221t
Adenosine triphosphate (ATP), 221t, 802t, 941
Adenosine triphosphate (ATP)–regulated potassium channel (K_{ATP}), 6
Adenosine triphosphate-sensitive potassium (KATP), 910
 diffuse form of, 910
 focal form of, 910
Adenylate cyclase, 548f
Adenyl cyclase (ADCY3), 223–226t
ADH. *See* Autosomal dominant hypercholesterolemia (ADH)
Adhesion G-protein coupled receptor A2 (ADGAR2), 223–226t
Adiopokines, 109
Adipocyte-, C1q-, and collagen domain-containing (Adiponectin), 223–226t
Adipocytes, 245–247
 in osteoblastogenesis, 246f
Adipocytokines, 955–956
Adiponectin, 955
ADIPOR1, 955
ADIPOR2, 955
Adipose tissue
 fasting metabolic systems, 829t
 insulin resistance, 953
 maturation of, 555–556
Adipsic hypernatremia, 380–381
Adolescence
 anovulation in, 562–563, 563f, 565f
 BMC in, 262t, 267–268t
 BMD in, 262t, 267–268t
 bone deposition in, 259t
 bone mineralization in, 750–768, 752–753t
 calcium (Ca) intake in, 228, 228t
 female reproductive system, 531–536, 533f, 534t, 535f
 GnRH secretion in, 531–533, 533f
 hyperandrogenism in, 588–600, 589b, 590f
 hypocalcemia in
 etiology of, 729t, 735–741
 evaluation of, 741–742, 742t
 management of, 742–744
 hypocalcemia in
 etiology of, 709–712t, 722–725
 evaluation of, 720f, 725–727
 management of, 727–728
 lipids in, 1015t
 with low bone mass, 782–785
 puberty in, 531–536, 533f, 535f
 sexual maturation in, 558–560
Adrenal cortex
 adrenal excess, 467–473
 Adrenal cortex (*Continued*)
 anatomy, 427–428
 clinical evaluation, 440
 development, 426–427
 embryology, 426–427
 fetal formation of, 426–427
 glucocorticoid therapy and withdrawal, 473–477
 history, 425–426
 insufficiency, 460–467
 laboratory evaluation, 440–446
 plasma steroids and disposal, 437–440
 response of, in maturation, 547f
 steroid hormone synthesis, 428–434
 steroidogenesis
 genetic lesions in, 446–460
 regulation, 434–437
 Adrenal cytoplasmic autoantibodies (ACA), 896
 Adrenal development, 129
 Adrenalectomy, for CAH, 459
 Adrenal enzyme autoantibodies, 897
 Adrenal function, clinical and laboratory evaluation of, 440–446
 Adrenal gland
 androgen secretion in, 437, 437f
 cytoplasmic autoantibodies in, 896
 enzyme autoantibodies in, 897
 excess of, 467–473, 467t
 function of
 clinical evaluation of, 440
 laboratory evaluation of, 440–446
 hypoplasia congenita of, 463
 tumors of, 469, 472
 weight of, 427f
 Adrenal hyperplasia
 congenital lipid, 144
 congenital syndromes, 20
 Adrenal hypoplasia congenita (AHC)
 autosomal forms of, 463
 IMAGe Syndrome, 463
 MCM4 mutations, 463
 MIRAGE syndrome, 463
 Triple A Syndrome, 463
 X-linked, 463
 Adrenal insufficiency
 ACTH causing, 466
 acute primary, 461, 461t
 causes of, 461, 461t
 chronic
 autoimmune disorders of, 461–462
 signs and symptoms of, 461t
 hypothalamic dysfunction causing, 466
 long-term steroid therapy for, 467
 metabolic disorders causing, 464–465

- Adrenal insufficiency (*Continued*)
 POMC disorders causing, 466–467
 secondary, 466–467
 signs and symptoms of, 461*t*
- Adrenarche
 adrenal androgen secretion in, 437, 437*f*, 546–547, 547*f*
 premature, 560–561, 673
 regulation of, 437
- Adrenocortical failure, 893
- Adrenocortical tumors, 509
- Adrenocorticotrophic hormone (ACTH), 1–2
 actions of, 435–436
 causing adrenal insufficiency, 466
 deficiency, 466
 ectopic syndrome, 469–470
 glucocorticoid feedback in, 436
 hypothalamic causes, 466
 -independent multinodular adrenal hyperplasia, 469
 in maturation, 547*f*
 metyrapone test, 446
 in pituitary gland, 435
 resistance syndromes, 464
 rhythms of, 436
 stimulation tests, 444–446, 571
 structure of, 435*f*
- Adrenocorticotropin deficiency, 923–924
- Adrenocorticotropin receptors, 36–37
- Adrenodoxin reductase (AdRed), 428–430, 429*f*
- Adrenoleukodystrophy (ALD), 464–465
- Adults
 care, 650–652
 female reproductive system in, 536–539, 536*f*
 fetal origins of disease in, 4
 height, prediction of, 313–314
 hypophosphatasia, 767
- Afferent system
 for energy balance, 941–943
 negative regulation, 942–943
 positive regulation, 941–942
 vagus nerve, 941
- Age
 aldosterone *vs.*, 459*f*
 body mass-index for, 950*f*
- Age-standardized incidence, of diabetes mellitus type 1, 818–819, 818*f*
- Aggrecan (Acan), 221*t*
- AGL gene, 920
- Agonist, 31
- Agranulocytosis, 404
- AgRP, 941
- AgRP/NPY neurons, 944
- AGTR2, 117
- AIA. *See* Anti-interferon autoantibodies (AIA)
- AITD, 893
- AKT. *See* Protein kinase B (PKB)
- AKT2 hypoglycemia, 913
- Alanine, 922
- Albright hereditary osteodystrophy (AHO), 328, 802*t*, 966
- Albumin, 220
- Alcohol-induced hypoglycemia, 925
- ALD3-CDG (CDG-Id), 913
- Aldo-keto reductase (AKR), 428.
See also 17 β -HSD type 5
- Aldosterone
 concentration of, age *vs.*, 459*f*
 endocrine renin-angiotensin system, 365–366
 glucocorticoid-suppressible hyper-, 460
 PHA, 473
 plasma concentration of, 441, 442–443*t*
 renin-angiotensin system, 364–366
 therapeutic, potency of, 457*t*
- Aldosterone-producing adenomas, 471–472
- Aldosteronism
 primary, cellular origins of, 472
 “Alimentary hypoglycemia,” 915
- Alkaline phosphatase, 223–226*t*, 233–234
 disorders of, 766–768
- Alkalosis, 836
- Allan-Herndon-Dudley syndrome, 207, 213
- Allgrove syndrome, 463
- Allosteric antagonist, 31
- Allosteric modulators, 31
- Alopecia, 244, 757–758, 885*t*, 892
- Alpha subunit of inhibitory GTP-binding protein (G $_{i\alpha}$), 221*t*
- Alpha subunit of stimulatory GTP-binding protein (G $_{s\alpha}$), 221*t*
- ALPL genes, 233, 766–768
- Alstrom syndrome, 865, 969–970
- Amenorrhea
 in APS, 892
 athletic, 585
 causes of, 580–581*f*
 differential diagnosis of, 574*b*, 580–581*f*
 functional hypothalamic, 585
 post-pill, 586
 psychogenic, 585
- Amino acid
 164-amino acid preprovasopressin, 359*f*
 aquaporin, 365*f*
 branched-chain, 962–963
 composition, of human natriuretic peptide system, 367*f*
 vasopressin, 363*f*
- 14-Amino acid peptide somatostatin, 304
- Amino acids (AA), 221*t*
- Amino acid tyrosine, 495, 496*f*
- Amino terminal cross-link telopeptide of collagen type 1 (NTX), 221*t*
- Amino terminal propeptide of collagen type III (PIIINP), 802*t*
- Amino terminal telopeptide of collagen type I (NTx), 802*t*
- Amiodarone-induced thyrotoxicosis (AIT), 408
- Amygdala, 947
- Amylin, 942
- Amylo-1,6-glucosidase deficiency, 920–921
- Analytic sensitivity, in analytic validation, 90
- Analytic specificity, in analytic validation, 90
- Anatomic defects, congenital, 377
- Anauxetic dysplasia, 799
- Andersen disease, 921
- Androgen action disorders, 148–149
- Androgen-dependent phase, 663
- Androgen insensitivity syndrome, 680
- Androgen receptor (AR), 59, 663
 for skeletal growth, 264
- Androgens, 264, 308
 action of
 in steroid metabolism, 552*f*
 anti-, inhibitors of, 458
 overproduction of, in
 hyperandrogenism, 594, 595*f*, 598*f*
 secretion of
 in adrenarche, 546–547, 547*f*
 regulation of, 546*f*
- Androstenedione, 431, 549, 668–669
- Anemia, 919
 autoantigens in, 887*t*
- Angiofibromas, 509, 510*f*
- Angiotensin
 endocrine renin-, -aldosterone, 365–366
 local renin-, systems, 366
 renin-aldosterone-, 364–366
- Anogenital distance, 130
- Anorchia, 666
- Anorexia nervosa, 980–982
 bone metabolism in, 981–982
 definition of, 980
 endocrine manifestations of, 981–982, 981*f*
 fat-derived hormones in, 982
 growth hormone-IGF-1 axis, 981
 hypogonadism in, 576
 hypothalamic-pituitary-adrenal axis in, 981
 hypothalamic-pituitary-gonadal axis in, 982
 hypothalamic-pituitary-thyroid axis in, 981
 treatment of, 982
- Anorexigenic peptide YY (PYY), 542
- Anosmia, 575–576
- Anosmin, 575–576
- Anovulation, 562–563, 563*f*, 565*f*
 disorders with, differential diagnosis of, 582–583*f*
 hypothalamic, 585–587
- Antenatal Bartter syndromes, 733
- Antenatal neonatal syndromes, 733
- Anteroventral periventricular (AVPV) region, 663–664
- Antiandrogen inhibitors, 458
- Antibody, islet cells, 823*t*
- Antigens
 auto-, 887, 887*t*
 in diabetes mellitus, 822
 self-, tolerance and, 888*f*
- Anti-interferon autoantibodies (AIA), 896

- Anti-Mullerian hormone (AMH), 639f, 663, 666
- Antithyroid drugs, placental transfer of, 204
- Antithyroid drug (ATD) therapy
duration and prediction of remission, 404
methimazole, 403–404
propylthiouracil, 403–404
- Aorta disease of, 640
- Aortic complications, 640
- Aortopathy, 640
- APC gene, 223–226t
- APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy). *See* APS I
- Apert syndrome, 248
- ApoB-100, familial ligand defective, 1007, 1007t
- ApoE pathways, 1009
- Apolipoproteins, 1005t
- Apparent athyreosis, 208–209
- Appetite
regulation, 940
stress and, 959–960
- APS. *See* Autoimmune polyglandular syndromes (APS)
- APS I
clinical aspects, 891–892
genetics of, 890–891
- APS II
clinical aspects, 893
genetics of, 890–891
- Aquaporin-2
mutations of, 381
structure of, 365f
- AR. *See* Androgen receptor (AR)
- Arachnoid cysts, 319–320
- Arginine vasopressin (AVP), 1–2
function of, 434–435
in hypothalamus, 434–435
- ARH. *See* Autosomal-recessive hypercholesterolemia (ARH)
- Aristaless-related homeobox (ARX) gene mutations, 131
- Aromatase, 432
inhibitors, 458
regulation of, 546f
- Aromatase deficiency, 343
- Aromatase inhibitors
short stature treatment, 342
- β -Arrestin, 237–238
- β -Arrestin 1, 223–226t
- Artificial hypoglycemia, 927–928
- “Artificial pancreas,” 815, 849–850
- Asherman syndrome, 565–566
- Astrocystoma, hypothalamic, 577f
- Atelosteogenesis, 798–799
- Atherosclerotic cardiovascular disease (ASCVD), 1004
- Atherosclerotic disease, premature, risk for, in Turner syndrome, 642
- Athletic amenorrhea, 585
- Athyreosis, apparent, 208–209
- Athyreotic human neonate, 207
- ATPase calcium channel (ATP2B1), 227
- ATPase, Ca (2⁺)-transporting
plasma membrane, 223–226t
- ATPase, Ca (2⁺)-transporting (*Continued*)
slow twitch, 223–226t
- Atresia, 539
- Atretic follicles, 538f
- Atrial natriuretic peptide (ANP)
anatomy and biochemistry of, 366, 367f
hyponatremia with, 375–376
regulation of secretion and action of, 367–368
- ATR-X syndrome, 135–136
- Audiogram, in Turner syndrome, 643f
- Autism spectrum disorder (ASD), 698
- Autoantibodies
adrenal cytoplasmic, 896
adrenal enzyme, 897
antiinterferon, 896
in APS, 896–898
to aromatic L-amino acid decarboxylase, 897
in hypoparathyroidism, 897
to IL-17A, IL-17F, and IL-22, 896
to isolated Addison disease, 897
steroidal cell, 897
to tyrosine hydroxylase, 898
- Autoantigens
in Addison disease, 887t
in anemia, 887t
in autoimmune diseases, 887–888
in celiac disease, 887t
- Autocrine action, 2–3
- Autocrine regulation, of childhood growth, 308–309
- Autoimmune disorders, 887–888
autoantigens in, 887–888, 887t
of chronic adrenal insufficiency, 461–462
with diabetes mellitus, 853–854
polyendocrine syndrome
type 1, 462
type 2, 462
tolerance defects in, 888–889
in Turner syndrome, 643
- Autoimmune hepatitis, 885t, 887t
- Autoimmune hypoglycemia, 914
- Autoimmune oophoritis, 575
- Autoimmune polyendocrine syndrome (APS), 802t
type I, 724
type II, 724–725
- Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), 724, 802t
- Autoimmune polyendocrinopathy syndrome
type I, 724
type II, 724
- Autoimmune polyglandular syndromes (APS), 884–898
Addison disease, 885t, 889–890, 892
amenorrhea in, 892
autoantibodies in, 896–898
classification of, 889–890, 891f
clinical aspects of, 891–894
diagnostic approach to, 894–896, 894f
follow-up to, 894–896, 894f
genetics of, 890–891
IPEX in, 893–894
- Autoimmune polyglandular syndromes (APS) (*Continued*)
tolerance and, 884
treatment of, 896
- Autoimmune regulatory (AIRE) gene, 724–725, 884–885, 896
- Autoimmune thyroid disease, 885t
autoantibodies in, 894–895
- Autoimmune thyroiditis, 406
- Autoimmunity
checkpoint inhibitors, 889
mechanisms for, 884–889, 885f, 885t, 888f
thyogastric, 893
in Turner syndrome, 634t, 643
- Autoradiography, 10
- Autosomal dominant
hypercholesterolemia (ADH), 1007, 1007t
- Autosomal dominant hyperthyroidism, 402
- Autosomal dominant hypoparathyroidism, 745–746
- Autosomal dominant hypophosphatemia with urolithiasis
type 1, 765
type 2, 765
- Autosomal dominant hypophosphatemic rickets (ADHR), 221t, 802t
- Autosomal-recessive hypercholesterolemia (ARH), 1007, 1007t
- Autosomal recessive hypophosphatemic rickets (ARHR), 221t, 802t
- AVP. *See* Arginine vasopressin (AVP)
- Axin, 248
- Axis inhibitor 1, 223–226t
- B**
- Barakat syndrome, 715–716
- Bardet-Biedl syndrome (BBS), 865, 969
- Bariatric surgery, 915
in pediatric obesity, 975–977
- Basal-bolus therapy, 840, 841f
- Basal insulin rates, 845–846
temporary, 846
verifying settings of, 846
- Basal insulin suspend, 846
- “Basal pattern,” 844
- Basic multicellular unit (BMU), 221t
- Basophilic cells, 1–2
- Bayley-Pinneau prediction, 313–314
- B cells, tolerance of, 887
- BDNF gene mutations/variants, 968
- Beckwith-Wiedemann syndrome (BWS), 189f, 281–282, 514, 912
- Benign hypophosphatasia, 767
- Beta-adrenergic receptors, 207
- Beta blockers, 499
- Beta cells
function of
genetic abnormalities in, 862t
genetic defects in, 861–864, 863t
history of, 819f
residual, 852

- Beta cells (*Continued*)
 insulin and
 resistance of, 815*f*
 secretion of, 280*f*
 pancreatic, 911
- Betamethasone, therapeutic, potency of, 457*t*
- Biased signaling, 31
- "Big IGF-2," 915
- Bilateral idiopathic hyperaldosteronism, 472
- Bilateral leg lengthening surgery, 342
- Bile acids, 943
 binding agents, 1014–1015, 1015*t*
- Bioinactive growth hormone, 323
- Bioinactive insulin-like growth factor-1, 325
- Biological sex, biological male/female, 696*b*
- Birth
 adaptations in endocrine function at, 5
 low, weight infants, 748–749
- Bisphosphonates, in low bone mass, 783–784
- Bivariate reference interval presentation, 104*f*
- Bladder, exstrophy of, 152
- Bladder-exstrophy-epispadias complex (BEEC), 152
- Blomstrand chondrodysplasia, 237–238, 799
- Blood glucose monitoring, 847
- Blood production rate, 549–550
- Bloom syndrome, 331
- "Blue diaper syndrome," 733
- BMC. *See* Bone mineral content (BMC)
- BMD. *See* Bone mineral density (BMD)
- BMP1 protein, 780–781
- BMP receptor 1A, 223–226*t*
- BMP receptor 2, 223–226*t*
- Body
 temperature of, maintenance of, 5
 weight
 glucose production *vs.*, 904–905, 905*f*
- Body mass index (BMI), 335*b*
 effect of, on pubertal timing, 670–671
- Body proportions, 311–313
- Bohr effect, 836
- Bolus insulin dosages, 846–847
 for correction of hyperglycemia, 846
 for food coverage, 846
- Bone
 age of, 313–314
 children with short stature, 335
 in sexually infantile girls, 574*b*
 calcium content of, 261
 deposition of
 in female child/adolescent, 259*t*
 in male child/adolescent, 259*t*
 development of, endochondral, 247
 differentiation of, 246*f*, 249
 disorders of, 747–795
 extracellular matrix, 256–259
 formation, 245–248, 246*f*, 258*f*, 259–265
 disorders of, 750–785
 exercise in, 261
- Bone (*Continued*)
 in mother/neonate, 258*t*
 PTH-rP in, 245–247, 246*f*
 increased bone mass, 750
 mass of, 261
 assessment of, 262*t*, 265–272, 268*t*, 269*f*
 bisphosphonates in, 783–784, 784*f*
 high, 786–793, 787–788*t*
 increased in infants, 750
 low, 770–778, 771*t*
 maturation of, 555
 metabolism, 706–708*t*
 of mineral homeostasis and, genes
 involved in, 223–226*t*
 mineralization, 259–265
 disorders in, 750–785
 glucocorticoid for, 263–265
 hormonal regulation of, 263–265
 Wnt signaling pathway, 248–249, 249–250*f*
 modeling of, 245–247
 morphogenic proteins, 245–247, 246*f*
 quality, assessment of, 265–272
 remodeling of
 sites for, 254–255
 resorption of, 258*f*
 in mother/neonate, 258*t*
 strength of, 260–261
 assessment of, 262*t*, 265–272, 268*t*, 269*f*, 270*t*
- Bone alkaline phosphatase (BAP), 221*t*
- Bone collagen subunit $\alpha 1(I)$ (COL1A1), 802*t*
- Bone metabolism, 981–982
- Bone mineral apparent density (volumetric BMD) (BMAD), 221*t*
- Bone mineral content (BMC), 221*t*, 261, 802*t*
 in child/adolescent, 262*t*, 267–268*t*
 hormones for, 263
 in infants, 262*t*
 for non-black/black male/female, with DEXA, 268*t*
- Bone mineral density (BMD), 221*t*, 266–267, 802*t*
 in child/adolescent, 262*t*, 267–268*t*
 in infants, 262*t*
- Bone morphogenetic protein (BMP), 221*t*, 802*t*
- Bone morphogenetic protein-1, 223–226*t*
- Bone morphogenetic protein-2, 223–226*t*
- Bone morphogenetic protein-4, 223–226*t*, 245–247
- Bone morphogenetic protein-7, 223–226*t*
- Bone remodeling unit (BRU), 221*t*
- Bone sialoprotein, 258–259
- Bone transmission time (BTT), 221*t*, 269–270
- BOREALIN, 210
- Bovine cartilage equivalent (BCE), 802*t*
- Brain
 damage, hypoglycemic, 181–182
 death, causing central diabetes
 insipidus, 378
- Brain (*Continued*)
 hypothalamic astrocytoma in, 577*f*
 inflammation and infiltration of, 318–319
 irradiation of, 320
 trauma of, 318
 tumors of, 319
- Brain-derived neurotrophic factor (BDNF), 944, 968
- Brain type 2 deiodinase, 203–204
- Breast
 development of, 559, 559*f*
 pubertal milestone attainment in, 559*t*
- "Brittle bone disease," 778–779
- "Brittle diabetes," 817
- Bromodomain adjacent to zinc finger domain, 1B (WSTF), 223–226*t*
- Bronchial neuroendocrine tumor, 509
- Brown adipose tissue thermogenesis, 207
- Bruck syndromes, 780–781
- BSND gene (Barttin), 223–226*t*
- C**
- Ca. *See* Calcium (Ca)
- Cachexia
 cancer, 979–980
 cytokines in, 979*b*
 starvation response *vs.*, 977
- CAH. *See* Congenital adrenal hyperplasia (CAH)
- Calbindin (9K), 223–226*t*
- CALCA, 238
- Calcilytics, 229–230
- Calcimimetics, 229–230, 744
- Calciopenic rickets, 753–759
- Calcitonin, 223–226*t*, 237–239, 739–740
 as class B receptors, 45
- Calcitonin-gene-related-peptide- α , 238
- Calcitonin receptor, 223–226*t*
- Calcitonin release-activated calcium modulator, 223–226*t*
- Calcitonin sensing receptor, 223–226*t*
- Calcitriol, 227, 239*f*, 240
 genes regulated by, 244*f*
 preparations of, 721*t*
 responses to, 245*f*
 VDR and, 241–242, 244*f*
- Calcium (Ca), 220–230, 221*t*
 absorption of, 227–228
 in bone, 261
 in gastrointestinal tract, 227
 homeostasis, 222*f*
 intake of, recommended, 227–228, 228*t*
 in muscles, 220–226
 obesity and, 964
 preparations of, 721*t*
 PTH regulating, 235
- Calcium channel, voltage dependent, L-type, alpha 1C subunit, 223–226*t*
- Calcium-ionized (Ca^{2+}), 221*t*
- Calcium-ionized, extracellular (Ca^{2+e}), 802*t*
- Calcium sensing receptor (CaSR), 45–47, 228–230, 723*f*, 736*f*, 802*t*
- Calmodulin, 227
- Calmodulin 1 (CALM1), 223–226*t*, 227

- Camurati-Engelmann disease.
See Progressive diaphyseal dysplasia
- Cancellous bone, 252
- Cancer
medicine, NGS and cell-free DNA, 25–26
medullary thyroid, 415–416
papillary thyroid, 408–410
survival, chemotherapy, radiation therapy and, 679–680
vasopressin and, 375*t*
- Candidal balanitis, 831
- Candidal vaginitis, 831
- Candidate gene-based studies, 669–670
- Carbohydrate
-deficient glycoprotein
hyperinsulinism, 912–913
dietary fat *vs.*, in obesity, 960
metabolism, abnormalities of, 644
- Carbohydrate-deficient glycoprotein hyperinsulinism, 912–913
- Carbohydrate-deficient glycoprotein syndrome, 576
- Carbonic anhydrase II, 223–226*t*, 786–789
- Carboxyl telopeptide of collagen type I (ICTP), 802*t*
- Carboxyl-terminal cross-link telopeptide of collagen type I (CTX), 802*t*
 α 1 chain, 221*t*
generated by matrix metalloproteinase (ICTP), 221*t*
- Carboxyl-terminal peptides, 235
- Carboxyl terminal propeptide of collagen type I (PICP), 802*t*
- Carboxypeptidase E (CPE), 967
- Carcinoembryonic antigen (CEA), 503
- Carcinoid tumors, 509
- Carcinoma
papillary-follicular, 396*b*
thyroid, 500–503
- Cardiac care, in Turner syndrome, 641–642
- Cardiovascular disease, 860
metabolism in, 1004–1008, 1005–1006*f*, 1005*t*
- Cardiovascular screening, Turner syndrome, 641
- Cardiovascular system
abnormalities, in Turner syndrome, 634*t*, 638–642
assessment and monitoring, 646–647
sex hormones in, 557
- Carney complex (CNC), 6, 411, 503–504, 503*f*
- Carney triad, 514
- Carpenter syndrome, 970
- CART, 941
- Cartilage, 245–247
disorders of, genetic causes of, 706–708*t*
epiphyseal, growth plate, 246*f*
- Cartilage-associated protein (CRTAP), 223–226*t*, 802*t*
- Cartilage matrix regulation, childhood growth, 309
- Cartilage oligomeric matrix protein (COMP), 221*t*, 223–226*t*
- CaSR, 228. See also Calcium sensing receptor (CaSR)
- Catch-up growth, 309–310
- Catecholamine-producing tumors, 491, 492*t*, 497
- Catecholamines, 207, 853
actions of, 495
biosynthesis of, 495
clinical presentation of, 492*t*, 495
complications of, 495
diagnosis of
biochemical, 496–497
radiographic studies in, 497–498
radiographic studies in, 497–498, 498*f*
- β -Catenin, 248, 249–250*f*
- β 1-Catenin, 223–226*t*
- Cathepsin K, 223–226*t*
- Caveolin 1 (CAV1), 223–226*t*
- Cayler cardiofacial syndrome, 715
- CCK. See Cholecystokinin (CCK)
- CD4, 886
- CDC73 gene, 737–738
- CDKN1C, 131–133
- cDNA. See Complementary deoxyribonucleic acid (cDNA)
- Celiac disease, 854
autoantigens in, 887*t*
in Turner syndrome, 644
- Cell-free deoxyribonucleic acid (cfDNA), 110
- Cell hypertrophy, 299
- Cell proliferation, 299
- Cellular signaling, 2*f*
- Cementoossifying fibromas, 505
- Central anabolic signals, 944–945
- Central catabolic signals, 943–944
- Central diabetes insipidus, 376–381
adipsic hypernatremia, 380–381
autoimmune disease causing, 377–378
brain death causing, 378
causes of, 376–381
congenital anatomic defects with, 377
drugs causing, 378
fluid therapy for, 378–379
genetic causes of, 376–377
increased vasopressin metabolism causing, 378
infectious disease causing, 377–378
infiltrative disease causing, 377–378
neoplasms with, 377
neurosurgical intervention for, 377
primary enuresis causing, 378
trauma and, 377
treatment of, 378–380
vasopressin analogs for, 379–380
- Central hypothyroidism, 213
- Central nervous system (CNS), 402
maturation of, 556–557
thyroid hormone actions in, 207
vasopressin and, 375*t*
- Central precocious puberty, 675
- Central processing, in energy balance, 943–945, 946*f*
- Centripetal migration theory, 427–428
- Cerebral coma, 831
- Cerebral edema, 837–838
- Cerebral glucopenia, 853
- Cerebral obtundation, 829–831
- Cerebral salt wasting, 375–376
treatment of, 376
- Chaperone proteins, 257
- CHARGE syndrome, 133
- CHD7 gene, 133
- Checkpoint inhibitors, 889
- Chemiluminescent moiety, 12–13
- Chemotherapy, radiation therapy and cancer survival, 679–680
- Childhood growth
biological basis of, 299
clinical evaluation
adult height prediction, 313–314
body proportions, 311–313
growth charts, 311
inheritance of stature, 314
measurement of, 310–311
skeletal maturation, 313
disorders of, 314
short stature, 314–342
tall stature, 342–347
growth plate function, 300*f*
regulation
autocrine/paracrine regulation of, 308–309
cartilage matrix regulation of, 309
catch-up growth, 309–310
cytokine regulation of, 308
endocrine regulation of, 300–308
growth plate senescence, 309
intracellular regulation of, 309
normal variation in stature, 310
nutritional regulation of, 308
- Childhood hypophosphatasia, 767
- Children
atherosclerotic cardiovascular disease in, 1010, 1010*b*
BMC in, 262*t*, 267–268*t*
BMD in, 262*t*, 267–268*t*
bone deposition in
female, 259*t*
male, 259*t*
bone mineralization in, 750–768, 752–753*t*
calcium (Ca) intake in, 228, 228*t*
congenital hypothyroidism, 400
coronary artery disease in, 1010
diencephalic syndrome in, 980
diffuse nontoxic goiter in, 396*b*
failure to thrive in, 977–979
glucose metabolism in, 904–906, 905*f*
Graves disease in, 403
hypercalcemia in
etiology of, 735–741
evaluation of, 741–742, 742*t*
management of, 742–744
hypocalcemia in
etiology of, 709–712*t*, 722–725
evaluation of, 720*f*, 725–727
management of, 727–728
hypoglycemia in, 904
definition of, 908
emergency treatment of, 928
fasting systems approach, 908–910
major causes of, 910–928

- Children (*Continued*)
 symptoms, signs, and effects, 906–908
 tests for diagnosis, 929
 hypoparathyroidism in, 710–712*t*, 713–719
 lipids in, 1015*t*
 with low bone mass, 782–785
 low-density lipoprotein apheresis in, 1016
 mineral homeostasis in, 705–747, 795–801
 sexual maturation in, 534*t*, 558
 tolbutamide in
 with neonatal diabetes mellitus, 285
 Turner syndrome in, 645*b*
- Chloride (Cl⁻), 221*t*
 Chloride channel, kidney, B, 223–226*t*
 Chloride channel 5, 223–226*t*
 Chloride channel 7, 223–226*t*
 Cholecalciferol, 239–240.
See also Vitamin D
 synthesis of, 239–242
- Cholecystokinin (CCK), 942
- Cholesterol, 428
 absorption of, inhibitors of, 1015
 biosynthesis of
 in mutations, 799–801, 800*t*, 801*f*
 high
 dietary additives for, 1016
 diet therapy for, 1012–1014, 1013*t*, 1013*b*
 supplements for, 1016
 25-hydroxylase, 223–226*t*
 low high-density lipoprotein, 1008
 metabolism of, disorders of, 1007–1008
 mitochondrial uptake of, 430
 in osteochondrodysplasias, 799–801
 and steroid biosynthesis, 141–144
 steroid biosynthesis from, 550*f*
 synthesis and trafficking, 446–448
- Cholesteryl ester storage disease, 446–447
- Chondroblasts, 245–247, 246*f*
- Chondrocytes, 245–247
 differentiation of, 247
 in estrogen synthesis, 264
 PTH-rP developing, 245–247, 246*f*
- Chondrodysplasia, 314.
See also Osteochondrodysplasias
- Chondrodysplasia punctata 2, 800
- Chondrogenesis, 245–248, 246*f*, 299
- Choriogonadotropin receptors, 38–39
- Chromobox Homolog 2 (CBX2), 137
- Chromogranin A, 497
- Chromosomal abnormalities
 short stature, 332
- Chromosomal origins, 627–628
 karyotype, in Turner syndrome, 628
- Chromosomes
 16p11.2, 969
 6q24, 281–282
 and molecular cytogenetics, 22–23
- Chronic kidney disease-mineral and bone disorder (CKD-MBD), 768–769, 802*t*
- Chronic lymphocytic thyroiditis, 853–854
- Chronic metabolic complications, diabetes, 856
- Chronic vascular complications, diabetes, 856
- Chylomicronemia syndrome, familial, 1007*t*, 1008
- Chylomicrons, 1005, 1005*t*
- Cinacalcet, 229–230
- Circulating steroids, 438–439
- Cisgender, 696*b*
- Cistrome, 242
- Claudin 2, 223–226*t*
- Claudin 10, 223–226*t*
- Claudin 14, 223–226*t*
- Claudin 16, 223–226*t*, 746–747
- Claudin 19, 223–226*t*, 232–233
- CLCN7 channel, 750, 786–789
- Closed-loop insulin delivery, 849–850
- Cocaine amphetamine regulated transcript (CART), 221*t*, 223–226*t*
- Cohen syndrome, 970
- COL1A1, 772–774*t*, 779
 in osteochondrodysplasias, 798
- COL2A1, mutations in, 772–774*t*
- COL10A1, mutations in, 328
- Collagen
 pyridinium of, 256*f*
 telopeptides of, 256–257, 256*f*
 type I, 256–257, 256*f*
- Collagenomas, 509, 510*f*
- Collagen triple helix repeat-containing protein 1, 223–226*t*
- Collagen type I(α1), 223–226*t*, 779–782
- Collagen type I(α2), 223–226*t*
- Collagen type II(α1), 223–226*t*
- Collagen type III(α1), 223–226*t*
- Collagen type IV(α1), 223–226*t*
- Collagen type IX(α1), 223–226*t*
- Collagen type X(α1), 223–226*t*
- Collagen type XI(α1), 223–226*t*
- Colony stimulating factor—Macrophage, 223–226*t*
- Compact bone, 252
- Complementary deoxyribonucleic acid (cDNA), 12–13, 17*f*
- Conception, 107
- Congenital abnormalities, pituitary, 322
- Congenital adrenal hyperplasia (CAH), 4–5, 431
 clinical and laboratory findings in, 447*t*
 lipoid, 448–449, 448*f*
 pathways to androgens in, 433*f*
 in puberty, 569
 syndromes, 20
 virilizing, 144–146
- Congenital adrenal hyperplasia-X (CAH-X) syndrome, 455
- Congenital anatomic defects, with central diabetes insipidus, 377
- Congenital autosomal nephrogenic diabetes insipidus, 381
- Congenital cardiovascular malformations (CVMs), 638–640
- Congenital disorders, 317
- Congenital disorders of glycosylation (CDG), 912
- Congenital hyperinsulinism, 184
 syndromic forms of, 912
- Congenital hyperthyroidism, 214–215
 Graves disease, 214–215
 nonautoimmune, 215
- Congenital hypothyroidism, 208–214
 diagnosis of, 212
 dysgenetic, 209*t*
 newborn screening for, 208, 400
 positive screening report for, 213–214, 214*f*
 transient, 212
 treatment of, 214
 with TSHR and transcription factor mutations, 209*t*
- Congenital lipoid adrenal hyperplasia, 144, 448–449, 448*f*
 disorders resembling, 449
- Congenital X-linked diabetes insipidus, 381, 382*f*
- Conn syndrome, 471–472
- Conradi-Hünermann-Happle syndrome.
See Chondrodysplasia punctata 2
- Constitutional delay, of growth and puberty, 678–679
- Consumptive hypothyroidism, 212
- Continuous glucose monitoring (CGM), 847–848
 clinical evidence for, 848
 practical deliberations, 848–849
- Copy number variation, 332
- Core binding factor, beta subunit, 223–226*t*
- Corpus callosum, 6
- Corpus luteum, 539
- Correction dose, 843
- Cortical porosity index, 270–271
- Corticosterone methyl oxidase deficiency, 431, 459–460
- Corticotrophin-releasing factor receptors, as class B receptors, 45
- Corticotropin-releasing factor (CRF)
 function of, 434–435
 in hypothalamus, 434–435
- Corticotropin-releasing hormone (CRH), 4–5, 109, 665
- Cortisol, 1–2, 436
 deficiency of, in hypoglycemia, 923–924
 plasma concentration of, 441, 442–443*t*
 rhythms of, 436
 stress and, 959–960
 therapeutic potency of, 457*t*
- Cortisone acetate, therapeutic, potency of, 457*t*
- Cortisone reductase deficiency, 11β-hydroxysteroid dehydrogenase-1-apparent, lesions in, 460
- Counterregulatory hormones, 5
 defects, 192
- Coxsackievirus B₄ virus, 864
- C-peptide, in diabetes mellitus, 824, 825*f*
- Craniopharyngiomas, 322–323
- CRISPR-Cas9 system, 19–20, 19*f*
- Crouzon syndrome, 248
- CRTAP genes, 780

- Cryptorchidism, 149–151, 680
 disorders associated with, 150–151*t*
 prevalence, 151
- CSF receptor-Macrophage (c-Fms),
 223–226*t*
- CSII-based treatment regimens,
 844–845, 844*b*
- CTLA-4, 886
- C-type natriuretic peptide (CNP),
 308
- C-type natriuretic protein, 223–226*t*
- CUL7, 330–331
- Cushing disease, 1–2, 425–426
- Cushing syndrome, 1–2, 439, 495, 503*f*,
 965–966
 adenoma in, 468, 504
 causes of, 469–470
 characteristics of, 467–471
 clinical findings, 467, 467*t*
 differential diagnosis of, 471–472
 endogenous, 470–471
 etiology of, 468*t*
- Cutaneous manifestations, 509
- Cyclic adenosine monophosphate
 (cAMP), 825–827
- Cyclic AMP response element-binding
 protein 3-like 1, 223–226*t*
- Cyclic AMP-responsive binding protein,
 223–226*t*
- Cyclic AMP-responsive element binding
 protein (CREB), 221*t*
- Cyclophilin B, 223–226*t*, 257
- CYP21A2 gene, 454–455
 causing 21-hydroxylase deficiency,
 455–456
- CYP24A1 genes, 732–733
- CYP27B1, 232, 235, 757
- Cyproterone acetate, 599
- Cystic fibrosis
 related diabetes, 915
- Cystic fibrosis-related diabetes (CFRD),
 863, 915
- Cystic hygroma, 632*f*, 633
- Cystic lesions, 319–320
- Cytochrome b₅, 430–431, 431*f*
 deficiency of, 146–148, 450–451
- Cytochrome P450, 428
 characteristics of, 428
 electron transport to mitochondrial
 forms of, 429*f*
- family 24, subfamily A, polypeptide 1
 (25OHD-1 α -hydroxylase),
 223–226*t*
- oxidoreductase deficiency, 147
- subfamily IIR, polypeptide 1 (25-
 hydroxylase), 223–226*t*
- subfamily XXVIIIB, polypeptide 1
 (25OHD-1 α -hydroxylase),
 223–226*t*
- Cytochrome P450 III A, 223–226*t*
- Cytokine receptors, 30
 structure and function of type 1,
 48
 transduce hormone action
 growth hormone receptors, 48–49,
 48*t*
- Cytokine regulation, of childhood
 growth, 308
- Cytokines
 adipo-, 955–956
 inflammatory, 955–956
- Cytosolic free calcium (Ca²⁺), 220–226
- D**
- Dairy, 964
- DAX1, 60–61
- dDAVP. *See* Desamino-d-arginine
 vasopressin (dDAVP)
- Dehalogenase defects, 211–212
- Dehydration, systemic, in hyponatremia,
 372–373
- 7-Dehydrocholesterol reductase
 (DHCR7), 133, 223–226*t*
- Dehydroepiandrosterone (DHEA), 429*f*,
 430, 668–669
 concentration of, 437
 pathway from cholesterol to, 434*f*
- Dehydroepiandrosterone sulfate
 (DHEAS), 546, 668–669
- Deiodinase, 396*f*
 type 1, 205
 type 2, 203–204
- Deiodination
 of T4, 205
 of thyroxine, 205
- Delayed puberty, 326, 675–680
 consequences of, 680
 etiologies of, 676, 676*f*
 evaluation of young man with,
 681–684
 additional evaluation, 684
 history, 681–682
 initial evaluation, 681
 physical examination, 682–683
 testing, 683–684
 history in, 681–682
 initial evaluation, 681, 681–682*t*
 investigations for, 681–682*t*
 medications used for, 685*t*
 persistent hypogonadotropic
 hypogonadism, 676–678
 physical examination in, 682–683
 self-limited, 678–679
 syndromes associated with, 680
 testing in, 683–684
 treatment of, 684–686
- Delayed skeletal maturation, 343
- Demeclocycline treatment, 382
- De Morsier syndrome, 317
- DEND syndrome, 285
- Denosumab, 783
- Dentin matrix acidic phosphoprotein 1
 (DMP1), 223–226*t*
- Dentin matrix protein (DMP), 221*t*
- Deoxypyridinoline (DPD), 221*t*, 257,
 257*f*, 802*t*
- Deoxyribonucleic acid (DNA), 3–4, 221*t*
 complementary, 12–13, 17*f*
 digestion of, 9–10
 fragments of, 10, 10*f*
 isolation of, 9–10
 probe, 10, 10*f*
 recombinant, pediatric endocrine
 disease therapy and, 27
 sequencing of, 15–19, 16*f*
- Deoxyribonucleic acid (DNA)
 (*Continued*)
 direct methods of, 15–19
 indirect methods of, 19
- Depo-Provera. *See* Medroxyprogesterone
 acetate (MPA)
- Desamino-d-arginine vasopressin
 (dDAVP), 357, 358*f*
- Desert hedgehog (DHH) gene, 137
- Desmoterolosis, 800
- Developmental delay, epilepsy, neonatal
 diabetes syndrome (DEND),
 281–282
- DEXA. *See* Dual-energy X-ray
 absorptiometry (DEXA)
- Dexamethasone (DEX), 207
 suppression test, 444
 test, 24
 therapeutic, potency of, 457*t*
- Dexamethasone androgen suppression
 test (DAST), 590, 596
- DHEA. *See* Dehydroepiandrosterone
 (DHEA)
- Diabetes
 cystic fibrosis related, 863
 genetic defects in, insulin action and,
 864–865
 gestational, 865
 lipotrophic, 864–865
 mitochondrial, 862
 neonatal, 865–866
 thiamine-responsive, 863
- Diabetes Control and Complications
 Trial (DCCT), 840
- Diabetes insipidus (DI), 319
 central, 376–381
 adipic hypnatremia, 380–381
 autoimmune disease causing,
 377–378
 brain death causing, 378
 causes of, 376–381
 congenital anatomic defects with,
 377
 drugs causing, 378
 fluid therapy for, 378–379
 genetic causes of, 376–377
 increased vasopressin metabolism
 causing, 378
 infectious disease causing, 377–378
 infiltrative disease causing, 377–378
 neoplasms with, 377
 neurosurgical intervention for, 377
 primary enuresis causing, 378
 trauma and, 377
 treatment of, 378–380
 vasopressin analogs for, 379–380
 evaluation of, 370*f*
 nephrogenic, 381–383
 causes of, 381, 382*f*
 congenital autosomal, 381
 congenital X-linked, 381, 382*f*
 treatment of, 382–383
- Diabetes mellitus, 814–851, 856–867
 antigens in, 822
 associated autoimmune diseases,
 853–854
 associated psychosocial problems, 854
 autoantibodies in, 894–895

- Diabetes mellitus (*Continued*)
 autoantigens in, 887*t*
 autoimmune polyglandular
 syndromes, 885*t*, 889–890, 893
 characteristics of, 815
 chemical-induced, 863
 classification of, 815–817, 816*b*, 816*t*
 clinical manifestations of, 830–831
 complications of, 856–860
 C-peptide in, 824, 825*f*
 cystic fibrosis related, 863, 915
 definition of, 814–815
 diagnosis of, 831
 drug-induced, 815–817, 863
 emerging digital world of, 850–851
 exercise in, 852
 HLA DQ phenotypes in, 819–820, 821*f*
 human leukocyte antigen DQ
 phenotypes in, 820*t*
 human leukocyte antigen DR
 phenotypes in, 820*t*
 hyperinsulinemic hypoglycemia as
 prodrome to onset of, 915
 hypoglycemia in, 852–853
 insulin in
 action of, 827–829
 receptors for, 827, 828*f*
 secretion of, 825–827, 826*f*
 insulin therapy, 1–2
 juvenile *vs.* maturity onset, 2–3
 lipotrophic, 864–865
 management during surgery, 855
 medical nutritional therapy, 851–852
 mitochondrial, 862
 morbidity of, 815
 mortality of, 815
 neonatal, 3–4
 outpatient care for, 854–855
 overview of, 814–815
 permanent neonatal, 285–288
 sick day management in, 853
 syndromic neonatal, 287–288
 thiamine-responsive, 863
 transient neonatal, 282–285
 treatment of, 835*t*
 glycemia and blood glucose, 844*b*
 goals of, 840
 type 1, 2–3, 279–280, 817–831
 age-standardized incidence of,
 818–819, 818*f*
 characteristics of, 816*t*, 817
 epidemiology of, 817–819, 818*f*
 etiology, pathogenesis and genetics
 of, 819–823, 819*f*, 820*t*, 821*f*
 immune intervention strategies in,
 823–824
 loci for, 820*t*
 non-autoimmune, 855
 pathophysiology of, 829–830, 829*t*,
 830*f*
 phenotypes in, 820*t*
 prediction and prevention of,
 823–824, 823*t*, 823*f*
 risk of, 820*t*, 822*t*
 treatment of, 840–843
 type 2, 2–3, 279–280, 817
 characteristics of, 816*t*, 817
 mutations causing, 862*t*
- Diabetes mellitus (*Continued*)
 typical, 860–861
 virus infections in, 864
 youth-onset, 863*t*
 Diabetic heterodimers, 823*t*
 Diabetic ketoacidosis (DKA), 831–833,
 835*t*
 aims of, 833*b*
 child with, 833–837
 clinical and biochemical monitoring,
 833–834
 complications of therapy, 837*b*
 fluid electrolyte therapy for, 835*t*
 hyperosmolar hyperglycemic syndrome,
 840
 management of, 833
 morbidity and mortality associated,
 838*b*
 pathophysiology, 831–840
 pathophysiology of, 830*f*
 synopsis of, 837*b*
 treatment of, 831–840
 Diabetic neuropathy (DN), 856–860
 Diabetic retinopathy, 858–859
 “Diabulimia,” 854
 1,2-Diacylglycerol (DAG), 221*t*
 Dialysis-based free hormone assays, 94
 Diaphyseal dysplasia, progressive, 793
 Diaphysis, 259–260
 Diarrheal illness, in hypoglycemia, 926
 Diazoxide, 910
 DICER1 syndrome, 411, 470, 514–515
 Dichlorodiphenyl dichloroethane
 (DDT), 671
 Diencephalic syndrome, in children,
 980
 Diet
 fat in, in obesity, 960
 intervention in, in childhood obesity,
 972
 therapy, for dyslipidemia, 1012–1014,
 1013*t*, 1013*b*
 Dietary phosphate deficiency,
 756–757
 Diethylstilbestrol (DES), 152
 Differentially methylated region (DMR),
 802*t*
 Differentiated thyroid cancer (DTC)
 etiology, 410–411
 follow-up, 414
 incidence, 410
 levothyroxine therapy, 414
 oncogene mutations, 411
 radioactive iodine therapy, 413–414
 surgical management, 411–413, 412*b*
 systemic therapy for children, 415
 thyroid tumorigenesis and progression,
 411
 DiGeorge syndrome (DGS), 714–715,
 802*t*
 DiGeorge syndrome chromosome region
 (DGCR), 802*t*
 Dihydrotestosterone (DHT), 27
 1,25-Dihydroxyvitamin D, 244–245
 1,25-Dihydroxyvitamin D₃ (calcitriol)
 (1,25(OH)₂D₃), 221*t*, 802*t*
 24,25-Dihydroxyvitamin D₃ (24R,25
 (OH)₂D₃), 221*t*
- Dikkopf, xenopus, homolog of,
 223–226*t*
 2,3-Diphosphoglycerate (2,3-DPG), 836
 Direct-to-consumer genetic testing
 (DCGT), 24
 Disheveled 1, 223–226*t*
 Disorders of sexual development (DSD),
 125, 156*f*
 in puberty, 564–565
 “Disposition index,” 815, 815*f*
 Disproportionate short stature, 750
 Distal-less 5, 223–226*t*
 Distal-less homeobox 5, 223–226*t*
 Distal tubule, structure of, 365*f*
 Diurnal rhythms, 4–5
 of adrenocorticotrophic hormone and
 cortisol, 436
 DMP1 protein, 764
 DNA damage repair syndromes, 331
 DOC acetate, therapeutic, potency of,
 457*t*
 Donohue syndrome, 6, 864, 913
 Doublesex and Mab-3 related
 transcription factor 1 (DMRT1),
 137
 Doublesex and Mab-3 related
 transcription factor 2 (DMRT2),
 137
 Down syndrome, 865
 Drug
 central diabetes insipidus from, 378
 diabetes mellitus induced by,
 815–817
 hypercalcemia from, 740
 impairing free water clearance,
 372*t*
 Dual-energy X-ray absorptiometry
 (DEXA), 221*t*, 262*t*, 266,
 268*t*, 802*t*
 estimation of BMC by, 748–749
 “Dumping syndrome,” 915
 Duodenopancreatic neuroendocrine
 tumors, 508
 DUOX2 defects, 211
 Dwarfism, 237
 primordial, 331
 Dysbetalipoproteinemia, 1007*t*, 1009
 Dysfunctional uterine bleeding, 587
 Dysgenetic congenital hypothyroidism,
 209*t*
 “Dysinsulinism,” 915
 Dyslipidemia
 diet therapy for, 1012–1014, 1013*t*,
 1013*b*
 pharmacologic management for,
 1014–1016, 1014*t*
 primary, 1007, 1007*t*
 secondary causes of, 1009–1010,
 1009*b*
 vascular changes and, 1010–1011,
 1010*b*
 Dysmenorrhea, 588
 Dysregulation, energy balance
 counterregulatory mechanisms,
 oppose weight loss, 948–949
 feed-forward mechanism, promote
 weight gain, 949
 leptin resistance, 947–948

E

- Early neonatal hypocalcemia, 709–712, 720–722
- Eating disorders, 698
- EBP genes (Emopamil-Binding Protein), 800
- Ectodermal dysplasia, in APS, 885*t*
- Ectodermal dystrophy, in APS, 892
- Ectodomain/exodomain, 32
- Ectonucleotide pyrophosphatase/phosphodiesterase 1, 223–226*t*
- Ectopic adrenocorticotrophic hormone syndrome, 469–470
- Ectopic calcification, 793–795
genetic causes of, 794*t*
- Ectopic thyroid gland, 398
- Edema, cerebral, 837–838
- Effector pathways
osmotic sensor and, 357–364, 358*f*, 360*f*
volume sensor and, 364–368, 367*f*
- Effector site-based selectivity, 553
- Efferent system
for energy balance, 945–947
vagus nerve, energy storage and, 945–947
- EGF, 233
- Ehlers-Danlos syndrome (EDS), 455
- EIF2AK3*, mutations in, 287
- Eiken chondrodysplasia, 717
- Elastin, 223–226*t*
- Electrolytes, 834–835, 835*t*, 839
- Electron transport
to P450c17, 430–431
to P450scc, 429*f*
- Embryogenesis, 244
in pituitary, 202
in thyroid system, 203
- Embryology, 426–427
- Embryonic stem (ES) cells, 303
- Emesis, 853
- Emiocytosis, 825–827
- EMX2* gene, 133
- Endocannabinoids (EC), in energy balance, 945
- Endochondral bone
development, 247
formation of, 247
- Endocrine deficiencies, 315–323
- Endocrine disorders
autoimmune disorders with, 461–462
genomic deletions causing, 15
with obesity, 964–966
pediatric, recombinant DNA technology in, 27
- Endocrine disrupting chemicals, effect of, 671
- Endocrine function
adaptations in, at birth, 5
test for, 370*f*
- Endocrine genetics, molecular
endocrinology and, 9–28
- Endocrine manifestations, anorexia nervosa, 981–982
- Endocrine neoplasia
APC-associated polyposis in, 514
Beckwith-Wiedemann syndrome in, 514
- Endocrine neoplasia (*Continued*)
Carney triad in, 514
DICER1 syndrome, 514–515
Li-Fraumeni syndrome in, 515
Lynch syndrome, 515
neurofibromatosis type 1 in, 515
Peutz-Jeghers syndrome in, 515–516
PTEN hamartoma tumor syndrome in, 516
tuberous sclerosis complex in, 516
- Endocrine placenta, structure and function, 108–110
- Endocrine regulation, of linear growth
growth hormone, 300–306
insulin-like growth factor-1, 306–307
- Endocrine renin-angiotensin-aldosterone system
anatomy and biochemistry of, 365
regulation of secretion of, 365–366
- Endocrinology. *See also* Pediatric endocrinology
positional genetics in, 20–22, 20*f*
- Endogenous retroviral family W, member 1, 223–226*t*
- Endonucleases, restriction, 10
- Endoplasmic reticulum, 782
- Energy
balance of
afferent system for, 941–943
central processing in, 943–945, 946*f*
disorders of, 939–982
dysregulation of, 947–949
efferent system for, 945–947
endocannabinoids, 945
food intake regulation, 940–941
genetic obesity disorders, 966–969
ghrelin in, 941–942
hedonic pathway, 947
homeostatic system for, 941–947, 941*f*
in hyperinsulinemia, 948*f*
leptin resistance in, 947–948, 948*f*
nonhomeostatic regulation of, 947
norepinephrine in, 944
orexins, 945
serotonin in, 944
study of, 939–941
total body energy stores, 940*f*
excess of, in obesity, 949–952
expenditure of, sympathetic nervous system (SNS) and, 945
storage of, efferent vagus and, 945–947
- Energy homeostasis, 941, 941*f*
- Enuresis, primary, causing central diabetes insipidus, 378
- Environmental factors, obesity, 959–960
- Enzymes, 426
cytochrome P450, 428
iodothyronine monodeiodinase, 398*f*
of steroid hormone synthesis, 428–434
steroidogenic, 426
human genes for, 426*t*
types of, 428–433
- Ependymomas, 505
- Ephrin-B1, 223–226*t*
- Ephrin-B2, 223–226*t*
- Ephrin receptor EPHA1, 223–226*t*
- Epidermal growth factor (EGF), 110, 223–226*t*
- Epigenetics, developmental
programming and, 958–959
- Epilepsy, in hypothalamic anovulation, 585
- Epinephrine, 1–2
deficiency, hypoglycemia, 924
nor-, in energy balance, 944
- Epiphyseal cartilage growth plate, 246*f*
- Epiphyseal fusion, 313
- Epiphysiodesis, 347
- Epithelium
site of, of PTH-rP, 237*t*
vaginal, 554*f*
- Epstein-Barr virus, 9
- Estradiol
in early puberty, 535*f*
in infants, 532*f*
in menstrual cycle, 533*f*
regulation of, 546*f*
sources of, 551*f*
testosterone-, binding globulin, 551*f*
transdermal, 584*t*
- Estrogen, 227–228, 249–250, 264.
See also Nonhypoestrogenic menstrual disturbances
mechanism of, model, 552*f*
synthesis, chondrocytes in, 264
in thyroid metabolism, 203*f*
- Estrogen receptor (ER), 223–226*t*
hypothalamic, 552*f*
for skeletal growth, 264
- Estrogen receptor α (ER α)
tall stature, 343
- Estrogens, 59–60, 307–308
- Estrone, 551*f*
- Ethanol, obesity and, 963–964
- Euglycemic ketoacidosis, 832
- Eukaryotic translation initiation factor 2 α -kinase 3 (*EIF2AK3*), 287
- European Nicotinamide Diabetes Intervention Trial (ENDIT), 823–824
- Euthyroid sick syndrome, 5
- Exaggerated adrenarche, 561
- Exercise
in bone acquisition, 261
in diabetes mellitus, 852
- Exercise-induced hyperinsulinemic hypoglycemia (EIH), 912
- Exercise-induced hyperinsulinism, 912
- Exocrine-duct lineage, 286–287
- Exon 1A transcript, of *GNAS*, 717
- Expressivity, 23
- Extracellular fluid (ECF), 223–226*t*
tonicity, 357
- Extracellular signal-regulated kinase (ERK), 223–226*t*
- Extracellular vesicles, 110
- Extrauterine thyroid adaptation, 204–205, 205*f*
- Ezetimibe, 1015

F

- Factitious hyperinsulinism, 914
- Factor in germline alpha (*FIGLA*), 141

- Failure to thrive
 in children, 977–979
 classification and etiology of, 977–978
 definition of, 977
 diagnosis and evaluation of, 978–979
 differential diagnosis of, 978b
 management of, 979
 prognosis of, 979
- Familial chylomicronemia syndrome, 1007t, 1008
- Familial combined hyperlipidemia (FCHL), 1007t, 1008
- Familial expansile osteolysis, 769–770
- Familial glucocorticoid deficiency (FGD), 22, 344
 type 1, 464
 type 2, 464
- Familial glucocorticoid resistance, 472–473
- Familial hyperaldosteronism, 472
- Familial hypercholesterolemia (FH), 1007, 1007t, 1011
- Familial hypertriglyceridemia (FHTG), 1007t, 1008
- Familial hypocalciuric hypercalcemia, 229
- Familial hypocalciuric hypercalcemia type 1 (HHC1), 735–736
- Familial isolated hyperparathyroidism type 1, 737–738
- Familial isolated pituitary adenomas (FIPA), 504
 AIP-associated, 504
- Familial isolated primary hyperparathyroidism (FIHP), 802t
- Familial medullary thyroid carcinoma (FMTC), 511. *See also* Medullary thyroid carcinoma (MTC)
- Familial nonmedullary differentiated thyroid cancer (FNMTTC), 411
- Familial paraganglioma syndrome, 492t
- Familial pituitary adenomas, 343
- Familial tumoral calcinosis, 795
- Family, intervention of, in childhood obesity, 973
- Family with sequence similarity, 20
 member A, 223–226t
 member C, 223–226t
- Fanconi anemia, 331
- Fanconi-Bickel syndrome, 284–285
 in hypoglycemia, 921–923
- Fanconi syndromes, 759–766
- Fasting, hypoglycemia in, 926–927
- Fasting adaptation, glucose metabolism, 908–910, 909f
- Fasting metabolic systems
 hormonal regulation, 179t
 in tissue, 829, 829t
- Fasting system approach, to hypoglycemia diagnosis, 908–910
- Fat
 -derived hormones, in anorexia nervosa, 982
 dietary, in obesity, 960
 saturated, diet therapy for, 1012–1014, 1013t, 1013b
- Fatty acid
 free, 942
 trans-unsaturated, 960
- Fatty acid oxidation
 defects of, 925
 hypoglycemia, 194–195, 194f, 195t
- Fatty streaks, 1010
- FBJ osteosarcoma oncogene (FOS), 223–226t
- FCHL. *See* Familial combined hyperlipidemia (FCHL)
- Feed-forward signaling, 949
- Female reproductive system
 in adolescent, 531–536, 533f, 534t, 535f
 in adults, 536–539, 536f
 fetal development of, 528–531, 530f
 infant development of, 531–532f, 544–545
- Feminization, and Turner syndrome, 649–650
- Feminizing adrenal tumors, 472
- Ferredoxin reductase, 428–430
- Ferriman-Gallwey hirsutism, 590f
- Fetal adrenal-placental crosstalk, 111–113
- Fetal genome, 118
- Fetal goiter, 203–204, 204f
- Fetal life, thyroid function in, 203
- Fetal maturation and parturition, 113–114
- Fetal-neonatal Graves disease, 406–407
- Fetal neuroendocrine development, 110–113
- Fetal origins, adult disease, 4
- Fetal thyroid hormone, 206
- Feto-placental system, steroid synthesis, 434f
- Fetus, 426–427
 adrenal cortex formation in, 426–427
 adrenal steroidogenesis in, 433–434
 characteristics of, 433–434
 female reproductive system in, 528–557, 530f
 neuroendocrine unit in, 528–529
 normal sexual maturation of, 557–558
 programming of, obesity and, 958–959
- FGD. *See* Familial glucocorticoid deficiency (FGD)
- FGF23. *See* Fibroblast growth factor 23 (FGF23)
- FGFR. *See* Fibroblast growth factor receptor (FGFR)
- FGFR3, 344
- FGF receptor substrate (FRS), 221t
- FH. *See* Familial hypercholesterolemia (FH)
- FHTG. *See* Familial hypertriglyceridemia (FHTG)
- Fiber, obesity and, 961
- Fibrates, 1015t, 1016
- Fibric acid derivatives, 1016
- Fibrillin-1 (FBN1), 344
- Fibroblast, 245–247, 246f
- Fibroblast-derived cultures, 9
- Fibroblast growth factor (FGF), 308, 802t
- Fibroblast growth factor-1, 223–226t
- Fibroblast growth factor-2, 223–226t
- Fibroblast growth factor-5, 223–226t
- Fibroblast growth factor-7, 223–226t
- Fibroblast growth factor-18, 223–226t
- Fibroblast growth factor 19, 943
- Fibroblast growth factor-21, 223–226t
- Fibroblast growth factor-23 (FGF23), 223–226t, 245–247
 in osteochondrodysplasias, 795
 in tumor-induced rickets, 764–765, 765f
- Fibroblast growth factor receptor (FGFR), 52–53, 109, 221t, 802t
 and phosphatonins, 231–232
- Fibroblast growth factor receptor-1, 53, 223–226t
- Fibroblast growth factor receptor-2, 223–226t
- Fibroblast growth factor receptor 2–4, 53
- Fibroblast growth factor receptor-3, 223–226t
- Fibroblast growth factor receptor-4, 223–226t
- Fibrodysplasia ossificans progressiva (FOP), 793–794, 802t
- Fibrous dysplasia, 785–786
- Fight/flight response, 4–5
- FIGLA. *See* Factor in germline alpha (FIGLA)
- Finasteride, 599
- Fine needle aspiration (FNA), thyroid nodule evaluation, 408–409
- FK506-binding protein, 223–226t
- Flat bones, 252
- Flavin adenine dinucleotide (FAD) moiety, 431f
- Flavin mononucleotide (FMN) moiety, 431f
- 9 α -Fludrocortisone, therapeutic, potency of, 457t
- Fluid
 and electrolyte maintenance requirements, in diabetes mellitus, 831t
 electrolyte therapy, 834–835, 835t
 osmotic regulation of, 359–361, 360f
 physiology of, 357–368
 of vasopressin secretion, 359–361, 360–361f
 therapy, for central diabetes insipidus, 378–379
- Fluorescent in situ hybridization (FISH), 627, 629–630f, 633, 802t
- Fluorescent 5' nuclease (TaqMan) assay, 14–15, 14f
- Flutamide, 599
- FMTC. *See* Familial medullary thyroid carcinoma (FMTC)
- Focal adenomatosis, 186–187
- Focal K_{ATP} hyperinsulinism, 186–187
- Follicle
 antral, 538f
 atretic, 538f
 autoamplification processes in, 535b
 classification of, 531f
 healthy, 538f
 ovarian of, 530f
- Follicle-stimulating hormone (FSH), 1–2, 665
 in hormone assays, 39–40
 in puberty, 529f, 531–533f, 535f
 secretion of, 544

- Follicle-stimulating hormone (FSH)
(*Continued*)
in sexually infantile girls, bone age in, 574*b*
- Follicular phase
hormone blood production rates in, 549*t*
of menstrual cycle, 536–539, 537–538*f*
- Follicular thyroid carcinoma (FTC), 408–410
diagnosis, 412–413
- Food intake
glucose concentration, 841*f*
regulation, 940–941
- Food reward, hedonic pathway of, 947
- Four-and-a-half Lim domains, 223–226*t*
- FOXA2 hyperinsulinism, 189
- FOXE1, 210
- FOXL2, 141
- FOXP3, 287, 724–725, 887
- Free fatty acid receptor 1, 42
- Free fatty acids (FFAs), 942
- Free hormone, tracer equilibrium dialysis, 94, 94*f*
- Free T4 (nondialysis), 94, 94*f*
- Frizzled, drosophila, homolog of, 223–226*t*
- Frizzled related protein-4 (FRP4), 223–226*t*, 232
- Fructose
1,6-diphosphatase deficiency, in hypoglycemia, 924
intolerance to hereditary, in hypoglycemia, 924–925
obesity and, 961, 962–963*f*
- FSH. *See* Follicle-stimulating hormone (FSH)
- Functional hypogonadotropic hypogonadism, 679
causes of, 677*t*
- Fundoplication, hypoglycemia, 191–192
- Furin, 235
- Furosemide, 228
- G**
- Gamma-amino butyric acid (GABA), 221*t*, 665
- Gamma-carboxyglutamic acid protein, bone (Osteocalcin), 223–226*t*
- Gastric bypass surgery, 927
- Gastric inhibitory polypeptide receptors, as class B receptors, 44–45
- Gastric neuroendocrine tumor, 509
- Gastrin, 238–239
- Gastrinoma, 506*t*, 508–509
triangle, 508
- Gastrointestinal bleeding, in Turner syndrome, 644
- Gastrointestinal disorders
bleeding, 644
inflammatory bowel disease, 644
liver disease, 643
- Gastrointestinal polypeptide (GIP)
pancreatic glucagon, 825–827
- Gastrointestinal surgery,
hypersinsulinism after, 915
- Gastrointestinal (GI) tract, 940
Ca in, 227
- GATA4, 137, 288
- GATA6, 288
heterozygous inactivating mutations of, 288
- GATA-binding protein 3, 223–226*t*
- GATA3, in PTH, 715–716
- GCM2, in PTH, 713
- Gender-affirming treatment, 696*b*
- Gender dysphoria (GD)
desistence *vs.* persistence, 698
eating disorders, 698
family support and treatment, 698
internalizing disorders and, 697–698
mental health comorbidities associated with, 697–698
natural history of, 698
suicidality and nonlethal self-harm, 698
traits of autism spectrum disorder, 698
- Gender expression, 696*b*
- Gender identity, biological determinants of, 695–697
- Gender identity disorder (GID), 695, 696*b*
- Gender identity/experienced gender, 696*b*
- Gender incongruence, 696*b*
- Gender labeling, 125–126
- Gender nonconforming youth
definitions and epidemiology, 695
delivery of care, challenges to, 701
emergence and developmental trajectories, 697
adolescence, 697
early childhood, 697
infancy, 697
- gender dysphoria
desistence *vs.* persistence, 698
eating disorders, 698
family support and treatment, 698
internalizing disorders and, 697–698
natural history of, 698
suicidality and nonlethal self-harm, 698
traits of autism spectrum disorder, 698
- gender identity, biological determinants of, 695–697
- medical treatment
current treatment models, 699–700
outcomes and potential adverse effects, 700–701
multidisciplinary care, 698–699
social transition, 699
- Gender reassignment, 696*b*
- Gender-reassignment surgery, 696*b*
- Gender role, 696*b*
- Gender variance. *See* Gender incongruence
- Gene conversions, causing salt-wasting 21-hydroxylase deficiency, 455–456
- Gene conversions, for CAH, 455–456, 456*f*
- Gene editing, 3–4
- Gene encoding PROP1, 320
- Genes, 395, 426
calcitriol regulating, 244, 244*f*
in mineral homeostasis, bone metabolism and, 223–226*t*
for oxytocin/vasopressin expression, 358*f*, 359
for steroidogenic enzymes, 426, 426*t*
for VDR, 244, 244*f*
X chromosomes, 628–631
- Gene therapy, 3–4
- Genetic abnormalities, GH production, 321–322
- Genetic counseling, 491–493
and testing, 491–493
- Genetic defects
of beta cell function, 861–864, 863*t*
with diabetes, insulin action and, 864–865
in insulin action, 864–865
- Genetic disorders, gluconeogenesis, 924–925
- Genetic issues, pheochromocytomas/paranglioma, 498–499
- Genetic lesions, steroidogenesis, 446–460
- Genetic obesity disorders, 966–969
- Genetic origins, 706–708*t*
- Genetics
of APS, 890–891
of Carney syndrome, 503–504
in clinical practice and next-generation sequencing applications, 24–26
endocrine, molecular endocrinology and, 9–28
of 21-hydroxylase locus, 454–455, 454*f*
positional
in endocrinology, 20–22, 20*f*
principles of, 20–21
of Turner syndrome, 627–633
of type 1 diabetes mellitus, 819–823, 819*f*, 820*t*, 821*f*
- Genetic syndromes
in insulin resistance, 865–866
of lipoatrophy, 865*t*
- Genetic testing
applications in, 24
children with short stature, 337–339
for lipid disorders, 1012
for MEN1, 505–508
for MEN2, 510–511
and presymptomatic screening, 509–510
principles of interpretation of, 23–24
for VHL, 516–517
- Genitalia
ambiguous
with asymmetry in 46, XY disorder patient, 142*f*
child with asymmetric, 154*f*
child with symmetric, 153*f*
diagnosis, 152–158
endoscopic studies, 157–158
genetic tests, 155
history, 153
hormone determinations, 155–157
laboratory studies, 154–155
mouse models, 130–131
physical examination, 153–154

- Genitalia (*Continued*)
 external
 structures of, differentiation of, 130
 structures development
 external, 130
 internal, 129–130
 treatment
 considerations with regard to surgery, 160–161
 gonadal tumors risks, 161
 long-term outcome studies, 161
 medical treatment, 159–160
 psychological and genetic counseling, 161–162
 sex of rearing, 158–159
 transition from pediatrics to adult care, 161
 in virilized female with 21-hydroxylase deficiency, 145f
- Genital tract, maturation of, 554, 554f
- Genitopatellar syndrome, 134
- Genome sequencing, 24
- Genome *vs.* exome sequencing, 25
- Genome-wide association studies (GWAS), 117, 670
- Genomic identification, “endocrine” genes, 21
- Genomic imprinting, Turner syndrome, 631
- Genomic medicine, 24
- Germ cell development, 128–129
- Germline *vs.* somatic mutation, 23
- Gestational diabetes, 865
- GH. *See* Growth hormone (GH)
- GHBP. *See* Growth hormone-binding protein (GHBP)
- GHD. *See* Growth hormone deficiency (GHD)
- GHDIA, 321
- GHR. *See* Growth hormone receptor (GHR)
- GH-releasing hormone (GHRH), 304
- GH-releasing peptides (GHRPs), 304
- Ghrelin, energy balance, 941–942
- Ghrelin receptors, 43–44
- GHRH. *See* GH-releasing hormone (GHRH)
- GHRH gene, 322
- GHRHR gene, 322
- GHRPs. *See* GH-releasing peptides (GHRPs)
- GH secretagogue receptor (GHS-R), 304
- GHS-R. *See* GH secretagogue receptor (GHS-R)
- GH stimulation tests, 336
- Giant hemangiomas, 402
- Gitelman syndrome, 747
- Gla protein, 221t
- Glasgow Coma Scale (GCS), 833, 834t
- Glial cell line-derived neurotrophic factor (GDNF), 221t
- Glial cells missing (GCM2), 713
- Glial cells missing, *Drosophila*, homologue of, 223–226t
- Glial-derived neurotrophic factor (GDNF), 802t
- Gli-Kruppel Family Member 3 (GLI3) gene, 133
- GLIS3, 210, 287
 etiology of, 287
 manifestations of, 287
 mutations in, 287
- Globulin, 220
 estradiol-testosterone binding, 551f
- GLP-1. *See* Glucagon-like peptide-1 (GLP-1)
- GLP-1 analogues, therapy, 975
- Glucagon, 238–239
 deficiency, in hypoglycemia, 924
- Glucagon, as class B receptors, 45
- Glucagon-like peptide (GLP), 825–827
- Glucagon-like peptide-1 (GLP-1), 45, 942–943, 946f
- Glucagon-like peptide 2 (GLP-2), 802t
- Glucagonoma, 509
- Glucagon stimulation test, hypoglycemia, 929
- Glucocorticoid excess, 965–966
 Cushing syndrome, 326
- Glucocorticoid receptors, 58–59
- Glucocorticoids, 227–228, 307, 362, 425–426
 for bone mineralization, 263–265
 -excess low bone mass, 775
 familial
 deficiency, 464
 resistance, 472–473
 feedback, adrenal, 436
 -suppressible hyperaldosteronism, 460
- Glucocorticoid therapy
 common preparations for, 475–476
 concentrations, 443t
 high-dose, complications of, 475t
 mechanisms of, 474
 mineralocorticoid replacement, 477
 pharmacological, 475
 replacement, 474–475
 stress doses of, 477
 and withdrawal, 473–477
 withdrawal of, 476–477
- Glucokinase, 286, 911
 activating mutations of, 286
 hyperinsulinism, 188–189, 911
 inactivating mutations of, 286
 homozygous, 286
 maturity-onset diabetes of the young (MODY2), 290
- Gluconeogenesis
 defects in, 906
 genetic disorders of, with hypoglycemia, 924–925
 renal, 927
- Glucose, 942
 clinical evidence, CGM, 848
 concentration, food intake and, 841f
 metabolism of
 adaptation to longer feeding intervals, 905–906
 fasting systems approach, 908–910
 hormonal regulation of, 906t
 physiologic development of, during infancy and childhood, 904–906
 monitoring
 blood, 847
 continuous, 847–848
 plasma concentrations of, 905f
- Glucose (*Continued*)
 production and utilization, 904–905, 905f
 self-monitoring of, 847
 tolerance, impaired, 866
- Glucose-alanine cycle, 922
- Glucose homeostasis, 5
- Glucose metabolism
 in fetus, 176
 newborn and infant, 175–176
 principles of, 175–176
- Glucose 6-phosphatase deficiency (GSD), 193, 917–918
 anemia, 919
 diagnostic studies, 918–919
 monitoring, 919–920
 neutropenia, 919
 renal disease, 919
- Glucose sensor of the beta cell, 286
- Glucose-stimulated insulin release (GSIR) threshold, 286
- Glucose tolerance test, 831
- Glucose transporter (GLUT)
 defects, newborn and infant, 195–196
 GLUT1 deficiency, 195
 GLUT2 deficiency, 195
 proteins, 175
- Glucosuria, renal, 831
- Glutamate dehydrogenase (GDH)
 hyperinsulinism with, 910–911
- Glutamate dehydrogenase-hyperinsulinism, 187–188
- GLUT2 glucose transporter, 284–285, 825
- Glycemic index, obesity and, 961
- Glycogen branching enzyme deficiency (type 4 GSD), in hypoglycemia, 921
- Glycogenolysis, 916–917
- Glycogenolysis and gluconeogenesis defects
 hypoglycemia, newborn and infant, 192–193
- Glycogen storage diseases (GSDs), 916–923, 916t
- Glycogen synthase deficiency, in hypoglycemia, 917
- Glycogen synthase kinase 3 (GSK3), 221t
- Glycogen synthase kinase 3-alpha, 223–226t
- Glycoprotein hormone receptor group
 follicle-stimulating hormone receptors, 39–40
 human chorionic gonadotropin and thyroid-stimulating hormone receptors during pregnancy, 41
 luteinizing hormone/choriogonadotropin receptors, 38–39
 thyroid-stimulating hormone receptors, 40–41, 41f
- Glycosylated hemoglobin (HbA_{1c}), 854–855
- Glycosylation, congenital disorders of, 912
- Gly-Lys-Arg, 358f
- GNAS, 717–718
 gene complex, first steps in, 718f

- GnRH. *See* Gonadotropin-releasing hormone (GnRH)
- Goiter, fetal, 203–204, 204f
- Gonadal dysgenesis, 574, 627, 680
XX (SRY-negative) individuals, 140t
- Gonadal hormone production
activin, 666
inhibin B, 666
sex steroids, 665–666
- Gonadarche, 661
- Gonad development, 126–127, 127f
- Gonadoblastoma, Turner syndrome, 637–638
- Gonadostat, 529f
- Gonadotrophin-inhibiting hormone (GnIH), 665
- Gonadotropic axis
circulating concentrations of, 667f
hormonal changes, 666–668
physiological mechanisms, 667–668
pubertal reactivation of, 666–668
somatic changes, 668–669
- Gonadotropin deficiency, 575–578, 580f, 582–583f
constitutional pubertal delay, 587b
- Gonadotropin-dependent form, of precocious puberty, 672
- Gonadotropin-independent form, precocious puberty, 672–673
- Gonadotropin-releasing hormone (GnRH), 6
in adolescents, 531–533, 533f
agonist therapy, 458–459
for precocious puberty, 572b
hypothalamic KNDy and, 663–665
in infants, 532f, 544
location of, 543f
in menstrual cycle, 537f
neuronal development, 676–678
neuronal network, development of, 661–662
pathways mediating, 548f
pulse generator, 662
receptor group, 41–42
regulation of, 540f
regulators of, 664f
secretion of, 543–544, 543f
short stature treatment, 342
- Gonadotropin resistance, 575
- Gonadotropins, 5
- GPR54, 664
as class A receptors, 42–43
- G-protein–coupled receptor kinase (GRK), 221t
- G-protein–coupled receptors (GPCR), 30–36, 32t, 228–229, 304, 802t
class A, B, and C, 36f
desensitization and recycling, 35f
structure and function, 33f
- G-protein gene disorders
activating mutations, 47–48
inactivating mutations, 47
- Granulosa cells, 546f
- Graves disease (GD), 214–215, 403, 887t
autoantibodies in, 896
in children, 403
radioactive iodine therapy, 405
somatic complications, 406
- Graves disease (GD) (*Continued*)
therapy in children for, 403, 403f
thyroidectomy and risks, 406
- Greulich and Pyle bone age, 313–314
- Greulich-Pyle method, 313–314
- Group specific component (DBP), 223–226t
- Growth
constitutional delay of, 678–679
factors, 109
for skeleton, 263
- Growth charts, 311
- Growth hormone (GH), 1–2, 221t, 223–226t, 227–228, 802t, 941–942, 974t
actions, 305–306
chemistry, 304
deficiency, 923, 966
growth hormone receptor/growth hormone-binding protein, 305
growth hormone signaling, negative regulation of, 305
hypersecretion, 504
-IGF-1 axis, in anorexia, 981
pituitary gland, 300–304
receptors, 48–49, 48t
secretion, 304–305
sulfation factor assay of, 3f
treatment with
for CAH, 458–459
in Turner syndrome, 649–650f
- Growth hormone-binding protein (GHBP), 305
- Growth hormone deficiency (GHD), 315–317
causes of, 317–323
- Growth hormone insensitivity (GHI), 323–324
associated with immune dysfunction, 324
- Growth hormone receptor (GHR), 223–226t, 305
- Growth hormone–releasing hormone receptor, as class B receptors, 44
- Growth hormone resistance
deficiency, in hypoglycemia, 923
- Growth hormone secretagogue receptor (GHSR), 941–942
- Growth hormone signaling, negative regulation of, 305
- Growth hormone treatment
indications for, 339b
recombinant human (*see* Recombinant human GH (rhGH) treatment)
- Growth plate, 313
senescence, 309
- Growth-promoting therapies, 648–649
- Gs α transcript, of GNAS, 717–718
- Guanine monophosphate (GMP), 221t
- Guanine nucleotide binding protein, alpha stimulating, 223–226t
- Guanine-nucleotide binding protein subunit alpha S (GNAS)
inactivation, 330
- Guanosine diphosphate (GDP), 802t
- Guanosine triphosphate (GTP), 221t, 802t
- Guide RNA (gRNA), 19–20
- Gynecomastia, 686
physiologic, 686
pubertal, 686
- ## H
- HAIR-AN. *See* Hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIR-AN)
- Hairless, 223–226t
- Hamartoma, hypothalamic, magnetic resonance imaging of, 567f
- Hand-foot-genital syndrome, 133–134
- Hashimoto thyroiditis, 400, 887t
antibodies in, 400
- Hashitoxicosis, 400–401
- hCG. *See* Human chorionic gonadotropin (hCG)
- HDL. *See* High-density lipoproteins (HDL), low, cholesterol
- HDL2, 1006–1007
- HDL3, 1006
- Healthcare provider, 299
- Heart failure, 440
- Heat shock protein (CBP) (HSP), 802t
- Heat shock protein (HSP), 221t
- Hedgehog acyl-transferase (HHAT) gene, 133
- Hedonic pathway, of food reward, 947
- Height, final, in Turner syndrome, 649–650, 650f
- Hematopoietically expressed homeobox protein (HHEX), 210
- Hematopoietic progenitor cells, 252–253
- Hematopoietic transcription factor PU.1, 223–226t
- Hepatic insulin resistance, 952
- Hepatic ketogenesis, 179f
- Hepatic phosphorylase complex
deficiency, 921
- Hepatitis, 892
- Hepatocyte nuclear factor 1 α (HNF1A)
hyperinsulinism, 911–912
- Hepatocyte nuclear factor 4 α (HNF4A)
hyperinsulinism, 911
- Hepatocyte nuclear factor 1 beta (HNF1 β), 284–285
mutations in, 284–285
- Hepatocyte nuclear factors and hyperinsulinism, 189
receptors, 58
- Hepatomegaly, GSD type 1, 193
- Hereditary endocrine neoplasia syndromes
Carney complex, 503–504, 503f
familial isolated pituitary adenomas, 504
hereditary paraganglioma-pheochromocytoma syndromes, 504–505
hyperparathyroidism-jaw tumor syndrome, 505
multiple endocrine neoplasia type 1, 505–508
multiple endocrine neoplasia type 2, 510–511
multiple endocrine neoplasia type 4, 513

- Hereditary endocrine neoplasia syndromes (*Continued*)
 X-linked acrogigantism, 513–514
- Hereditary fructose intolerance (HFI), 924–925
- Hereditary hypocalciuric hypercalcemia (HHC), 802*t*
- Hereditary nonpolyposis colon cancer, 515
- Hereditary paraganglioma-pheochromocytoma syndromes (HPS), 504–505
- Hermaphroditism, true, 564–565
- Heterogeneous nuclear ribonucleoprotein D (HNRPD), 221*t*, 223–226*t*
- Heterophilic antibody and human antianimal antibody (HAAA), 99–100
- Heterotopic bone formation, 793–795
 genetic causes of, 794*t*
- Heterozygous mutations, in *LHX4*, 320
- Hexokinase D, 286
- Hexokinase 1 hyperinsulinism, 911
- Hexokinase IV, 286
- High bone mass, 786–793, 787–788*t*
 mutations of, 787–788*t*
- High-density lipoproteins (HDL), low, cholesterol, 1008
- High-mobility group (HMG) domain, 127–128
- High-resolution peripheral quantitative computed tomography (HRpQCT), 221*t*, 265–266, 270–271, 271*t*, 272*f*
- High-throughput NGS/massively parallel sequencing, 17–19
- High voltage dependent calcium channels, 220–226
 “Hirata disease,” 914
- Hirsutism, 590*f*, 598–599
- Histone cell cycle regulation (HIRA)* gene, 714–715
- HLA DQ phenotypes, in diabetes mellitus, 819–820, 820*t*, 821*f*
- HLA DR phenotypes, in diabetes mellitus, 820*t*
- HMG-CoA reductase inhibitors, 1015, 1015*t*
- HNF1A/4A-MODY*, 290–291
- HNF1alpha and HNF4alpha-hyperinsulinism, 189
- HNF1B-MODY*, 291–292
- HNF1 homeobox B, 223–226*t*
- HNRNPC genes, 759
- Holoprosencephaly, 202
- Homeostasis
 alkaline phosphatase, 233–234
 calcium, 222*f*
 magnesium, 232–233
 phosphate, 222*f*
 PTH in, 234–236
 vasopressin in, 357
- Homeostatic energy system, 941–947, 941–942*f*
- Homovanillic acid (HVA), 496–497
- Homozygosity, for *PROPI* mutations, 320–321
- Honeymoon period, 842, 852
- Hook (prozone) effect, 99, 99*f*
- Hormonal and metabolic systems, fasting adaptation
 hypoglycemia, newborn and infant, 178–180, 178*f*, 179*t*
- Hormonal assay methods
 accuracy/method comparison, 90
 analytic sensitivity, 90
 analytic specificity, 90
 carryover, 91
 impact of, 2–4
 interferences, 90
 precision, 89–90
 recovery, 90
 sample types and matrix effects, 90
 stability, 91
- Hormone replacement therapy, in congenital adrenal hyperplasia, 159
- Hormones. *See also* Peptide hormones; Steroid hormones
 blood production rates of, in mid-follicular phase women, 549*t*
 for BMC, 263
 deficiency, 923–924
 fat-derived, 982
 future directions, 600
 receptor types of, 31*t*
 secretion and action of, acquisition of patterns, 4–5
 secretion of, in children/infants, 531–532*f*, 544–545
 signaling, abnormalities of, 3–4
 for skeleton, 263
- Hormone treatment, 696*b*
- HOXA135*, 133–134
- 17 β -HSD type 5, 549
- Human antimouse antibody (HAMA), 99–100
- Human chorionic gonadotropin (hCG)
 during pregnancy, 41
 in thyroid metabolism, 203*f*
- Human energy balance, 940
- Human growth, 299
- Human leukocyte antigen (HLA) linkage, 454*f*, 455
- Human LH choriogonadotropin receptor (LHCGR), 672
- Human regular insulin, 840–841
- Hunger, 941–942
 “Hungry bone syndrome,” 756
- Hybridization, 10
- Hydatidiform mole-associated and imprinted transcript (*HYMAI*), 282
- Hydrogen ion (H⁺), 221*t*, 802*t*
- Hydroxyapatite, 220
- β -Hydroxybutyrate, 830
- 17 α -Hydroxylase, 146
- 11 β -Hydroxylase deficiency (11OHD), 146, 459
- 17 α -Hydroxylase deficiency, 450
- 21-Hydroxylase deficiency (21OHD), 451–459
 adrenalectomy for, 459
 characteristics of, 144–146
 clinical forms of, 453–454
CYP21A2 gene causing, 455–456
- 21-Hydroxylase deficiency (21OHD) (*Continued*)
 diagnosis of, 456–457
 gene conversions causing, 455–456, 456*f*
 genetics of, 454–455, 454*f*
 hypertensive form of, 459
 incidence of, 454
 microconversions causing, 455–456, 456*t*
 mutations causing, 456
 newborn screening programs, 456
 nonclassic, 453–454, 456
 pathophysiology of, 452–453, 452*f*
 postnatal
 diagnosis, 456–457
 treatment of, experimental, 458–459
 prenatal
 diagnosis of, 456
 treatment of, experimental, 458
 salt-wasting, 453, 455–456
 simple virilizing, 453, 456
 treatment of, 457–458
- 3 β -Hydroxysteroid dehydrogenase, 430
 characteristics of, 430
 deficiency, 146, 449–450
- 11 β -Hydroxysteroid dehydrogenase
 -1-apparent cortisone reductase deficiency, lesions in, 460
 -2-apparent mineralocorticoid excess, lesions in, 460
 isozymes of, lesions in, 460
- 17 β -Hydroxysteroid dehydrogenase (17 β -HSD), 147, 431–432, 549
- 3 α -Hydroxysteroid dehydrogenase
 isozyme deficiencies, 146–147
- 11 β -Hydroxysteroid dehydrogenases, 432–433
- Hydroxysteroid dehydrogenases (HSDs), 428
 characteristics of, 428
- 3 α -Hydroxysteroid dehydrogenases (3 α HSDs), 433
- 5-Hydroxytryptamine (5-HT), 944
- 25-Hydroxyvitamin D₃ (calcidiol) (25OHD₃), 221*t*, 802*t*
- Hyperaldosteronism
 bilateral idiopathic, 472
 child with, 472
 familial, 472
 primary, 471–472
- Hyperandrogenism
 in adolescence, 588–600, 589*b*, 590*f*
 adrenal, 590
 androgenic drugs, 594
 androgen overproduction in, 594, 595*f*, 598*f*
 antiandrogens for, 599
 clinical manifestations, 589
 combination oral contraceptive pills for, 599
 differential diagnosis of, 594
 functional ovarian, 590*f*, 593–594
 initial laboratory workup for, 597*f*
 insulin-lowering treatments for, 600
 laboratory manifestations, 590
 management of, 596–600
 menstrual irregularity, 599

- Hyperandrogenism (*Continued*)
 Metformin for, 600
 polycystic ovarian syndrome causing, 588–594, 589b
 progestin monotherapy for, 599
 tumors of, 594
- Hyperandrogenism, insulin resistance, and acanthosis nigricans (HAIR-AN), 52
- Hypercalcemia, 238
 in adolescence
 etiology of, 729t, 735–741
 evaluation of, 741–742, 742t
 management of, 742–744
 causes of, 729t
 in children
 etiology of, 729t, 735–741
 evaluation of, 741–742, 742t
 management of, 742–744
 drugs causing, 740
 etiology of, 728–733, 729–731t
 evaluation of, 733–735, 734f
 genetic variants, 740
 in infants, 728–744, 729t, 734f
 management of, 733–735
 in neonates, 728–744, 729t, 734f
 oncogenic, 740
- Hypercalciuric hypocalcemia, 713–714
- Hypercholesterolemia, 401
 autosomal dominant, 1007, 1007t
 autosomal-recessive, 1007, 1007t
 familial, 1007, 1007t
- Hyperfunctioning nodules, 408
- Hyperglycemic clamps, 861
- Hyperglycemic hyperosmolar state (HHS), 832
- Hypergonadotropic hypogonadism, 679–680
- Hyperinsulinemia
 algorithm for, 948f
 energy balance in, 948f
 negative feedback pathway in, 948f
- Hyperinsulinemic hypoglycemia, 280–281
 associated with insulin receptor defects, 913
 diagnostic criteria for, 910t
 as prodrome to onset of diabetes mellitus, 915
- Hyperinsulinism, 910–916
 adenosine triphosphate sensitive potassium channel, 910
 after gastrointestinal surgery, 915
 carbohydrate-deficient glycoprotein, 912–913
 cause of, 184
 congenital, 184, 912
 diagnosis of, 183t, 908–910
 diffuse, 186
 exercise-induced, hypoglycemia, 912
 factitious, 914
 FOXA2, 189
 with GDH, 910–911
 glucokinase, 188–189, 911
 hepatocyte nuclear factors and, 189
 hexokinase 1, 911
 HNF1A, 911–912
 HNF4A, 911
- Hyperinsulinism (*Continued*)
 hyperammonemia syndrome, 187–188
 hypoglycemia, newborn and infant, 184–196
 in Kabuki syndrome, 190
 K_{ATP}, 185–187
 monogenic, 184–189
 newborn and infant, 183t
 perinatal stress-induced, 184
 protein-sensitive, oral protein tolerance test for, 929
 SCHAD, 188, 911
 and similar disorders, 183–184
 syndromic forms of, 189–190, 189f
 in toddler and child, 910–928
 transient, 184
 in Turner syndrome, 190
 uncoupling protein-2, 189, 912
- Hypermagnesemia, 747
- Hypernatremia, 368–371, 369f
 adipsic, 380–381
- Hyperosmolality, 360–361, 361f, 829–830
- Hyperosmolar hyperglycemic syndrome and diabetic ketoacidosis, 840
 principles of, 840b
 treatment of, 839
- Hyperosmolar nonketotic coma, 832
- Hyperostosis, 786
- Hyperostosis hyperphosphatemia syndrome, 795
- Hyperparathyroidism
 jaw tumor syndrome, 505
 neonatal
 primary, 736
 secondary, 731
 primary, 507–508, 511
- Hyperparathyroidism-jaw tumor (HPT-JT) syndrome, 505
- Hyperphosphatemia, 709t, 712–713
- Hyperphosphatemic familial tumoral calcinosis, 795
- Hyperplasia
 ACTH-independent multinodular adrenal, 469
 C-cell, 501, 502f
- Hyperprolactinemia, 577–578
- Hypertension, in Turner syndrome, 634t, 640–641
- Hyperthyroid, 5
- Hyperthyroidism, 403–406
 antithyroid drug therapy, 403–404
 autosomal dominant, 402
 causes of, 407–408
 clinical features, 403
 Graves disease, 403
 transient, 400
- Hyperthyrotropinemia, 209–210
 transient neonatal, 212
- Hypertriglyceridemia
 disorders of, 1008
 familial, 1007, 1007t
- Hypervitaminosis D, 728
- Hypoalphalipoproteinemia, 1007t
- Hypobetalipoproteinemia, 1009
- Hypocalcemia, 238, 705–728
 in adolescence
 etiology of, 722–725
- Hypocalcemia (*Continued*)
 management of, 727–728
 causes of, 709t
 in children, 722–728
 etiology of, 722–725
 evaluation of, 720f, 725–727
 management of, 719–722, 727–728
 and infant, 705–722
 in neonates, 705–722
 causes of, 709t
 early, 709–712
 late, 712–713
 management of, 719–722
- Hypoglycemia, 5
 adrenocorticotropin deficiency in, 923–924
 AKT2, 913
 alcohol-induced, 925
 alimentary, 915
 artifactual, 927–928
 autoimmune, 914
 cortisol deficiency in, 923
 critical sample of, 904, 929
 in cystic fibrosis related diabetes, 915
 definition of, 908
 in diabetes mellitus, 852–853
 diagnostic fasting test, 929
 diarrheal illness in, 926
 emergency treatment, 928
 epinephrine in, 924
 exercise-induced, hyperinsulinism, 912
 Fanconi-Bickel syndrome in, 921–923
 in fasting, starvation, illness, and stress, 926–927
 fasting system approach to, 908–910
 fatty acid oxidation defects in, 925
 fructose 1,6-diphosphatase deficiency in, 924
 glucagon deficiency in, 924
 glucagon stimulation test, 929
 gluconeogenesis disorders in, 924–925
 glycogen branching enzyme deficiency (type 4 GSD), 921
 in glycogen storage diseases, 916–923
 glycogen synthase deficiency in, 917
 growth hormone resistance and IGF-1 deficiency in, 923
 in hereditary fructose intolerance, 924–925
 hyperinsulinemic, 280–281
 induced by exogenous agents, 925–926
 infections in, 926
 insulin signaling pathway, 191
 in intensive care unit, 927
 ketotic, 922–923
 malaria in, 926
 newborn and infant
 abnormalities of transition, 177
 after fundoplication, 191–192
 classification of causes, 183–190, 183b
 clinical symptoms and signs associated with, 182
 counterregulatory response defects, 192
 definition, 180–182, 180t
 diagnostic approach, 182–183, 182f
 diagnostic glucose threshold, 181

- Hypoglycemia (*Continued*)
 differential diagnosis, 180t
 fatty acid oxidation, 194–195, 194f
 glucose metabolism, principles of, 175–176
 glucose transporters defects, 195–196
 glycogenolysis and gluconeogenesis defects, 192–193
 hormonal and metabolic systems, fasting adaptation, 178–180, 178f, 179t
 hyperinsulinism, 184–196
 hypoglycemic brain damage, 181–182
 hypoketotic, 191
 in infants of diabetic mothers, 184
 management in first 24 to 48 hours, 177–178
 neonates at risk of persistent, 177b
 signs in neonates, 182b
 therapeutic threshold, 181
 treatment, 196
 nonislet tumor, 915–916
 in organ system disease, 926–927
 phosphoenolpyruvate carboxykinase deficiency in, 924
 postprandial, 915
 oral glucose tolerance tests for, 929
 with prolonged exercise, 926
 pyruvate carboxylase deficiency in, 924
 reactive, 927
 from salicylate intoxication, 925–926
 sepsis in, 926
 during starvation and malnutrition, 926
 symptoms, signs and effects of, 906–908, 907b
 tests for diagnosis of, 929
 in toddler and child, 904
- Hypoglycemia-associated autonomic failure (HAAF), 853, 908
- Hypoglycin A, 926
- Hypogonadism
 causes, 573–578, 574t
 differential diagnosis of, 574t, 577–578f, 578–583
 hypergonadotropic, 679–680
 hypogonadotropic, 151–152
 functional, 679
 mutations in, 576t
 persistent, 676–678
 hypothalamic, 152t
 management of, 583–585
 menstrual cycle in, 573–585
- Hypogonadotropic hypogonadism
 functional, 679
 mutations in, 576t
 persistent, 676–678
- Hypokalemia, 733
- Hypoketotic hypoglycemia, newborn and infant, 191
- Hypolipidemias, 1008–1009
- Hypomagnesemia, 745–747
 magnesium metabolism disorders, 745–747
 mutations associated with, 746t
- Hypomagnesemia type 1 with secondary hypocalcemia, 233
- Hypomethylation of imprinted loci (HIL), 282–283
- Hyponatremia, 368, 368f
 ANP with, 375–376
 cancer and, 375t
 central nervous system and, 375t
 organic osmolyte changes in, 374f
 plasma volume in, decreased effective, 373
 sodium chloride, primary loss of, in, 373
 systemic dehydration in, 372–373
 treatment of, 372–373
 emergency, precautions in, 374, 374f
 true/factitious, causes of, 376
 vasopressin in
 abnormal regulation of, 374–376, 375t
 with decreased secretion, 371–372
 with increased secretion, 372–374
 with normal regulation, 371–374, 372t
- Hypoparathyroidism, 713–719, 727, 885t, 887t, 892
 autoantibodies in, 897
 causing hypocalcemia, 709t
 in children, 710–712t, 713–719
 deafness, renal disease syndrome (Barakat disease) (HDR), 802t
 deafness, renal disease syndrome (HDR), 802t
 genetic variants, 740
 in infants, 710–712t, 713–719
 retardation, dysmorphism syndrome (HRD), 802t
- Hypophosphatasia (HPP), 733, 751t
- Hypophosphatemia, 839
- Hypophosphatemic rickets
 in children, 759
 with hypercalciuria, 764–765
 PHEX in, 759–760, 761f
- Hypophysectomy, 3f
- Hypophysitis, 318–319
- Hypopituitarism
 congenital, undescended testes in, 192f
 growth hormone deficiency and, 923
- Hypoplasia
 congenita of
 in adrenal gland, 463
 X-linked, 463
 Leydig cell, 143–144
- Hypospadias, 149, 671
- Hypothalamic anovulation, 585–587
 causes, 585
 differential diagnosis of, 585–586
 management of, 586–587
- Hypothalamic astrocytoma, in brain, 577f
- Hypothalamic development, congenital disorders of, 317
- Hypothalamic dysfunction, 577
 causing adrenal insufficiency, 466
- Hypothalamic hamartoma, magnetic resonance imaging of, 567f
- Hypothalamic hypogonadism, 152t
- Hypothalamic KNDy, 663–665
- Hypothalamic obesity, 970–971
- Hypothalamic paraventricular nuclei, magnocellular components of, 359, 360f
- Hypothalamic-pituitary-adrenal axis, 434–436
 anorexia, 981
- Hypothalamic-pituitary dysfunction, 402
- Hypothalamic-pituitary-gonadal (HPG) axis, 661, 663f
 anorexia, 982
- Hypothalamic-pituitary hypothyroidism, 402
 diagnosis of, 402
 in infants, 402
- Hypothalamic-pituitary-thyroid axis, 5
 in anorexia, 981
- Hypothalamus, 434–435
 in AVP, 434–435
 conditions affecting, 678
 in CRF, 434–435
 inflammation and infiltration of, 318–319
 irradiation of, 320
 trauma of, 318
 tumors of, 319
 vasopressin cells in, 360f
- Hypothyroidism, 6, 325, 400–402, 964–965
 cancer survivors in, 402
 central, 213
 congenital, 208–214
 diagnosis of, 212
 dysgenetic, 209t
 newborn screening for, 208, 400
 positive screening report for, 213–214, 214f
 transient, 212
 treatment of, 214
 with TSHR and transcription factor mutations, 209t
 consumptive, 212
 hypothalamic-pituitary, 402
 diagnosis of, 402
 in infants, 402
 iodine-induced, 401–402
 primary, 212
 sexual precocity caused by, 568f
 subclinical, 401
 transient neonatal, 212
- Hypothyroxinemia, transient, of prematurity, 212
- I**
- IBD. *See* Inflammatory bowel disease (IBD)
- Idiopathic hyperandrogenism, 594
- Idiopathic juvenile osteoporosis, 778
- Idiopathic pituitary growth hormone deficiency, 323
- Idiopathic short stature
 constitutional delay, 333
 etiologies, 332
 of postnatal onset, 332–333
- IGF. *See* Insulin-like growth factor (IGF)
- IGF1 defects, 325
- IGF-1 receptor, 223–226t

- ¹³¹I iodine-avid pulmonary metastases, 415
- IKBKG genes, 786
- Illness, hypoglycemia in, 926–927
- ILs. *See* Interleukins (ILs)
- IMAGe syndrome, 131–133, 463
- Imaging studies
magnetic resonance, of hypothalamic hamartoma, 567f
in pheochromocytoma, 492t
- Immune dysregulation,
Polyendocrinopathy, Enteropathy, and X-linked inheritance (IPEX), 887
- Immune intervention strategies, in diabetes mellitus type 1, 823–824
- Immunoassay
analytical specificity via extraction and chromatography, 93f
free hormone assays, 92–94
free T4 by direct measurement, 93, 93f
vs. immunometric (sandwich) assay, 91–92, 92f
limitations of, 92
potential confounders in, 99–100
standardization/harmonization, 100–101, 100f
- Immunoglobulins, placental transfer of, 208–209
- Immunoreceptor tyrosine-based motif (ITAM), 221t
- Immunotherapy-related autoimmune disease, 462
- Implantation, 107
docking sites, 107
trophoblast cells proliferation, 108
- Imprinting, disorders of, 23
- Inborn errors
of metabolism, 4–5
of thyroid hormone synthesis, 6
- Increased bone mass, 750
- Indian hedgehog (IHH), 221t, 223–226t, 245–247, 309
- Infant Cushing disease, 470
- Infantile hypophosphatasia, 767
- Infants
adenosine triphosphate sensitive potassium channel hyperinsulinism, 910
BMC in, 262t
BMD in, 262t
bone mineralization disorders in, 748–750
calcium (Ca) intake in, 228, 228t
with congenital hypothyroidism, treatment of, 400
female reproductive system in, 531–532f, 544–545
glucose metabolism in, physiologic development of, 904–906
GnRH secretion in, 532f, 544
hypercalcemia in, 728–744, 729t, 734f
hyperinsulinism in, 184–196, 910–916
hypocalcemia, 705–722
hypoglycemia in, 910–928
abnormalities of transition, 177
after fundoplication, 191–192
- Infants (*Continued*)
classification of causes, 183–190, 183b
clinical symptoms and signs
associated with, 182
counterregulatory response defects, 192
definition, 180–182, 180t
diagnostic approach, 182–183, 182f
diagnostic glucose threshold, 181
differential diagnosis, 180t
fatty acid oxidation, 194–195, 194f
glucose metabolism, principles of, 175–176
glucose transporters defects, 195–196
glycogenolysis and gluconeogenesis defects, 192–193
hormonal and metabolic systems, fasting adaptation, 178–180, 178f, 179t
hypoglycemic brain damage, 181–182
hypoketotic, 191
in infants of diabetic mothers, 184
management in first 24 to 48 hours, 177–178
neonates at risk of persistent, 177b
signs in neonates, 182b
therapeutic threshold, 181
treatment, 196
hypoparathyroidism in, 710–712t, 713–719
hypothalamic-pituitary
hypothyroidism in, 402
increased bone mass in, 750
low birth weight, 748–749
low bone mass, 748–750
mineral homeostasis in, 705–747, 795–801
osteopetrosis in, 750
premature, thyroid dysfunction syndromes, 207
thyroid function in, 203
vitamin deficiency in, 754f, 756
- Infection
causing central diabetes insipidus, 377–378
causing diabetes mellitus, 864
in hypoglycemia, 926
- Infiltrative disease, causing central diabetes insipidus, 377–378
- Inflammation
brain and/or hypothalamus, 318–319
cytokines in, 955–956
- Inflammatory bowel disease (IBD), 327, 644
- Inflammatory disorders, 326–327
- Inhibin B, 666
- Inhibins, 549
- Inhibitor of kappa light polypeptide gene enhancer, 223–226t
- Inner ring monodeiodination, 206, 206f
- Inositol-1,4,5-triphosphate (IP₃), 221t
- Inositol triphosphate receptor, 223–226t
- INS gene, 284
- Insulin, 1–2, 308
action of
acquired defects in, 865
genetic defects in, 864–865
active, 846
basal
rates, 845–846
suspend, 846
beta cells and
resistance of, 815f
secretion of, 280f
biosynthesis of, 824–825, 825f
closed-loop delivery, 849–850
as a determinant of fetal growth, 280–281
in diabetes mellitus
action of, 827–829
receptors for, 827, 828f
secretion of, 825–827, 826f
human regular, 840–841
hypersecretion of, 970
suppression of, 975
initiating, therapy, 842–843
injections, 836–837
intermediate-acting, 842
long-acting, analogues, 842
muscle, 953
negative regulation, 942
nonalcoholic fatty liver disease (NAFLD) and, 957
premixed, 842
pump therapy, 843–844
acute complications of, 846–847
in kindergarten and school, 847
specific considerations in, 846–847
rapid-acting analogues, 841–842
receptors, structure of, 828f
receptor tyrosine kinase family, 50–51, 51f
regimens for, 843–849
resistance to, 2–3, 952–953
nonalcoholic fatty liver disease and, 957
polycystic ovarian syndrome (PCOS) and, 957–958
type B, 864–865
secretion of
action and, 815
pancreatic beta cells in, 280f
signaling pathway
AKT2-hypoketotic hypoglycemia, 191
PI3K-hypoketotic hypoglycemia, 191
syringes *versus* pens, 842
therapy, 835, 839
types of, 840–843, 841f
utero deficiency of, 280–281
- Insulin gene mutations, 286
- Insulin-like factor 3 (INSL3), 663
- Insulin-like growth factor (IGF), 109, 221t, 308–309, 802t
insensitivity, 325
- Insulin-like growth factor-1 (IGF-1), 223–226t, 263
androgens, 308
deficiency, in hypoglycemia, 923
deficiency/resistance, 324–325
estrogens, 307–308

- Insulin-like growth factor-1 (IGF-1)
(Continued)
glucocorticoids, 307
IGFBPs, 307
insulin, 308
serum concentrations of, 306
structure and molecular biology, 306
targeted disruption of components,
307
thyroid hormone, 307
type 1 IGF receptor, 306–307
- Insulin-like growth factor (IGF)-1 and 2,
1–2, 52
- Insulin-like growth factor-binding
proteins (IGFBPs), 307
- Insulinoma, 508, 914–915
- Insulin promoter factor 1 (*IPF1*),
286–287
- Insulin receptor, 51–52
- Insulin resistance, 439, 952–953
adipose tissue, 953
assessment of, 953
comorbidities related to, 958
hepatic, 952
- Insulin secretion
glucokinase, 286
 K_{ATP} channel and, 283–284, 284*f*
- Insulin sensitivity factor (ISF), 843
- Insulin-to-carbohydrate (ICR) ratio, 843
- Integrin α v, 223–226*t*
- Integrin β 3, 223–226*t*
- Integrin-binding sialoprotein (*IBSP*),
223–226*t*, 232
- Intensive care unit, hypoglycemia in,
927
- Interferences, 90
- Interferon induced transmembrane
protein family 5, 223–226*t*
- Interferon-induced transmembrane
protein-5 or bone-restricted
ITIFM5-like protein (IFITM5),
780–781, 802*t*
- Interleukin-6, 223–226*t*
- Interleukin 6-dependent DNA binding
protein, 223–226*t*
- Interleukins (ILs), 221*t*, 802*t*
- Interleukins-1-beta, 223–226*t*
- Intermediate-acting insulin, 842
- Intermediate DEND syndrome (iDEND),
285–286
- Internalizing disorders and gender
dysphoria, 697–698
- Internal secretions, 2*f*
- International Osteoporosis Foundation
(IOF), 770
- International Osteoporosis Society, 802*t*
- International Society for Clinical
Densitometry (ISCD), 770, 802*t*
- International Society for Pediatric and
Adolescent Diabetes (ISPAD), 840
- Intersex, 564–565
- Intestinal microbiome, in obesity, 964
- Intolerance, carbohydrate, in Turner
syndrome, 644
- Intracellular regulation, of childhood
growth, 309
- Intrauterine growth retardation, 280–281
- Inverse agonist, 31
- Iodide/sodium symporter defects, 396*f*
- Iodine, 401
deficiency, 212, 402
excess, 212
- Iodothyronine, 203*f*, 402
enzymes in, 396*f*, 398*f*
maturation of thyroid hormone
metabolism, 206–207, 206*f*
monodeiodinase, 398*f*
sulfated, 206
- Iodotyrosine deiodinase, 396*f*
- Ionizing radiation, abdominal, 864
- IPEX syndrome, 287, 725, 893–894
- Islet cells, antibody, 823*t*
- Islet transplantation, pancreas and, 866
- ITPR1*, 220–226
- J**
- Jackson-Weiss syndrome, 248
- JAG1*, 210
- Jamaican vomiting sickness, 926
- Jansen metaphyseal chondrodysplasia,
238
- JIA, 326–327
- Jod-Basedow effect, 408
- “Juvenile diabetes,” 817
- Juvenile hypothyroidism, acquired, 401
Hashimoto thyroiditis in, 401
- Juvenile Paget disease, 769–770
- K**
- Kabuki syndrome, 190, 912
- Kallmann syndrome, 676.
See also Anosmia
- Karyotypes
testing, 632, 632*f*
in Turner syndrome, 628
- Katacalcin, 238
- KAT6B*, 134
- K_{ATP} channel, 279–280
and insulin secretion, 283–284, 284*f*
normal state of, 283–284
role of, 280*f*
- K_{ATP} gene mutations, 285–286
- K_{ATP} hyperinsulinism
classification, 185–187
clinical features of infants with, 186
clinical presentation, 185–186
diffuse, 186*f*
focal, 186–187
histologic forms of, 186
pathophysiology, 186
- KCNJ11* gene, 6, 285–286
- Kenny-Caffey syndrome (KCS), 802*t*
- Kenny-Caffey syndrome type 1 (KCS1),
716
- Ketoacidosis, 831
- Ketoconazole, 573
for hypercalcemia, 744
- Ketogenesis, 835
- Ketonemia, 847
- Ketones, 830
- Ketoneuria, 836–837
- “Ketosis-prone diabetes,” 817
- 17-Ketosteroid reductases, 431
- 17-Ketosteroids (17-KS), 528
- Ketotic hypoglycemia, 922–923
- Kidney, vasopressin action in, 363–364,
364*f*
- Kisspeptin, 663–664
- KISS1 receptor/GPR54, 42–43
- Klinefelter syndrome, 344, 680, 865
- Klotho, 223–226*t*
- α Klotho, 228, 231
- Koch’s postulates, 864
- Kussmaul respiration, 831
- L**
- Laboratory evaluation, of adrenal gland
function, 440–446
- Laboratory methods
laboratory terminology, 87*t*
in pediatric endocrinology, 86–87
analytic validation of, 89–91
clinical sensitivity, 87
clinical specificity, 87
interpretation of test results, 98
negative predictive value (NPV), 87,
88*f*
overview of, 86
positive predictive value (PPV), 87,
88*f*
statistics, 87–89, 88*f*
- Langerhans cell histiocytosis (LCH),
319
- Laparoscopic adjustable gastric banding
(LAGB), for pediatric obesity, 976
- Laparoscopic sleeve gastrectomy (LSG),
976
- Laron dwarfism, 923
- Laron syndrome, 2–3
- Late neonatal hypocalcemia, 712–713
- Lathosterolosis, 800
- Lawson Wilkins Pediatric Endocrine
Society (LWPES), 2
- LCH. *See* Langerhans cell histiocytosis
(LCH)
- LDL. *See* Low-density lipoprotein (LDL)
- Leiomyomas, 514
- LEOPARD syndrome, 330
- Lepreca. *See* Prolyl 3-hydroxylase 1
(P3H1)
- Leprechaunism, 864, 913
- LEPRE genes, 781
- Leptin, 207, 223–226*t*, 265, 955, 974*t*
deficiency of, 967
negative regulation, 943
in puberty, 542
receptor, 49–50
deficiency of, 967
isoforms, 49*f*
for skeletal growth, 265
resistance, 947–948, 948*f*
therapy, 975
- Leptin-melanocortin pathway defects,
966–969
- Leri-Weill dyschondrosteosis (LWD),
329, 629, 634–635
- Levothyroxine, 203–204, 204*f*
- Levothyroxine therapy, 326
differentiated thyroid cancer, 414
- Leydig cells, 665–666
- LH. *See* Luteinizing hormone (LH)

- LH choriogonadotropin receptor (LHCGR), 672
- LHX4*, heterozygous mutations in, 320
- LHX3*, recessive mutations in, 320
- Lifestyle modification, in childhood obesity, 972–973
- Li-Fraumeni syndrome (LFS), 515
- Ligand, 31
- Ligand defective apoB-100, familial, 1007, 1007*t*
- Lightbulb sign, 497–498
- Limited joint mobility (LJM), 856
- Linearity, in analytic validation, 89, 89*f*
- Lipids
- disorders of
 - in children and adolescents, 1004–1010, 1012–1014
 - genetic testing for, 1012
 - hypolipidemia, 1008–1009
 - routine screening for, 1011–1012
 - partitioning of, 953–954, 954*f*
- Lipoatrophy, 854
- diabetes, 864
 - genetic syndromes of, 865*t*
- Lipopalin 2, 223–226*t*
- Lipolysis, 905–906
- Lipomas, 510*f*
- Lipoprotein lipase (LPL), 1005
- Lipoproteins
- disorders of, pediatric, 1007*t*
 - HDL, 1008
 - low-density lipoprotein apheresis in, 1016
 - metabolism of, 1004–1008, 1005–1006*f*, 1005*t*
 - normal levels of, in children/adolescents, 1011*t*
 - subclasses of, 1005*t*
 - VLDL, 1008
- Liver
- disease, in Turner syndrome, 643
 - fasting metabolic systems, 829*t*
 - fatty, insulin resistance in, 957
- Local renin-angiotensin system, 366
- anatomy and biochemistry of, 366
 - regulation of secretion of, 366
- Long-acting insulin analogues, 842
- Long bones, 245–247
- Long-term steroid therapy, 467
- Lorenzo's oil, 465
- Low birth weight (LBW), 802*t*
- Low bone mass, 770–778, 771–774*t*
- children and adolescents, 782–785
 - disorders, 748–750
 - in infants, 748–750
 - neonate and infant, 748–750
- Low-density lipoprotein (LDL), 1005–1006, 1005*t*
- apheresis, in children, 1016
- Low-density lipoprotein receptor-related protein (LRP), 221*t*
- Low-density lipoprotein receptor-related protein 2 (Megalyn), 223–226*t*
- Low-density lipoprotein receptor-related protein 5, 223–226*t*, 769–770
- Low-density lipoprotein receptor-related protein 6, 223–226*t*
- LPL. *See* Lipoprotein lipase (LPL)
- LRP5* mutations, 248
- LSG. *See* Laparoscopic sleeve gastrectomy (LSG)
- Luminal membrane, 365*f*
- Luteal phase defects, 587–588
- Luteal phase ovary, 539
- Luteinizing hormone (LH), 1–2, 38–39
- choriogonadotropin receptor gene, 143–144
- peak from, 532*f*
 - in puberty, 529*f*, 531–533*f*, 535*f*
 - regulation of, 545, 546*f*
- 17,20-Lyase deficiency, 146, 450
- Lymphatic obstruction, of Turner syndrome, 627, 633–634, 634*t*
- Lymphedema, 633–634, 634*f*
- Lynch syndrome, 515
- Lysinuric protein intolerance, 749–750
- M**
- Macroorchidism, 672–673
- Macrophage-colony stimulating factor (M-CSF), 221*t*, 802*t*
- Macrovascular disease, 860
- Madelung deformity, 329*f*, 634–635, 634*t*
- Magnesium (Mg), 221*t*, 232–233
- homeostasis, 745*t*
 - metabolism disorders, 744–747
 - hypermagnesemia, 747
 - hypomagnesemia, 745–747
 - preparations of, 721*t*
 - renal excretion of, 228
- Magnesium (Mg²⁺), 802*t*
- Magnetic resonance imaging (MRI), 6, 221*t*, 269–270
- bone volume assessment, 271–272
 - of hypothalamic hamartoma, 567*f*
- Major histocompatibility complex (MHC), 884–885
- Malabsorption, 885*t*, 892
- Malan syndrome, 344–345
- Malaria, in hypoglycemia, 926
- Male, puberty in, disorders of
- delayed puberty, 675–680
 - treatment of, 684–686
 - young man with, 681–684
- gonadotropin-releasing hormone neuronal network, 661–662
- gynecomastia, 686
- physiology, 663–669
 - pituitary development, 662
 - precocious puberty, 671–675
 - regulation of timing, 669–671
 - testicular development, 663
- Male reproductive endocrine axis, disorders
- cryptorchidism, 680
 - gynecomastia, 686
 - hypospadias, 671
- Malnutrition, 314–315
- Mammary gland, maturation of, 554–555
- “Marble bone disease.” *See* Osteopetrosis
- Mass spectrometry (MS)
- detection of ions, 95
 - direct measurement of intact proteins, 94
- Mass spectrometry (MS) (*Continued*)
- fingerprint peptide fragment, 96
 - high resolution mass, 97*f*
 - ion traps, 95
 - liquid chromatography-tandem, 95*f*
 - predicted vs. actual high-resolution, 97*f*
 - quadrupole mass filtration, 95, 95*f*
 - quantitation of testosterone, 96*f*
 - time of flight (TOF), 95
 - top-down proteomics, 97
 - triple quadrupole configuration, 96*f*
- Mastermind-like domain containing 1 (*MAMLD1*), 137
- Maternal factors, of antithyroid drugs, 212
- Maternal hyperandrogenism, 148
- Maternofetal transfers, 203–204
- Matrilin 3 (*MATN3*), 221*t*, 223–226*t*
- Matrix extracellular phosphoglycoprotein (MEPE), 221*t*, 231–232, 802*t*
- Matrix gamma carboxyglutamic acid (Gla protein), 221*t*, 223–226*t*
- Matrix metalloproteinase (MMP), 221*t*
- Matrix metalloproteinase 9, 223–226*t*
- Maturity-onset diabetes of the young (MODY), 279–280, 294*b*
- clinical criteria, 289–290
 - clinical presentation, 289–290
 - genes associated with, 289–290, 291*t*
 - with renal cysts, 291–292
 - transcription factor, 290–291
- Mauriac syndrome, 856
- Mayer-Rokitansky-Kuster-Hauser syndrome, 565–566
- McCune-Albright syndrome (MAS), 2–3, 6, 26, 343–344, 407, 568–569, 672
- MCM4* mutations, 463
- MC2R* mutations, 464
- Mediator complex subunit 4 (Vitamin D interacting protein), 223–226*t*
- Medical nutrition therapy, 851–852
- Medications, in obesity, 964
- Medroxyprogesterone acetate (MPA), 573, 802*t*
- Medullary carcinoma of thyroid (MCT), 802*t*
- Medullary thyroid carcinoma (MTC), 415–416, 500–503.
- See also* Familial medullary thyroid carcinoma (FMTC)
 - causes of, 415
 - C-cell hyperplasia, 502*f*
 - clinical presentation, 502
 - evaluation, 502–503
 - management, 502–503
 - metastatic, 416
 - in multiple endocrine neoplasia 2A, 511
 - in multiple endocrine neoplasia 2B, 511–513
 - prognosis, 503
 - thyroid lobes, 501, 501*f*
- Megalyn, 235, 240
- Melanin-concentrating hormone (MCH)
- in energy balance, 944–945
 - receptors, 44
- Melanocortin-2 receptor (*MC2R*), 36–37

- Melanocortin-3 receptor (MC3R)
mutation in, 968
- Melanocortin-4 receptor (MC4R), 37
mutation in, 968
- Melanocortin receptor accessory protein 2 (MRAP2), 968
- Melanocortin receptors, 944
- MELAS syndrome, 292
- Membrane-associated rapid response steroid binding protein (MARRS), 221*t*
- Membrane-associated rapid response steroid-binding protein (MARRS-BP), 221*t*, 244–245
- Membrane-bound transcription factor protease site 2, 223–226*t*
- MEN. *See* Multiple endocrine neoplasia (MEN)
- Menarche
pubertal milestone attainment
in, 559*t*
in rhesus monkey, 533*f*
weight at, 578*f*
- Mendelian randomization and causative pathways, 117
- Menin, 738–739
- Meningiomas, 505
- Menstrual cycle
abnormalities in, 565*f*
estradiol in, 537
failure of, 566
follicular phase of, 536–539, 537–538*f*
GnRH in, 537*f*
in hypogonadism, 573–585
interval between, 563*f*
luteal phase of, 539
normal, 536–539
sex steroids in, 540*f*
steroidogenesis in, 537*f*
- Menstrual irregularity, 599
- Mental health, in multidisciplinary care, 698–699
- MEPE, 760
- Mesenchymal sites
of PTH-rP, 237*t*
stem cell, 253*f*
- Messenger RNA (mRNA), 9
VDR, 242
- Metabolic changes, in cachexia, 979*b*
- Metabolic clearance rate (MCR), 4–5
- Metabolic syndrome, 599–600
in obesity, 954*f*, 956–957
- Metaiodobenzylguanidine (MIBG), 498
- Metanephrine testing, 496–497
- Metaphase chromosomes, 23*f*
- Metformin, 973–974, 974*t*
for hyperandrogenism, 600
- Methimazole, 403–405
- Methyl-CpG-binding domain protein 4, 223–226*t*
- Methyl prednisolone, therapeutic
potency of, 457*t*
- Metyrapone test, 446
- Metyrosine, 499–500
- Mg. *See* Magnesium (Mg)
- MGP, 260
- MHC. *See* Major histocompatibility complex (MHC)
- Microalbuminuria screening, 858
- Microconversions
for CAH, 455–456, 456*t*
causing salt-wasting 21-hydroxylase deficiency, 455–456
- Microfinite element (mFE), 221*t*
- Micro-magnetic resonance imaging (mMRI), 265–266
- Microphthalmia-associated transcription factor (MITF), 221*t*
- MicroRNA, 15
- Microsomal triglyceride transfer protein (MTP), 1004–1005
- “Midline defects,” 202
- Mineral homeostasis
bone disorders, 747–795
in children and adolescence, 705–747, 795–801
genes involved in, bone metabolism and, 223–226*t*
genetic causes of, 706–708*t*
hypercalcemia, 728–744
hypocalcemia, 705–728
in low birth weight infant, 748–749
magnesium metabolism, disorder of, 744–747
osteochondrodysplasias, 795–801
PTH effects on, 235
- Mineralocorticoids, 425–426
concentrations, 443*t*
excess of, 11 β -hydroxysteroid dehydrogenase-2-apparent, lesions in, 460
receptors, 60
replacement, in glucocorticoid therapy, 477
secretion of, 436–437
- Minifostin motif, 232
- Minipuberty, 662, 666
of infancy, 5
of newborn, 544
- MIRAGE syndrome, 134, 463
- Missense mutations, 321
- Mitchell-Riley syndrome, 287–288
- Mitochondria, 716
- Mitochondrial cholesterol uptake, 430
- Mitochondrial diabetes, 292, 862
- Mitochondrial disorders, 465
- Mitochondrial P450scc, 428
- Mitochondrial RNA-processing endoribonuclease, RNA component of, 223–226*t*
- Mitogen-activated kinase kinase kinase 1 (MAP3K1), 137–138
- Mitogen-activated protein kinase (MAPK), 221*t*, 802*t*
- Mixed renal osteodystrophy, 768
- MNX1* mutations, 288
- MODY syndromes, 817, 861–862, 862*t*
- Molecular biology, impact of, 2–4
- Molecular cytogenetics, chromosome analysis and, 22–23
- Molecular genetic investigation, for children with short stature, 337*f*
- Molecular karyotyping, 346
- Molecular mimicry, 888–889
- Monocarboxylate transporter 8 (MCT8), 207
- Monogenic diabetes, 279, 862–864
forms of, 292–293
mitochondrial diabetes, 292, 862
Rogers Syndrome, 292–293
Wolfram syndrome 1, 292
- Monogenic hyperinsulinism, 184–189.
See also Hyperinsulinism
- Morbidity
of atherosclerotic cardiovascular disease, 1004
of diabetes mellitus, 815, 838*b*
- Mortality, of diabetes mellitus, 815, 838*b*
- Mosaicism
somatic genetic defects detection, 26
in Turner syndrome, 628
- Mother, bone resorption/formation in, 258*t*
- Mothers against decapentaplegic, *drosophila*, homolog of, 2, 223–226*t*
- MPI-CDG (CDG-Ib), 912–913
- MRAP mutations, 464
- 3-M syndrome, 330–331
- MTC. *See* Medullary thyroid carcinoma (MTC)
- MTP. *See* Microsomal triglyceride transfer protein (MTP)
- Mucocutaneous candidiasis, 885*t*, 887*t*, 889–890
- Mucopolidosis type II, 731
- Mullerian duct abnormalities, 149
in 46,XX individuals, 149
- Mullerian inhibitory hormone (MIH), 129
- Multidisciplinary care, mental health in, 698–699
- Multiple daily injection (MDI) regimens, 843, 844*b*
advantages of, 843
- Multiple endocrine neoplasia (MEN), 20, 802*t*
MEN1, mutations in, 739*f*
types of, 738*t*
- Multiple endocrine neoplasia 1 (MEN1)
clinical presentation of, 505–507
dermatologic manifestations of, 510*f*
duodenopancreatic neuroendocrine tumors, 508
genetic testing, 509–510
management, 505–507
penetrance of, 507*f*
pituitary adenomas, 508
presymptomatic screening, 509–510
primary hyperparathyroidism, 507–508
two-hit theory of tumorigenesis as exemplified by, 507*f*
- Multiple endocrine neoplasia 2 (MEN2), 510–511
MEN2A, 511
medullary thyroid carcinoma in, 511
MTC in, 511
pheochromocytoma in, 510–511
primary hyperparathyroidism in, 511
MEN2B, 511–513
marfanoid body habitus in, 511
medullary thyroid carcinoma in, 511–513

- Multiple endocrine neoplasia 2 (MEN2) (*Continued*)
 MTC in, 511–513
 pathognomonic clinical phenotype of, 512*f*
 pheochromocytoma in, 513
 thyroidectomy in, 513
 timing of thyroidectomy in, 513
- Multiple endocrine neoplasia type 4 (MEN4), 513
- Multiple endocrine neoplasia type 1 (MEN1) syndrome, 914
- Multiple pituitary-specific transcription factors, 301–302
- Munchausen by proxy syndrome, 914
- Murk-Jansen metaphyseal chondrodysplasia, 731–732
- Muscle, 556
 fasting metabolic systems, 829*t*
 insulin resistance, 953
- Musculoskeletal system, Ca in, 220–226
- Mutations
 activating, of G protein gene disorders, 47–48
 aquaporin-2, 381
 for CAH, 456
 carboxypeptidase E, 967
 cholesterol biosynthesis in, 799–801, 800*t*, 801*f*
 detection of, 15–19
 for diabetes mellitus type 2, 862*t*
 germline, 23
 in *GLIS3*, 287
 in high bone mass, 787–788*t*
 inactivating, G protein gene disorders, 47
 in insulin gene, 286
 in K_{ATP} gene, 285–286
LRP5, 248
MCM4, 463
MC2R, 464
MRAP, 464
 nicotinamide nucleotide transhydrogenase, 464
 in osteogenesis imperfecta, 771–774*t*, 778–782
 POMC deficiency, 967
 somatic, 23
 thyroid-stimulating hormone receptor, 207–208
 thyroid transcription factor, 210
 TSHR and transcription factor, 209*t*
- Myasthenia gravis, 887*t*, 893
 autoantibodies in, 896
- Mycotoxin, 671
- Myoblasts, 245–247, 246*f*
- Myokines, 955
- N**
- National Cholesterol Education Program (NCEP), 1009, 1011
- Natriuretic hormone type C (CNP), 221*t*
- Natriuretic peptide precursor C, 223–226*t*
- Natriuretic peptide receptor B, 223–226*t*
- Natriuretic peptide receptor C, 223–226*t*
- Natriuretic peptide system, 366–368, 367*f*
- Nausea, vasopressin secretion and, 360
- NCEP. *See* National Cholesterol Education Program (NCEP)
- NDI. *See* Nephrogenic diabetes insipidus (NDI)
- Necrotizing enterocolitis, 748
- Negative regulation, afferent system, 942–943
 insulin in, 942
- Neonatal diabetes, 865–866
- Neonatal diabetes mellitus (NDM), 279–280, 293*b*
 clinical presentation of, 281*f*
 with congenital hypothyroidism, 287
 definition of, 279–280
 diagnosis and treatment of, 288–289
 future directions in, 293
 genetic diagnosis and management of, 281*f*
 incidence of, 280
 molecular diagnosis of, 288
 with pancreatic and cerebellar agenesis, 287
 permanent, 285–288
 syndromic, 287–288
 transient, 282–285
 transition to oral therapy, 289*t*, 293
- Neonatal hyperthyrotropinemia, transient, 212
- Neonatal hypothyroidism, 2–3
 transient, 212
- Neonatal severe hyperparathyroidism (NSHPT), 728–731, 802*t*
- Neonatal thyrotoxicosis, 406–407
- Neonates
 athyreotic human, 207
 bone resorption/formation in, 258*t*
 diabetes in, 865–866
 diabetes mellitus, 3–4
 epigenetics and developmental programming, 958–959
 Graves disease in, 207
 hypercalcemia in, 728–744, 729*t*, 734*f*
 hyperparathyroidism, 731
 hypocalcemia, 705–728, 709*t*
 hypoglycemia
 at risk of persistent, 177*b*
 signs in, 182*b*
 mineral homeostasis in, 705–747, 795–801
 disorders of, 705–722
 neuroendocrine unit, 544–545
 transient hyperthyrotropinemia, 212
- Neoplasms, with central diabetes insipidus, 377
- Nephrogenic diabetes insipidus (NDI), 381–383
 acquired causes of, 381–382
 genetic causes of, 381, 382*f*
 treatment of, 382–383
- Nephrogenic syndrome of inappropriate antidiuresis (NSAID), 375
- Nervous system, 402
 central
 maturation of, 556–557
 vasopressin and, 375*t*
 sympathetic, energy expenditure and, 945
- Nervous system (*Continued*)
 thyroid hormone actions in, 207
- NESP55 transcript (neuroendocrine secretory protein-55), of GNAS, 717
- NETRIN*, 210
- Neuroactive hormones, 109
- Neuroactive steroids, 553
- Neuroectoderm, 301
- Neuroendocrine-ovarian axis
 maturation of, 528–539, 530*f*, 532*f*
 in normal puberty, 528–539, 529*f*
 regulation of, 539–543, 540–541*f*
- Neuroendocrine unit
 in fetus, 528–529
 in neonates, 544–545
- Neurofibromatosis type 1 (NF1), 515
- Neurogenin 3 (*NEUROG3*), 288
 homozygous mutations in, 288
- Neurohypophysis, 357
- Neurokinin B, mutations of, 664–665
- Neuron-specific insulin receptor knockout (NIRKO), 829
- Neuropathy, diabetic, 859–860
- Neurophysin, 358
- Neurosteroids, 553
- Neurosurgical intervention, for central diabetes insipidus, 377
- Neutropenia, glucose-6-phosphatase deficiency, 919
- Newborn
 hyperinsulinism, 184–196
 hypoglycemia
 abnormalities of transition, 177
 after fundoplication, 191–192
 classification of causes, 183–190, 183*b*
 clinical symptoms and signs
 associated with, 182
 counterregulatory response defects, 192
 definition, 180–182, 180*t*
 diagnostic approach, 182–183, 182*f*
 diagnostic glucose threshold, 181
 differential diagnosis, 180*t*
 fatty acid oxidation, 194–195, 194*f*
 glucose metabolism, principles of, 175–176
 glucose transporters defects, 195–196
 glycogenolysis and gluconeogenesis defects, 192–193
 hormonal and metabolic systems, fasting adaptation, 178–180, 178*f*, 179*t*
 hyperinsulinism, 184–196
 hypoglycemic brain damage, 181–182
 hypoketotic, 191
 in infants of diabetic mothers, 184
 management in first 24 to 48 hours, 177–178
 neonates at risk of persistent, 177*b*
 signs in neonates, 182*b*
 therapeutic threshold, 181
 treatment, 196
 screening of
 for congenital hypothyroidism, 208
 for hypothyroidism, 6

- Newborn Ovary Homeobox (*NOBOX*) gene, 141
- Newborns, 398
screening of
for congenital hypothyroidism, 400
hypothyroidism, 6
- Newborn screening programs (NBS), 157
21-hydroxylase deficiency, 456
- Next-generation sequencing (NGS), 3–4
applications, 24–26
and cell-free DNA, 25–26
mosaicism, 26
- NFκB. *See* Nuclear factor-κB (NFκB)
- Niacin, 1015*t*, 1016
- Nicotinamide adenine dinucleotide (NAD), 221*t*
- Nicotinamide adenine dinucleotide phosphate (NADPH), 221*t*
- Nicotinamide nucleotide transhydrogenase (NNT), 464
- Niemann-Pick C1-like 1 (NPC1L1), 1004
- Niemann-Pick type C disease, 447–448
- Nissen fundoplication, 915, 929
- Nivelon-Nivelon-Mabille syndrome, 133
NKX2.1, 210
NKX2.5, 210
NKX2.2 homozygous mutations, 288
- NOBOX*. *See* Newborn ovary homeobox (*NOBOX*) gene
- Noggin, mouse, homolog of, 223–226*t*
- Nonalcoholic fatty liver disease, insulin resistance and, 957
- Nonautoimmune congenital hyperthyroidism, 215
- Nonautoimmune hyperthyroidism, 215
- Nonclassic 21-hydroxylase deficiency, 453–454
mutations causing, 456
- Nonendocrine tall stature treatment, 346–347
- Nonhomeostatic regulation of energy balance, 947
- Nonhypoestrogenic menstrual disturbances
dysfunctional uterine bleeding, 579*b*, 587
hypothalamic anovulation, 585–587
perimenstrual syndromes, 588
- Noninvasive prenatal testing (NIPT), 24–25
- Nonislet tumor hypoglycemia, 915–916
- Nonlethal self-harm, 698
- Nonosmotic regulation
of thirst, 361–362, 361*f*
of vasopressin, 361–362, 361*f*
- Nonsyndromic disorders, sex development, 136*t*, 137–139
- Nonsyndromic XX disorders, sex development, 139
- Noonan syndrome (NS), 329–330, 680
diagnosis, 330
facial dysmorphic features, 329–330, 330*f*
prevalence, 329–330
PTPN11 pathogenic variants, 330
Turner syndrome *vs.*, 632–633
- Norepinephrine (NE), in energy balance, 944
- Northern blotting, 12–13
- Notch, drosophila, homolog of 1, 223–226*t*
- NOTCH signaling pathway, 302–303
- NPC1L1. *See* Niemann-Pick C1-like 1 (NPC1L1)
- NPY, 941
- NR2F2* gene, 134
- NTRK2 mutation, 969
- Nuclear factor I X (*NFIX*), mutations in, 344–345
- Nuclear factor-κB (NFκB), 221*t*, 253*f*, 802*t*
subunit 1, 223–226*t*
- Nuclear factor-κB, inhibitor (IKB), 223–226*t*
- Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (NFATC1), 221*t*, 223–226*t*
- Nuclear glucocorticoid receptor, 264–265
- Nuclear receptor coactivators, 553
- Nuclear receptors, 30, 53–54
and clinical conditions, 57*t*
ligand-induced activation of transcription, 56*f*
phylogenetic tree, 55*f*
structure, 54
subfamily 0, 60–61
subfamily 2, 58
subfamily 3, 58–60
- Nuclear receptor subfamily 0, group B, member 1 (NROB1), 138
- Nucleic acid bases, 10
- Nucleus accumbens, 947
- NUP107*, 141
- Nutritional deficiencies, 314–315
- Nutritional regulation, of childhood growth, 308
- Nutrition therapy, diabetes mellitus, 851–852
- O**
- Obesity, 439, 599–600, 939–982
body mass-index-for-age percentiles for, 950*f*
cause of tall stature, 344
cortisol and, 959–960
definition of, 949
dietary fat in, carbohydrate *vs.*, 960
disorders of, 964–971
endocrine disorders with, 964–966
energy excess in, 949–952
environmental factors in, 959–960
epidemic of, factors associated with, 958–964
epidemiology of, 949–951
fetal programming and, 958–959
fiber and, 961
fructose and, 961, 962–963*f*
genetics, 958
glycemic index and, 961
hypothalamic, 970–971
intestinal microbiome, 964
medications in, 964
metabolic syndrome in, 954*f*, 956–957
- Obesity (*Continued*)
neonatal programming and, 958–959, 959*f*
in Prader-Willi syndrome, 969
predictive factors for, 952
prevalence of, 949–951
global, 951
racial/ethnic considerations in, 951–952
sleep deprivation and, 960
stress and, 959–960
syndromic disorders, 969–970
television viewing and, 960
vascular changes in, 954–955
- Octreotide, 974*t*, 975
- Odontohypophosphatasia, 767
- Olfactory-genital dysplasia. *See* Anosmia
- Oligomenorrhea, 563
- OMIM. *See* Online Mendelian Inheritance in Man (OMIM)
- Oncogenic hypercalcemia, 740
- One disease, multiple genes (phenocopy), 24
- One gene, multiple diseases, 24
- ONH. *See* Optic nerve hypoplasia (ONH)
- Online Mendelian Inheritance in Man (OMIM), 221*t*, 802*t*
- Open-source movement, 851
- OPG, 253–254, 253*f*
- Optic gliomas, 319
- Optic nerve hypoplasia (ONH), 6
- Optimal fat mass, 586
- ORAI1, 226
- ORAI calcium release-activated calcium modulator 1, 223–226*t*
- Oral ectoderm, 301
- Oral glucose tolerance tests (OGTTs), 927
for postprandial hypoglycemia, 929
- Oral protein tolerance tests, for protein-sensitive hyperinsulinism, 929
- Oral sulfonylurea, inpatient transition to, 289*t*
- Orchidism
crypt-, 680
macro-, 672–673
- Orexin receptors, 43
- Orexins, in energy balance, 945
- Organic osmolyte, in hyponatremia, 374*f*
- Organification complex, 396*f*
- Organ system disease, hypoglycemia in, 926–927
- Orlistat, 973, 974*t*
- Orthostatic ligands, 31
- Orthosteric antagonist, 31
- Osmotic diuresis, 829
- Osmotic regulation
physiology of, 357–368
of thirst, 359–361, 360*f*
of vasopressin secretion, 359–361, 360–361*f*
- Osmotic sensor, effector pathways and, 357–364, 358*f*, 360*f*
- Osseous heteroplasia, progressive, 719
- Ossification, 793–794
- Osteoblastogenesis, 248–252
adipocytes in, 246*f*
- Osteoblasts, 246*f*
maturation of, 248

- Osteocalcin, 248, 258–259
- Osteochondrodysplasias
Blomstrand, 799
characterization of, anatomic, 795f
cholesterol in, 799–801
COL1A1 in, 798
FGF3 in, 796–798
FGF23 in, 795
genetic variants with, 796–797t
PTH in, 799
- Osteoclast, 245–247
biochemical defects in, 789f
differentiation of, 252–253, 253f
maturation of, 253, 253f
- Osteoclast-associated receptor (OSCAR), 221t, 223–226t
- Osteoclastogenesis, 252–256, 253f
- Osteocrin, 223–226t
- Osteocytes, 245–247
calcitriol in, 245–247
- Osteogenesis imperfecta
classification of, 771t, 778–779
collagen type 1 process, 779–782
endoplasmic reticulum, 782
low bone mass, 782–785
mutations in, 771–774t, 778–782
osteoblast differentiation, maturation, function, 779
type I, 780–781
type II, 781
type III, 781
type IV, 781
type V, 781
type VI, 781
type X, 782
type XI, 781–782
type XII, 779
type XIII, 780–781
type XIV, 782
- Osteogenesis imperfecta congenita (OIC), 780–781, 802t
- Osteomalacia/rickets, tumor-induced, 764–765, 765f
- Osteonectin, 258–259
- Osteopenia, 770
- Osteopetrosis
genes mutations in, 787–788t
in infants, 750, 789–790
- Osteopetrosis-associated transmembrane protein-1 (OSTM1), 221t, 223–226t, 802t
- Osteopontin, 241–242, 258–259
- Osteoporosis, low bone mass, 784
- Osteoporosis-pseudoglioma syndrome, 248, 778
- Osteoprogenitor pluripotent stromal mesenchymal stem cells, 248
- Osteoprotegerin (OPG), 221t, 248, 253–254, 253f
- Osteosclerosis, 750, 787–788t
- Osterix, 779
- OSTM1 genes, 750
- Otitis, in Turner syndrome, 643
- Otologic disorders, and Turner syndrome, 643
- Ovarian failure, 893
autoantibodies in, 894–895
- Ovarian follicles, 530f
- Ovarian insufficiency, 636–637
- Ovaries. *See also* Neuroendocrine-ovarian axis
development, 128
diagram of, 531f
fetal development of, 528–531, 530f
follicular phase of, 536–539, 537–538f
luteal phase of, 539
secretion of, 545–546, 546f
steroidogenesis in, in menstrual cycle, 537f
testosterone secretion in, 549
- Ovotesticular disorder of sex development, 139
- Ovulatory dysfunction, 587
- Oxandrolone treatment, 342
- 17-Oxidoreductases, 431
- Oxyntomodulin, 942–943
- Oxytocin, 35
biochemistry of, 357–359, 358f
peptide products of, 359f
receptor of, 363f
structure of, 358f
- P**
- Paired box gene 1, 223–226t
- Paired Box Gene 8 (PAX8), 210
- Pallister-Hall syndrome, 133
- Pancreas
beta cells in
insulin secretion by, 280f
exocrine, disorders of, 863–864
insulin secretion by beta cell, 185f
islet transplantation and, 866
- Pancreas transcription factor 1 (PTF1), 286–287
- Pancreatectomy, 864
- Pancreatic duodenal homeobox 1 (PDX1), 286–287
- Pancreatic neuroendocrine tumors
functional, 509
nonfunctioning, 509
- Pancreatic polypeptide, 943
- Pancreatic transcription factor 1 α (PTF1A), 287
mutations in, 287
- Pancreozymin, 238–239
- Papillary-follicular carcinoma, 396b
- PAPSS2 deficiency, 460
- Paracellin 1. *See* Claudin 16
- Paracrine action, 2–3
- Paracrine effects, 2f
- Paracrine regulation, of childhood growth, 308–309
- Parafibromin, 737–738
- Paraganglioma (PGL)
biochemical diagnosis, 496–497
catecholamines, biosynthesis and actions of, 495
clinical presentation, 495
disorders and genes associated with, 492t
follow-up, 500
genetic issues, 498–499
history of, 493f
management, 499–500
pediatric, diagnosis of, 498f
- Paraganglioma (PGL) (*Continued*)
pheochromocytoma and, 493–500, 493f
preoperative medical management of, 500t
prognosis, 500
radiographic studies, 497–498
and SDHB mutation, 494f
surgery, medical preparation for, 499–500
surgical therapy, 499
- Parathyroid adenoma, 736–737
with Technetium-99m Sestamibi scanning, 744f
- Parathyroid gland (PTG), 802t
- Parathyroid hormone (PTH), 1–2, 221t, 223–226t, 234–236, 705, 802t
in Ca homeostasis, 222f
in mineral homeostasis, 222f
in osteochondrodysplasias, 799
and parathyroid hormone-related peptide receptors, 45
receptors related to, 237–238
regulating calcium, 235
regulation of, 714f
- Parathyroid hormone-related protein (PTH-rP), 221t, 236–237, 237t, 309, 802t
in bone formation, 245–247
receptors related to, 237–238, 237t
types of, 237
- Paraventricular nucleus, 359, 360f
- P450aro, 432
characteristics of, 432
- Pars distalis, 300–301
- Partial agonists, 31
- Parturition
fetal maturation and, 113–114
hormonal control of human, 114–116, 115f
- Parvocellular neurons, role of, in vasopressin synthesis, 359
- Patched 1, 223–226t, 245–247
- Pathobiological Determinants of Atherosclerosis in Youth (PDAY), 1010
- PBX1 (Pre-B-cell-leukemia factor 1), 138
- P450c17
characteristics of, 430
deficiency of, 450–451
electron transport to, 429f, 430–431, 431f
- P450c21, 431
causing CAH deficiency, 426t, 429f, 431
- P450c11AS, 431
- P450c11 β , 431
- P450c11, isozymes of, lesions in, 459–460
- PCR. *See* Polymerase chain reaction (PCR)
- PDAY. *See* Pathobiological Determinants of Atherosclerosis in Youth (PDAY)
- Peak height velocity (PHV), 264
- PEDEF, 781

- Pediatric endocrine disease
 diagnosis and management of,
 principles of interpretation of
 genetic tests in, 23–24
 therapy for, recombinant DNA
 technology and, 27
- Pediatric endocrinology
 laboratory methods for, 86–87
 overview and principles of
 evaluating endocrine disorders in
 infancy and childhood, 6
 historical background of, 1–2
 impact of hormonal assays and
 molecular biology to, 2–4
 unique aspects of, 4–5
- Pediatric obesity
 bariatric surgery for, 975–977
 diagnostic approach in, 971, 971*t*
 disorders in, classification of, 965*b*
 energy inadequacy in, 977–982
 laparoscopic adjustable gastric banding
 in, 976
 lifestyle modification in, 972–973
 metabolic impact of, 952–958
 pharmacotherapy for, 973–975, 974*t*
 future of, 975
 Roux-en-Y gastric bypass in, 976
- Pediatric thyroid nodules, somatic driver
 mutations in, 412*b*
- Pelvic ultrasound studies, in Turner
 syndrome, 636, 638*f*
- Pendred syndrome, 211
- Penetrance, 23
- Penis, small. *See* Cryptorchidism
- Pentagastrin, 238–239
- P450 enzymes, 439
- Peptide hormones
 metabolism of, 547–554
 synthesis of, 547–549
- Peptide hormones peptide YY (PYY),
 942–943
- Peptide receptor group
 adrenocorticotropin and
 melanocortin-2 receptors, 36–37
 melanocortin-4 receptor (MC4R), 37
 vasopressin receptors, 38
- Peptidyl-prolyl isomerase B (CypB),
 223–226*t*
- Perinatal asphyxia, 6
- Perinatal hypophosphatasia, 767
- Perinatal trauma, 318
- Periosteum, 252
- Peripheral precocity, 672
- Peripheral quantitative computed
 tomography (pQCT), 269–270,
 270*t*
 non-dominant distal radius, 270*t*
- Pelzman syndrome, 345
- Permanent neonatal diabetes mellitus
 (PNDM), 285–288
 cause of, 286
 epidemiology of, 285
 genetic forms of, 286–287
 glucokinase, 286
PDX1, 286–287
 insulin gene mutations in, 286
K_{ATP} gene mutations in, 285–286
- Pernicious anemia, 885*t*, 887*t*
- Peroxisome biogenesis disorders (PBDs),
 465
- Peroxisome proliferator activator
 receptor gamma (PPAR γ), 58, 221*t*,
 223–226*t*
- Persistent hyperinsulinemic
 hypoglycemia of infancy, 184
- Persistent Mullerian duct syndrome
 (PMDS), 149
- Peutz-Jeghers syndrome (PJS), 515–516
- Pfeiffer syndrome, 248
- PHA. *See* Pseudohypoadosteronism
 (PHA)
- Pharmacogenomics (PGx), 24
- Phenocopy, 24
- Phenolic ring, 206
- Phenotypic features, in Turner syndrome,
 633, 633*f*
- Phenoxybenzamine, 499
- Pheochromocytomas (PHEOs), 493–500
 biochemical diagnosis, 496–497
 catecholamines, biosynthesis and
 actions of, 495
 clinical presentation, 495
 disorders and genes associated with,
 492*t*
 follow-up, 500
 genetic issues, 498–499
 history of, 493*f*
 management of, 499–500
 in multiple endocrine neoplasia 2A,
 511
 in multiple endocrine neoplasia 2B,
 513
 paraganglioma and, 493–500
 pediatric, diagnosis of, 498*f*
 preoperative medical management of,
 500*t*
 prognosis, 500
 radiographic studies, 497–498
 surgery, medical preparation for,
 499–500
 surgical therapy, 499
 surgical treatment of, 499
- Phosphatidylinositol-3-kinase
 hypoglycemia, 913
- Phosphatase and tensin homolog (PTEN)
 hamartoma tumor syndrome, 411
- Phosphate, 221*t*, 230–232, 836
 administration of, 720–722
 homeostasis, 222*f*
 late neonatal hypocalcemia, 712–713
 preparations of, 721*t*
 renal tubular reabsorption of, 230
 in sodium transport, 230
- Phosphate-regulating endopeptidase
 homolog, X-linked (PHEX), 221*t*,
 223–226*t*, 802*t*
 in hypophosphatemic rickets,
 759–760, 761*f*
- Phosphatonins, 222*f*, 231–232, 760
- Phosphodiesterase 3A, cGMP-inhibited,
 223–226*t*
- Phosphodiesterase 4D, cAMP-specific,
 223–226*t*
- Phosphoenolpyruvate carboxykinase
 (PEPCK) deficiency, 924
 in hypoglycemia, 924
- Phosphoinositide-3-kinase (PI3K), 221*t*
- Phospholipase C (PLC), 221*t*
- Phospholipase C β -1, 223–226*t*
- Phosphomannomutase 2 (PMM2)-CDG
 (CDG-1a), 912
- Phosphomannomutase deficiency.
See Carbohydrate-deficient
 glycoprotein syndrome
- Phosphopenic rickets, 758*f*, 759–766,
 761*f*
- PHV. *See* Peak height velocity (PHV)
- Physical activity intervention, in
 childhood obesity, 972–973
- Pigment epithelium derived factor
 (PEDF), 802*t*
- Pilosebaceous unit, maturation of, 555,
 555*f*
- PIT-1 deficiency, 202
- Pituitary adenomas, 508
- Pituitary deficiency, degree of, 320
- Pituitary development, 662
 genes involved in, 678
- Pituitary gland, 202, 434–436
 ACTH action in, 435, 435*f*
 conditions affecting, 678
 congenital abnormalities of, 322
 tumors involving, 322–323
- Pituitary gonadotropins, 665
- Pituitary hormone deficiency, 320–321
- Pituitary, posterior, disorders of,
 357–383
- Pituitary tumors, 1–2
- PKR-like endoplasmic reticulum kinase
 (PERK), 287
- Placenta, 530–531
 in sex hormones, 530–531
- Placental aromatase deficiency, 147–148
- Plasma
 concentration, of cortisol, 441,
 442–443*t*
 volume, decreased effective, in
 hyponatremia, 373
- Plasma adrenocorticotrophic hormone,
 444
- Plasma glucose
 concentration, 181*b*
 responses, 188*f*
- Plasma renin activity (PRA), 441–444
- Plasma steroids
 adrenal, responses of, 445*t*
 catabolism of, 439–440
 circulating types of, 438–439
 and disposal, 437–440
 nomenclature of, 437–438
 structure of, 437–438, 438*f*
- Pleckstrin homology domain containing
 protein, family M, member 1,
 223–226*t*
- Pleomorphic adenoma gene-like 1
 (PLAGL-1), 282
- 11p14 microdeletion, 968
- PMM2-CDG (CDG-1a), 913
- Podosomes, 255
- Polybrominated biphenyls (PBBs), 671
- Polycystic ovarian syndrome (PCOS)
 causing hyperandrogenism, 588–594,
 589*b*
 clinical manifestations of, 590*f*

- Polycystic ovarian syndrome (PCOS)
(*Continued*)
etiology, 593
insulin resistance in, 957–958
pathogenesis of, 591–592*f*
pathophysiology, 591
- Polydipsia, 368–371, 369*f*, 830
- Polymerase chain reaction (PCR),
9–12, 12*f*
competitive RT-, 13–14
qRT-, 13
RT-, 13–14
- Polyphagia, 830
- Polyposis, APC associated, 514
- Polyuria, 368–371, 369*f*, 830
- POMC
deficiency and splice site mutation, 967
disorders, causing adrenal
insufficiency, 466–467
gene, 435
- POMC/CART neurons, 943–944
- Porous bone. *See* Osteopenia
- Portal circulatory system, 302
- Positional genetics
in endocrinology, 20–22, 20*f*
principles of, 20–21
- Positive regulation, afferent system,
941–942
- Posterior pituitary, disorders of, 357–383
differential diagnosis of disorders of
water metabolism, 368–371
osmotic sensor and effector pathways,
357–364
overview of, 357
physiology of osmotic and volume
regulation, 357–368
sites of vasopressin action, 362–364
volume sensor and effector pathways,
364–368
- Postnatal life
CAH in, 458–459
Silver-Russell Syndrome, 462
- Postnatal thermogenesis, 207
- Postprandial hyperinsulinemic
hypoglycemia after
fundoplication, 191–192
- Postprandial hypoglycemia, 915
oral glucose tolerance tests for, 929
- Potassium, 221*t*
deficits, 839
replacement, 835–836
- Potassium channel, inwardly rectifying,
subfamily J, member 10, 223–226*t*
- Potassium channel, inwardly rectifying,
subfamily 1, member 1, 223–226*t*
- Potassium channel, voltage-gated,
Shaker-related subfamily, member
1, 223–226*t*
- POU1F1*, 321
- P450 oxidoreductase (POR), 430–431,
431*f*
deficiency, 451
- 11p paracentric inversion, 968
- Prader-Willi syndrome (PW), 331–332,
680, 865
obesity in, 969
- Precision, in analytic validation, 89–90,
90*f*
- Precision medicine, 26–27
- Precocious puberty, 671–675
central, 675
consequences of, 673–674
evaluation of boy with, of secondary
sexual characteristics, 674
gonadotropin-releasing hormone-
dependent, 672
gonadotropin-releasing hormone-
independent, 672–673
peripheral, 675
premature adrenarche, 673
treatment of child with, 674–675
- Prednisolone, therapeutic, potency of,
457*t*
- Prednisone, therapeutic, potency of, 457*t*
- Pregnancy
and birth, evolution of, 106–107
establishment of, 107–108
genetics and genomics study, 116
human gestation, 106
integrative steroidogenesis during,
111–113
thyroid function during, fetal, 203
- Pregnancy-associated plasma protein A2
(PAPP-A2), 324–325
- Pregnane X receptor, 223–226*t*
- Pregnenolone, 428, 430
regulation of, 429*f*, 547*f*
- Prehypertension, 858
- Premature adrenarche, 673
- Premature gonadal failure, 885*t*,
887*t*, 892
- Premature ovarian failure protein 1B
(POF1B), 630–631
- Premenstrual syndrome, 588
- Premixed insulin, 842
- Prenatal testicular development, 663
- Prenatal testing, 25–26
- Preproopiomelanocortin (POMC), 435,
435*f*
- Prepuberty, 662
- Presymptomatic screening, genetic
testing and, 509–510
- Preterm birth
causes of, 106
GWAS, 117
progesterone, 115–116
rare variant analysis, 117
- Primary growth impairment, short
stature
chromosomal abnormalities, 332
copy number variation, 332
disorders involving
autocrine/paracrine factors, 327–328
cartilage extracellular matrix,
328–329
C-type natriuretic peptide signaling,
328
fibroblast growth factor signaling,
328
Indian hedgehog signaling, 328
insulin-like growth factor-2, 328
intracellular factors and/or
fundamental cellular process,
329–331
parathyroid hormone-related protein
signaling, 328
- Primary growth impairment, short
stature (*Continued*)
idiopathic short stature, 332–333
imprinting disorders
Prader-Willi syndrome, 331–332
Silver-Russell syndrome, 331, 331*f*
Temple syndrome, 332
- Primary hyperparathyroidism (PHPT),
507–508
in multiple endocrine neoplasia 2A,
511
- Primary IGF-1 deficiency (PIGFD),
336–337
- Primary insulin-like growth factor
deficiency, 325–326
- Primary ovarian failure, 573–575
- Primary pigmented nodular
adrenocortical disease (PPNAD),
503–504, 503*f*
- Primordial dwarfisms, 331, 333
- Procollagen-lysine,2-oxoglutarate
5-dioxygenase 1, 223–226*t*
- Procollagen-lysine,2-oxoglutarate
5-dioxygenase 2, 223–226*t*
- Procollagen type I amino-terminal
propeptide (PINP), 221*t*
- Procollagen type I carboxyl-terminal
propeptide (PICP), 221*t*
- Procollagen type III amino-terminal
propeptide (PIIINP), 221*t*
- Progesterone receptor isoform
production, 116*f*
- Progesterin monotherapy, 599
- Progressive diaphyseal dysplasia, 793
- Progressive osseous heteroplasia (POH),
719, 794–795, 802*t*
- Prohormone convertase-1 deficiency,
967
- Proinsulin, 824–825
- Prolactin, 42
secretion of, 546
in steroidogenesis, 546
structure of, 549
- Prolactin-growth hormone (PRL-GH)
family, 108–109
- Prolonged exercise, hypoglycemia with,
926
- Prolyl 3-hydroxylase 1 (P3H1), 221*t*,
223–226*t*, 780, 802*t*
- Proopiomelanocortin (POMC), 824
peptides, 444
in pituitary gland, 435
- Prophet of Pit1 (PROP1), 302–303
PROP1 mutations, 320–321
- Prostaglandin E₂, 248
- Protein disulfide isomerase, family A,
member 3 (MARRS-BP), 223–226*t*
- Protein kinase A (PKA), 221*t*, 223–226*t*
- Protein kinase B (PKB), 221*t*
- Protein phosphatase 3, catalytic subunit,
alpha isoform (calcineurin A),
223–226*t*
- Protein phosphatase 1, regulatory
subunit 1B, 223–226*t*
- Protein-sensitive hyperinsulinism, oral
protein tolerance test for, 929
- Proteins, in bones, 246*f*
- Provocative growth hormone tests, 336*t*

- P450_{sc}, 428
 characteristics of, 428
 deficiency, 449
 electron transport to, 428–430, 429f
- Pseudoautosomal region 1 (PAR1), 628–629
- Pseudocyesis, in hypothalamic anovulation, 585
- Pseudohermaphrodite true hermaphrodite, 125–126
- Pseudohermaphroditism
 female, 564–565
 male, 564–565
- Pseudohypercalcemia, 733–734
- Pseudohypoadosteronism (PHA), 60
 causes of, 473
- Pseudohypoparathyroidism (PHP), 2–3, 712–713, 802t
- Pseudohypoparathyroidism type 1a (PHP-1a), 966
- Pseudohypophosphatasia, 767
- Pseudopseudohypoparathyroidism (PPHP), 328, 802t
- Pseudotumor cerebri, 958
- Pseudovitamin D-deficiency rickets (PDDR), 802t
- Psychogenic amenorrhea, 585
- Psychosocial deprivation, 327
- Psychosocial problems, with diabetes mellitus, 854
- PTEN hamartoma tumor syndrome (PHTS), 516
- Pterygium colli, 633–634
- PTH. *See* Parathyroid hormone (PTH)
- PTH/PTHrP receptor 1 (PTHrP-1R), 802t
- PTH/PTHrP-1 receptor (PTH/PTHrP-1R) (PTH1R), 221t
- PTH1R, 237, 247
- PTHrP, 238
- PTH 1 receptor, 223–226t
- PTH 2 receptor, 223–226t
- PTH related/like protein, 223–226t
- PTH-rP. *See* Parathyroid hormone-related protein (PTH-rP)
- Pubarche, 560–561
- Pubertal development
 constitutional delay of, 561–562
 normal, 560–564
 premature, 560–561
- Pubertal induction, and Turner syndrome, 649–650
- Pubertal milestone attainment, 559t
- Pubertal transdermal estradiol replacement, 584t
- Puberty
 abnormal, 564–600
 adolescent development of, 531–536, 532–533f
 autoamplification process in, 535b
 baseline and follow-up protocol during induction of, 700b
 constitutional delay of, 678–679
 delayed, 6, 561–562, 675–680
 constitutional, gonadotropin deficiency, 587b
 etiologies of, 676
 persistent hypogonadotropic hypogonadism, 676–678
- Puberty (*Continued*)
 disorders of, in female, 528–600
 DSD in, 564–565
 estradiol in, 532f, 545
 FSH in, 529f, 531–533f, 535f
 leptin in, 542
 normal, 528, 529f
 obesity and metabolic syndrome, 599–600
 physiology of, 663–669
 precocious
 causes of, 566–570, 566t, 567–569f
 complete of, 566–568
 differential diagnosis of, 570–571, 571b
 GnRH agonist therapy for, 572b
 gonadotropin-dependent, 566t
 incomplete of, 568–570
 LH in, 529f, 531–533f, 535f
 management of, 571–573
 onset of, 539–543, 540–541f
 precocious, 566–568
 in rhesus monkey, 533f
 protocol induction of, 700b
 regulation of the timing, 669–671
 candidate gene-based studies, 669–670
 genetic contributions to variation, 669–670
 genome-wide association studies, 670
 secular trends, 669
 and Turner syndrome, 649–650
- Pubic hair
 development of, 559, 559f
 pubertal milestone attainment in, 559t
- Pycnodysostosis, 792
- Pyogenic skin infections, 831
- Pyridinium, of collagen, 256f
- Pyridinoline (Pyr), 221t, 802t
 urine, 257f
- Pyrophosphate, 766–768
- Pyrosequencing, 17–19, 18f
- Pyruvate carboxylase deficiency, in hypoglycemia, 924
- Q**
- Quantitative computed tomography (QCT), 221t, 269–270, 802t
- Quantitative real-time reverse transcription PCR (qRT-PCR), 13
- Quantitative ultrasonography (QUS), 221t, 269–270, 802t
- R**
- Rabson-Mendenhall syndrome, 6, 864, 913
- Radiation exposure, ionizing, abdominal, 864
- Radiation therapy, chemotherapy and cancer survival, 679–680
- Radioactive iodine (RAI) therapy, 405
 in children, 404
 differentiated thyroid cancer, 413–414
 Graves disease, 405
 risks in children, 405–406
- Radioautography, 10
- Radioimmunoassay (RIA), 824
 development of, 2–3
 principles of, 2–3
- RAI-refractory (RAIR) disease, 415
- Raloxifene, 553
- RANK-ligand (RANKL), 221t, 223–226t, 235, 802t
 in osteoclast differentiation, 253–254, 254f
- Rapid-acting analogues, 841–842
- Rapid-onset gender dysphoria (ROGD), 701
- Rapid onset obesity, hypoventilation, hypothalamic, autonomic and thermal dysregulation (ROHHD), 970–971
- Rare variant analysis, 117
- RASopathies, 329–330
- Rathke's pouch, 300–303
- Reactive hypoglycemia, 927
- Reactive oxygen species (ROS), 956, 956f
- Rearranged during transfection (RET), 500–503
- Receptor action, principles of, 31
- Receptor activator of nuclear factor κ B (RANK), 221t, 223–226t, 802t
- Receptor-based selectivity, 553
- Receptors. *See also Specific hormone i.e.*
 Adrenocorticotrophic hormone (ACTH)
 calcium sensing, 228–230
 selectivity of, in steroid hormones, 552
 vitamin D, 242–245
- Receptor tyrosine kinase (RTK), 50, 50f, 50t
- Recessive mutations, in *LHX3*, 320
- Recombinant deoxyribonucleic acid, 27
- Recombinant DNA technology, pediatric endocrine disease therapy and, 27
- Recombinant human GH (rhGH)
 treatment
 benefits, 339–340
 on-treatment monitoring, 341
 potential adverse effects, 340–341
- Recommended dietary allowance (RDA), 221t
- Recovery, 90
- Recurrent laryngeal nerve (RLN) injury, 413
- 5 α -Reductases, 432
 deficiency, 147
- Reference interval (RI), 101–104, 102–103f
- Regulators of G protein signaling (RGs), 32–33
- Renal anomalies, in Turner syndrome, 642–643
- Renal cascade, of vasopressin function, 363–364, 364f
- Renal disease, 440
 glucose-6-phosphatase deficiency, 919
 GSD type 1, 193
- Renal excretion
 of Ca, 228
 of Mg, 228
 of phosphate, 228

- Renal free water clearance
decreased, 371–372
drugs impairing, 372*t*
thyroid hormone in, 372
- Renal gluconeogenesis, 927
- Renal glucosuria, 831
- Renal osteodystrophy, 768
- Renal tubular reabsorption
of calcium, 228
of phosphate, 230
- Renin-angiotensin-aldosterone system, 364–365
- Renin-angiotensin system, 436–437
local, 364–366
- Reportable range, in analytic validation, 89
- Reproductive endocrine system, development of, 661–663
- Reproductive system
development of
anogenital distance, 130
external genital structures
development, 130
germ cell development, 128–129
human adrenal development, 129
human ovary development, 128
human testicular development, 127–128
internal genital structures
development, 129–130
sexual differentiation of brain, 130
urogenital ridge and bipotential gonad development, 126–127, 127*f*
female
in adolescent, 531–536, 533*f*, 534*t*, 535*f*
in adults, 536–539, 536*f*
fetal development of, 528–531, 530*f*
infant development of, 531–532*f*, 544–545
- Restriction, 10
- Restriction endonucleases, 10
- Restriction fragment length polymorphism (RFLP), 10, 11*f*
- RET genes, 739–740
- Retinoid X receptor (RXR), 58, 221*t*
- Retinoid X receptor α , 223–226*t*
- Retinopathy, diabetic, 858–859
assessment of, 859
treatment for, 859
- Reverse T3 (rT3), 206
- Reversion-inducing cysteine-rich protein with kazal motifs (RECK), 223–226*t*
- RFLP. *See* Restriction fragment length polymorphism (RFLP)
- Rfx6, 287–288
- Rhesus monkey, prepubertal, 533*f*
- rhIGF-1 treatment, 341–342
- RIA. *See* Radioimmunoassay (RIA)
- Ribonucleic acid (RNA), 3–4, 9, 221*t*
analysis, 12–15
denatured, 12–13
- Rickets
calciopenic, 753–759
FGF23 in, 764–765, 765*f*
in infants, 751*t*
- Rickets (*Continued*)
laboratory data for, 755*t*
phosphopenic, 758*f*, 759–766, 761*f*
tumor-induced osteomalacia and, 764–765, 765*f*
vitamin D-deficient, 754*f*
- RIEG gene, haploinsufficiency of, 321
- Ring X chromosome (rX), 644
- Risk
of atherosclerotic cardiovascular disease, in children, 1010*b*
of diabetes mellitus, type 1, 820*t*, 822*t*
of low bone mass, 770–778
- RMRP, 247
- RNA. *See* Ribonucleic acid (RNA)
- Roche-Wainer-Thissen (RWT) method, 313–314
- Rogers syndrome, 292–293, 863
- ROHHAD. *See* Rapid onset obesity, hypoventilation, hypothalamic, autonomic and thermal dysregulation (ROHHAD)
- ROS. *See* Reactive oxygen species (ROS)
- Rotterdam criteria, for PCOS, 588–589
- Roux-en-Y gastric bypass (RYGB), for pediatric obesity, 976
- R-spondin 1 (RSPO1) gene, 134
- RTHalpha, 326
- RTHbeta, 325–326
- RTK. *See* Receptor tyrosine kinase (RTK)
- Runt-related transcription factor 2, 223–226*t*, 247–248
- RUNX2, 247–248
- S**
- Salicylate intoxication, in hypoglycemia, 925–926
- Salivary glands, 1–2
- Salt-wasting 21-hydroxylase deficiency, 453
gene conversions and microconversions causing, 455–456
- SAMD9, 134
- Sandwich immunometric assays, 91–92, 92*f*, 99
- Sanger sequencing, 18*f*
- Sanjad-Sakati syndrome, 716
- Saturated fat, diet therapy for, 1012–1014, 1013*t*, 1013*b*
- SCA. *See* Steroidal cell autoantibodies (SCA)
- Scaffolding protein involved in DNA repair (SPIDR), 141
- Scavenger receptor B1 (SR-B1), 1004
- Schaaf-Yang syndrome, 969
- SCHAD hyperinsulinism, 188
- School intervention, in childhood obesity, 972–973
- Sclerosteosis type 1, 792–793
- Sclerostin, 223–226*t*, 245–247, 249–250, 249–250*f*, 769–770
- Scoliosis, in Turner syndrome, 635, 648
- SDHB mutation, paraganglioma and, 494*f*
- Seckel syndrome, 331
- Secondary follicles, 530
- Secondary growth impairment, short stature
abnormal extracellular fluid, 327
endocrine deficiencies, 315–323
hypothyroidism, 325
inflammatory disorders, 326–327
nutritional deficiencies, 314–315
psychosocial deprivation, 327
thyroid hormone resistance, 325
- Secretagogue receptor group, 42
- Secreted frizzled-related protein 1, 223–226*t*
- Secreted phosphoprotein 1 (osteopontin) (SPP1), 221*t*, 223–226*t*, 232
- Secreted protein, acidic, cysteine-rich (osteonectin), 223–226*t*
- Selective estrogen receptor modulator (SERM), 802*t*
- Self-antigens, tolerance and, 888*f*
- Self-limited delayed puberty, 678–679
- Self-monitoring, of glucose, 847
- Sensor-augmented pump (SAP)
practical deliberations for, 849
therapy, 849
- Sepsis, in hypoglycemia, 926
- Septooptic dysplasia (SOD), 6, 202, 317
- Septum pellucidum, 6
- Sequence variants, 157*t*
- Serotonin, in energy balance, 944
- Serpentine region, 32
- SERPINF1, 779, 781–782
- SERPINH1, 782
- Serpin peptidase inhibitor, clade H, member 1 (Heat shock protein 47), 223–226*t*
- Sertoli cells, 663, 665–666
- Serum concentration, of cortisol, 441
- Serum frizzled related protein-4 (sFRP4), 802*t*
- Serum IGF-1 concentrations, 335
- Serum osmolality, 830
- Serum pH, 220–230
- Serum phosphate, 230
- SESAME syndrome, 233
- Seven-transmembrane receptors, 31
- Sex, 696*b*
designated at birth, 696*b*
determination, 126
development disorders, classification of, 132*t*
- Sex chromosomal disorders, sex development, 126*t*
- Sex chromosomes
analysis of, 629–630*f*
disorders, 131
- Sex hormones
gonadotropins and, 5
in menstrual cycle, 536*f*
regulation of, 541*f*
target organs of, maturation of, 554–557, 554–555*f*
- Sex steroid exposure, 326
premature/excess, 326
- Sex steroids, 1–2, 665–666
biosynthesis defects, 146–148
concentrations by age, 442*t*
short stature treatment, 342

- Sexual differentiation of brain, 130
- Sexually infantile girls, bone age in, with normal FSH levels, 574*b*
- Sexual maturation
adolescence, 558–560, 559*f*
in children, 534*t*, 558
normal
in fetus, 557–558
in neonate, 557–558
- Sexual orientation, 696*b*
- SF1/NR5A1* gene, 136–137
- S100G*, 227
- SH2B1 haploinsufficiency, 969
- Short-chain dehydrogenase/reductase (SDR) family, 428
- Short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) hyperinsulinism, 911
- Short integrin-binding ligand-interacting glycoprotein (SIBLING), 231–232, 802*t*
- Short stature, 314
causes of
primary growth impairment, 327–329
secondary growth impairment, 314–315
children with nonspecific skeletal abnormalities, 338*f*
diagnostic approach to patient with, 333
anthropometrics, 334–335
bone age, 335
follow-up laboratories and imaging, 336–337
genetic testing, 337–339
IGF-1 concentrations, 335
inheritance pattern, 334
medical history and physical examination, 334
screening laboratory investigations, 335
time course, 333–334
genes associated with isolated, 338*t*
surgical leg lengthening, 342
treatment of, 339
aromatase inhibitors, 342
C-type natriuretic peptide agonists for achondroplasia, 342
GnRH agonist, 342
growth hormone, 339–341
insulin-like growth factor-1, 341–342
sex steroids, 342
surgical treatment, 342
in Turner syndrome, 634–636, 635*f*
- SHOX gene, 631*f*, 632–633
chromosomal location, 329
defects in, 329
- Shprintzen velocardiofacial syndrome, 715
- Side chain cleavage cytochrome P450 enzyme, 144
- Side-chain cleavage cytochrome P450sc, 428
- Signal transducers and activators of transcription (STAT-3), 943
- Signal transduction and transcription (STAT), 221*t*
- Signal transduction pathways, 2–3
- Silver-Russell Syndrome (SRS), 331, 331*f*
- SIM-1 mutation, 968
- Simple virilizing 21-hydroxylase deficiency, 453
mutations causing, 456
- Simpson-Golabi-Behmel syndrome type 1 (SGBS1), 344
- Single-nucleotide polymorphisms (SNPs), 24
- Single nucleotide variants (SNVs), 25
- Sirtuin 1, 223–226*t*
- Sitosterolemia, 1004–1005, 1007*t*, 1008
- Skeletal dysplasia, 314
- Skeletal growth, disturbances of, in Turner syndrome, 634*t*, 635*f*
- Skeletal maturation, 313
- Skeleton
growth factors for, 263
hormones for, 263
metabolism disorders of, 245–272
mineral metabolism, disorders of, 246*f*, 249–250*f*, 253–258*f*, 259*t*, 262*t*, 267–268*t*, 269*f*, 270–271*t*
- SLC8A1*, 228
- SLC26A2* genes, 798–799
- SLC19A*, mutations in, 292
- Sleep deprivation, obesity and, 960
- Small for gestational age (SGA), 333
- Small integrin-binding ligand, N-glycosylated (proteins) (SIBLING), 221*t*
- Smart pump, 844, 846
- Smith-Lemli-Opitz syndrome (SLOS), 133, 465
- Smith-Magenis syndrome (SMS), 970
- Smoothed protein, 223–226*t*
- Social transition, 699
- Sodium, 221*t*
- Sodium chloride, primary loss of, in hyponatremia, 373
- Sodium-hydrogen exchanger regulatory factor (NHERF), 221*t*
- Sodium hydrogen exchanger regulatory factor 1 (NHERF1), 802*t*
- Sodium/iodide symporter defects, 396*f*
- Sodium-iodine symporter defects, 211
- Sodium phosphate cotransporter Solute carrier family 34, member 1 (NPT2a/NaPi-IIa), 223–226*t*
- Sodium phosphate cotransporter Solute carrier family 34, member 2 (NPT2b/NaPi-IIb), 223–226*t*
- Sodium phosphate cotransporter Solute carrier family 34, member 3 (NPT2c/Na-Pi-IIC), 223–226*t*
- Sodium/phosphate transporter 2 (NPT2), 221*t*
- Solute carrier family 4 (anion exchanger), member 2, 223–226*t*
- Solute carrier family 8 (sodium-calcium exchanger), member 1, 223–226*t*
- Solute carrier family 8 (sodium-calcium exchanger), member 2, 223–226*t*
- Solute carrier family 9, member 3, 223–226*t*
- Solute carrier family 12 (sodium/chloride transporter), member 3, 223–226*t*
- Solute carrier family 12 (sodium/potassium/ chloride transporter), member 1, 223–226*t*
- Solute carrier family 20 (phosphate transporter), member 2, 223–226*t*
- Solute carrier family 9, member 3, regulator 1 (NHERF1), 223–226*t*
- Somatic changes, in puberty, 668–669
- Somatic maturation, advancement of, 567
- Somatic mutation, germline *vs.*, 23
- Somatomedin, 1–2
- Somatomedin-C, 306
- Sonic hedgehog (SHH), 223–226*t*
signaling pathway, 317
- Sorting nexin 10 (*SNX10*), 223–226*t*
- Sotos syndrome 2, 344–345
- Southern blotting, 9–10, 10*f*
- SOX8, 137
- SOX9, 134–135, 247–248
- Speed of sound (SOS), 221*t*, 802*t*
measurement of, 269–270
- Sphingosine-1-phosphate (*S1PR1*), 223–226*t*
- Sphingosine-1-phosphate lyase (SGPL1) deficiency, 465
- Spironolactone, 439, 599
- Spleen tyrosine kinase (SYK), 221*t*
- “Split-bolus,” 846
- Spondyloepimetaphyseal dysplasia, 329
- Spondyloepimetaphyseal dysplasia/brachyolmia type 4, 798–799
- SRC oncogene (*Src*), 221*t*
- SRY-box 9, 223–226*t*
- SRY-Box (SOX), 221*t*
- SRY gene, 138
- SRY gene-specific sequence, 13*f*
- Standard deviation score (SDS), 802*t*
- StAR. *See* Steroidogenic acute regulatory protein (StAR)
- Starvation, 207
hypoglycemia in, 926–927
and malnutrition, hypoglycemia during, 926
- Starvation response, 947–948
cachexia *vs.*, 977
- STAT5B* gene, 324
- Statins. *See* HMG-CoA reductase inhibitors
- Stature
inheritance of, 314
normal variation in, 310
- Stem cells, 303
- Steroidal cell autoantibodies (SCA), 897
- Steroid biosynthesis, 111*f*
- Steroid hormones, 108–110
metabolism of, 550–551, 552*f*
origins of, 550
pathways of, 549–554, 550*f*
receptor selectivity in, 552
relationship among, 551*f*
- Steroidogenesis, 426
disorders, 142*t*, 143*f*, 145*f*
enzymes of
human genes for, 426*t*
types of, 428–433

- Steroidogenesis (*Continued*)
 fetal adrenal, 433–434
 genetic lesions in, 446–460
 morphologic zonation and, 113*f*
 placental and fetal adrenal, 112*f*
 during pregnancy, integrative, 111–113
 prolactin in, 546
 regulation of, 434–437, 434*f*
 types of, 428–433
- Steroidogenic acute regulatory protein (StAR), 430
 in mitochondrial cholesterol uptake, 430
- Steroidogenic enzymes
 cytochrome P450, 428
 genes for, 426, 426*t*
 17 β -hydroxysteroid dehydrogenase, 431–432
 hydroxysteroid dehydrogenases, 428
 3 α -hydroxysteroid dehydrogenases, 433
 11 β -hydroxysteroid dehydrogenases, 432–433
 mitochondrial cholesterol uptake, 430
 P450aro, 432
 P450c17, 430–431
 P450c21, 431
 P450c11AS, 431
 P450c11 β , 431
 P450scc, 428–430
 5 α -reductases, 432
 steroid sulfotransferase and sulfatase, 432
- Steroidogenic factor 1 deficiency, 449
- Steroid receptor coactivator (SRC), 221*t*
- Steroid receptors, 58–60
- Steroids, 426
 in adrenal gland, 426
 hormone synthesis in, 428–434
 catabolism of, 439–440
 hormone synthesis of, 428–434
 enzymes for, 428–434
 steps for, 428
 laboratory evaluation, 440–446
 measurement techniques, 440–441
 metabolism, 439–440
 plasma, 437–440
 plasma/serum concentrations of, 441
 sulfotransferase and sulfatase, 432
 therapeutic, potency of, 457*t*
- Sterol receptor binding protein (SREBP), 1006
- STIM1, 226
- Stress, 439
 cortisol and, 959–960
 doses, of glucocorticoid therapy, 477
 hypoglycemia in, 926–927
 in neonatal diabetes mellitus, 286
 obesity and, 959–960
 response, 947
- Stretch receptors, 942
- Stromal interaction molecule 1, 223–226*t*
- Structural variation (SV), 25
- Subacute thyroiditis, 396
- Subtle systemic acquired disease, 299
- α Subunit of stimulatory G-protein (G $_s\alpha$), 802*t*
- Suicidality, 698
- Sulfatase, 432
- Sulfated iodothyronines, 206
- Sulfation factor-somatomedin hypothesis, 1–2, 3*f*
- Sulfokinase enzyme, 206
- Sulfonylurea receptor, 825–827
- Sulfotransferase, 432
- Sumoylation, 226–227
- Super agonist, 31
- Supraoptic nuclei, magnocellular components of, 359, 360*f*
- Suprarenal glands, 427
- Surgery
 diabetes mellitus during, 855
 for pheochromocytomas, 499–500
- Sympathetic nervous system, energy expenditure, 945
- Syncytin-1, 255
- Syndromic disorders associated with disorder of sex development, 132*t*
- Syndromic neonatal diabetes mellitus, 287–288
 GATA4, 288
 GATA6, 288
 GLIS3, 287
 IPEX syndrome, 287
 MNX1, 288
 NEUROG3, 288
 NKX2.2, 288
 Rfx6, 287–288
 Wolcott-Rallison syndrome, 287
- Syndromic obesity disorders, 969–970
- T**
- T3. *See* Triiodothyronine (T3)
- T4. *See* Thyroxine (T4)
- TAC3 gene mutations, 677
- TACR3 gene mutations, 677
- Takao velocardiofacial syndrome, 715
- Tall stature, 342–343, 347*f*
 complementary diagnostic tests, 346*t*
 diagnostic approach, 345–346
 disorders involving
 cartilage extracellular matrix/fluid, 344
 intracellular factors, 344–345
 endocrine causes of
 delayed skeletal maturation, 343
 estrogen receptor α deficiency, 343
 familial glucocorticoid deficiency, 344
 familial pituitary adenomas, 343
 growth hormone/insulin-like growth factor-1 excess, 343
 McCune-Albright syndrome, 343–344
 sex steroid excess, 343
 thyrotoxicosis, 343
 treatment for, 346
 X-LAG syndrome, 343
 medical history and physical examination of children with, 345*t*
 nutritional obesity, 344
 treatment of, 346–347
- Tamoxifen, 553
- Tanner genital stage, 667–668, 671*f*
- Tanner staging, 102
- TaqMan (fluorescent 5' nuclease) assay, 14–15, 14*f*
- Target organs, sex hormone, maturation of, 554–557, 554–555*f*
- Tartrate resistant acid phosphatase (TRAP), 221*t*, 223–226*t*, 802*t*
- Tartrate resistant acid phosphatase type 5b isoform (TRAP5b), 221*t*
- TBG. *See* Thyroxine-binding globulin (TBG)
- T-Box 1, 223–226*t*
- TBX3, 135
- T cell factor/lymphoid enhancement factor, 223–226*t*
- T cell factor/lymphoid enhancer binding factor, 221*t*
- T cell immune regulator 1 (TCIRG1), 223–226*t*
- T cells
 activation of, 886*f*
 central, tolerance of, 885–886, 885*f*
 ignorance, 887
 peripheral, 884–887, 885*f*
 receptor, 819–820, 821*f*
 tolerance of, 885–887, 885*f*
 peripheral, 884–887
- TCIRG1 genes, 750
- T4, deiodination of, 205
- Television viewing, obesity and, 960
- Telopeptides
 amino-, 257*f*
 of collagen, 257*f*
- Temple syndrome, 332
- Tenascin-X (TNX), 455
- Teriparatide (PTH^{1–34}), 785
- Testes
 cryptorchidism in, 149–151, 680
 disorders associated with, 150–151*t*
 prevalence, 151
 descent of, 663
 development, 663
 puberty, and delayed, 677*t*
 undescended, 149–151
- Testicular descent
 androgen-dependent phase in, 663
 transabdominal phase in, 663
- Testicular development, 127–128, 663
- Testicular regression syndrome, 141
- Testosterone, 5
 estradiol-binding globulin, 551*f*
 in ovaries, 549
- Testotoxicosis, 2–3, 672
- Tg. *See* Thyroglobulin (Tg)
- TGF- β . *See* Transforming growth factor- β (TGF- β)
- Thanatophoric dysplasia
 type I, 796–798
 type II, 796–798
- Th1 cells, 887
- Th2 cells, 887
- The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), 408–409
- Theca cells, 546*f*
- Thelarche, premature, 560, 560*f*

- Thermogenesis
 brown adipose tissue, 207
 postnatal, 207
- Thermogenin, 207
- Thiamine-responsive diabetes mellitus, 863
- Thiamine-responsive megaloblastic anemia and diabetes, 292–293
- Thiazide diuretics, 382–383
- Thick ascending loop of Henle (TALH), 221*t*, 802*t*
- Thioredoxin binding protein-2 (TBP-2), 221*t*
- Thirst
 nonosmotic regulation of, 361–362, 361*f*
 osmotic regulation of, 359–361, 360*f*
- THR. *See* Thyroid hormone receptors (THR)
- Thymic neuroendocrine tumor, 509
- Thymus, 234
- Thyrogastric autoimmunity, 893
- Thyroglobulin (Tg), 396*f*, 397, 397*t*, 888
 defects of, 211
 synthesis of, 211, 397
- Thyroid adaptation, extrauterine, 204–205, 205*f*
- Thyroid disorders, in newborn and infant, 202
 congenital hyperthyroidism, 214–215
 congenital hypothyroidism, 208–214
 embryology, physiology and pathophysiology of, 202–208
 overview of, 202
 thyroid hormone transport and, 215
- Thyroid dysfunction syndromes
 in neonates, with Graves disease, 207
 in premature infants, 207
 prevalence of, 208
- Thyroid dysgenesis, 203, 208–210
 prevalence of, 208, 209*t*
- Thyroid dysmorphogenesis, 210–212
 dehalogenase defects, 211–212
 DUOX2 defects, 211
 features of, 210–211
 pendred syndrome, 211
 sodium-iodine symporter defects, 211
 thyroglobulin defects, 211
 thyroperoxidase defects, 211
 TSH resistance of, 210
- Thyroid gland, 202–203, 205, 395
 biochemical evaluation of, 396*b*, 398–400
 development of, 203
 ectopic, 398
 function of, 397
 in infants, 398
 perinatal changes in, 207–208
 regulation of, 397, 399*f*
 test parameters for, in human fetus, 205*f*
- Thyroid hormone, 1–2, 307, 395–397, 439
 action of, 207, 395–397, 396*f*, 397–398*t*
 cell membrane transporters and receptors, 207
- Thyroid hormone (*Continued*)
 fetal, 206
 in growth, 414
 metabolism, 398*t*, 398*f*
 defects in, 213
 maturation of, 206–207, 206*f*
 receptors, defects in, 213
 in renal free water clearance, 372
 resistance, 325, 402–403
 syndromes of, 396, 396*b*
 signaling pathways, defects in, 212–213
 synthesis and secretion, 204–206
 transport
 into cells, defects in, 213
 disorders of serum, 215
- Thyroid hormone receptor alpha (TR α), 326
- Thyroid hormone receptors (THR), 54–57, 221*t*, 402
 for skeletal growth, 263–264
- Thyroid hormone releasing hormone (THRH), 397, 399*f*
- Thyroiditis, 407
 Hashimoto, 400
 in acquired juvenile hypothyroidism, 400
 antibodies in, 400
 infectious, 396, 396*b*
 infectious bacterial, 407
 subacute, 407–408
 suppurative, 396, 396*b*
- Thyroid neoplasia
 in children, 396, 396*b*
 in CNC patients, 504
 MTC in, 415–416
- Thyroid nodule, thyroid cancer and, 408–416
 in children, 408–409
 evaluation of, 408–409
- Thyroid-stimulating hormone (TSH), 397
vs. free T4 by equilibrium dialysis, 104*f*
 producing adenomas, 407
 in thyroid follicular cell, 397, 399*f*
 in thyroid regulation, 397
- Thyroid-stimulating hormone receptors, 40–41, 41*f*
 activating mutations of, 407
 mutations, 207–208
 during pregnancy, 41
- Thyroid system
 embryogenesis in, 203
 maturation, 206–207, 206*f*
- Thyroid transcription factor mutations, 210
- Thyroid transcription factors-1 and -2, 203
- Thyroperoxidase defects, 211
- Thyrotoxicosis, 343, 406
 neonatal, 406–407
- Thyrotrophic axis, development of, 202–203
- Thyrotropin-releasing hormone (TRH), 202, 203*f*, 402
 defects of, 212–213
 fetal, 205–206, 205*f*
 receptors, 42
- Thyroxine (T4), 1–2, 5, 395, 402
 deiodination, 206*f*
 deiodination of, 205–206
 replacement therapy, 402
 in thyroid adaptation, 205*f*
 in thyroid maturation, 205–206, 206*f*
- Thyroxine-binding globulin (TBG), 203*f*
 deficiency of
 complete, 396
 partial, 396
- TIP39, 238
- Tissue-nonspecific alkaline phosphatase (TNSALP), 221*t*, 233–234, 802*t*
- TNF receptor associated factor (TRAF), 221*t*
- TNF-related activation induced cytokine (TRANICE), 221*t*
- Toddler, hypoglycemia in, 904
 major causes of, 910–928
- Tolerance
 autoimmune polyglandular syndromes and, 884
 of B cells, 887
 defects in, causing autoimmune diseases, 888–889
 impaired glucose, 866
 self-antigens and, 888*f*
 of T cells, 885–887
- Toxic multinodular goiters, 408
- Traits of autism spectrum disorder, 698
- Transabdominal phase, 663
- Transcatheter, 244–245
- Transcription factor, mutations, congenital hypothyroidism with, 209*t*
- Transcription factor Sp7 (Osterix), 223–226*t*
- Transcription factor 3/VDR interacting repressor, 223–226*t*
- Transdermal estradiol, 584
- Transforming growth factor (TGF), 221*t*, 802*t*
- Transforming growth factor- β (TGF- β), 109–110, 248
- Transforming growth factor, beta 1, 223–226*t*
- Transgender, care of
 definitions and epidemiology, 695
 delivery of care, challenges to, 701
 emergence and developmental trajectories, 697
 adolescence, 697
 early childhood, 697
 infancy, 697
 gender dysphoria
 desistence *vs.* persistence, 698
 eating disorders, 698
 family support and treatment, 698
 internalizing disorders and, 697–698
 natural history of, 698
 suicidality and nonlethal self-harm, 698
 traits of autism spectrum disorder, 698
 gender identity, biological
 determinants of, 695–697
 medical treatment
 current treatment models, 699–700

- Transgender, care of (*Continued*)
 outcomes and potential adverse effects, 700–701
 multidisciplinary care, 698–699
 social transition, 699
- Transgender, definition, 696b
- Transgender male, 696b
- Transgender woman, 696b
- Transient hypothyroxinemia of prematurity (THOP), 212
- Transient neonatal diabetes mellitus type 1 (TNDM1), 282–285
 causes of, 282
 hypomethylation of imprinted loci in, 282–283
 manifestations of, 282–283
 mechanisms for differential methylation patterns in, 282, 283f
 transgenic mouse model in, 282
 treatment of, 283
- Transient neonatal diabetes mellitus type 2 (TNDM2), 283–285
 causes of, 283–284
 comparison to TNDM1, 284, 285t
 relation between K_{ATP} channel activity and insulin secretion, 284f
- Transient neonatal hypothyroidism, 212
- Transient receptor potential cation channel 6 (*TRPV6*), 227
- Transient receptor potential cation channel, subfamily M, member 6, 223–226t
- Transient receptor potential cation channel, subfamily M, member 7, 223–226t
- Transient receptor potential cation channel, subfamily V, member 4, 223–226t
- Transient receptor potential cation channel, subfamily V, member 5, 223–226t
- Transient receptor potential cation channel, subfamily V, member 6, 223–226t
- Transient receptor potential channel (TRP), 221t
- Transition, 696b
- Transitional hypophosphatasia.
See Benign hypophosphatasia
- Transition phase
 abnormalities of, 177
 changes at birth, 176–178
- Transmembrane protein 38B, 223–226t
- Transmembrane receptors, 31
- Transsexual, 696b
- Transthyretin variants, 396
- Trans-unsaturated fatty acids, 960
- Trauma, central diabetes insipidus and, 377
- Traumatic brain injury (TBI), 318
- Tregs, 887
- TRH. *See* Thyrotropin-releasing hormone (TRH)
- Triamcinolone, therapeutic, potency of, 457t
- Triggering receptor expressed on myeloid cells-2 (TREM2), 221t, 223–226t
- TRIGR study, 824
- Triiodothyronine (T3), 5, 203f, 247–248, 395
 in thyroid adaptation, 205f
 in thyroid maturation, 205–206, 206f
- Triple A Syndrome, 463
- Trisomy 21, 865
- Troglitazone, 439
- Tropomyosin-related kinase B (TrkB) receptor, 944
- TRPM6, 233
- TRPV5, 228
- TSH. *See* Thyroid-stimulating hormone (TSH)
- Tuberoinfundibular protein (hypothalamic) (TIP), 221t
- Tuberoinfundular peptide 39, 223–226t
- Tuberous sclerosis complex (TSC), 516
- TUBULIN, 210
- Tumor induced osteomalacia (TIO), 221t
- Tumor necrosis factor (TNF), 221t, 802t
- Tumor necrosis factor- α , 223–226t
- Tumor necrosis factor-associated factor 6, 223–226t
- Tumor necrosis factor ligand superfamily, member 11 (RANKL, OPLG, TRANCE), 223–226t
- Tumor necrosis factor receptor superfamily, member 11A, 223–226t
- Tumor necrosis factor receptor superfamily, member 11B (osteoprotegerin), 223–226t
- Tumors
 adrenal, 469
 feminizing, 472
 virilizing, 472
 -induced osteomalacia/rickets, 764–765, 765f
- Turner, Henry, 627
- Turner syndrome (TS), 6, 13f, 865, 912
 autoimmune disorders, 643
 carbohydrate metabolism, abnormalities of, 644
 cardiac care in, 641–642
 cardiovascular screening in, 641
 cardiovascular system anomalies, 638–642
 chromosomal origins, 627–628
 chromosomes in, karyotypes of, 628
 clinical characteristics and comorbidities, 633–645
 clinical features of, 633–634, 634t
 differential diagnosis, 632–633
 epidemiology, 628
 gastrointestinal disorders in, 643–644
 genetics of, 627–633
 genomic imprinting in, 631
 gonadoblastoma in, 637–638
 growth failure, 634–636
 height and height velocity in, 637f
 historical background, 627
 hyperinsulinism in, 190
 hypertension in, 634t
 indications for karyotype testing, 632
 initial evaluation, 645–646
 lymphatic obstruction, 633–634, 634t
 lymphedema, 633–634, 634f
 management, 190–196
- Turner syndrome (TS) (*Continued*)
 medical management, 645–650
 monitoring in, 645–647
 neurocognition and psychological features, 644–645
 Noonan syndrome *vs.*, 631–632
 otitis in, 643
 outpatient care and treatment, 651–652
 ovarian insufficiency, 636–637
 phenotypic variability in, 633f
 prenatal diagnosis of, 633
 puberty, 649–650
 renal anomalies, 642–643
 short stature, 634–636
 skeletal anomalies, 634–636
 stigmata in, 633f
 transition to adult care, 650–652
 treatment, 190–196, 191f
 14-week-old fetus with, 632f
 X chromosome genes in, 628–631
 X chromosome genomic imprinting and, 631
 Y chromosome in, 647–648
- Type A insulin resistance, 864
- Type B insulin resistance, 864–865
- Type 1 collagen, 256–257, 256–257f
- Type 1 diabetes mellitus (T1DM), 279–280, 817–831
 age-standardized incidence of, 818–819, 818f
 characteristics of, 816t, 817
 epidemiology of, 817–819, 818f
 etiology, pathogenesis and genetics of, 819–823, 819f, 820t, 821f
 immune intervention strategies in, 823–824
 loci for, 820t
 non-autoimmune, 855
 pathophysiology of, 829–830, 829t, 830f
 phenotypes in, 820t
 prediction and prevention of, 823–824, 823t, 823f
 risk of, 820t, 822t
 treatment of, 840–843
- Type 2 diabetes mellitus (T2DM), 279–280, 817
 characteristics of, 816t, 817
 mutations causing, 862t
 typical, 860–861
 virus infections in, 864
 youth-onset, 863t
- Type 1 IGF receptor, 306
- Type 2 IGF receptor, 306–307
- Tyrosine hydroxylase, autoantibodies to, 898
- Tyrosine kinase inhibitors (TKIs), 415
- Tyrosine kinase receptors (RTKs), 30
- Tyrosyl ring, 206
- U**
- Ubiquitin fusion degradation 1-like (UFD1L), 714–715
- UDP-N-acetyl-alpha-D-galactosamine:
 Polypeptide N-acetylgalactosaminyl-transferase 3, 223–226t

- Ullrich-Turner syndrome, 627
 Ulnar-Mammary syndrome, 135
 Ultrasound, abdominal, of adrenal adenoma, 570f
- Uncoupling
 protein-2, 207
 protein-3, 207
- Uncoupling protein-1 (UCP1) gene, 945
 Uncoupling protein-2 (UCP2)
 hyperinsulinism, 189, 912
- Upper-to-lower body segment ratio, 311–312
- Urinary steroid excretion, 441
 Urine, pyridinoline, 257f
- Urogenital ridge and bipotential gonad development, 126–127, 127f
- U.S. Diabetes Prevention Trial for T1DM (DPT1), 822–823
- V**
- VACTERL association (VA), 152
 Vaginal epithelium, 554f
 Vagus nerve
 afferent, 941
 efferent, 945–947
- van Buchem disease type 1, 792–793
 Vanillylmandelic acid (VMA), 496–497
 Vanishing testes, 141
 VanWyk-Grumbach syndrome, 325, 568, 672–673
- Variant interpretation, 25
- Vascular changes
 dyslipidemia and, 1010–1011, 1010b
 in obesity, 954–955
- Vascular endothelial growth factor (VEGF), 221t, 223–226t, 237, 245–247, 781, 802t
 family, 109
- Vasopressin
 action of, 364f
 analogs of, for central diabetes insipidus treatment, 379–380
 arginine, 1–2
 biochemistry of, 357–359, 358f, 360f
 cells, in hypothalamus, 360f
 dDAVP, 357, 358f
 in homeostasis, 357
 hyponatremia with abnormal regulation of, 374–376, 375t
 decreased, 375–376, 382f
 increased, 374–375
 in kidney, 363–364, 364f
 metabolism of, 362
 increased, central diabetes insipidus with, 378
 nausea and, 360
 nonosmotic regulation of, 361–362, 361f
 normal regulation of, 371–374, 372t
 origin of, 358
 osmotic regulation of, 359–361, 360–361f
 parvocellular neurons role in, 359
 peptide products of, 359f
 pre-, peptide, structure of, 359f
 receptors, 38, 362, 363f
 renal cascade of, 363–364, 364f
- Vasopressin (*Continued*)
 SIAD, 374–375, 375t, 382f
 sites of action of, 362–364
 structure of, 358f
- Vasopressin V2 receptor (SIAD)
 causes of SIAD, 374–375, 375t
 treatment of, 375
- VDR. *See* Vitamin D receptor (VDR)
- VEGF. *See* Vascular endothelial growth factor (VEGF)
- Velocity charts, 311
- Ventral tegmental area (VTA), 947
 Venus flytrap domain, 35
- Very low birth weight (VLBW), 802t
- Very low density lipoproteins (VLDLs), 1005, 1005t
 disorders of, 1008
- V-Fos FBJ murine osteosarcoma, 223–226t
- Virilizing congenital adrenal hyperplasias, 144–146
- Virilizing tumors, 472
- Virtual bone biopsy, 265–266
- Virus infections, in diabetes mellitus, 864
- Vitamin D, 239–245, 239f
 binding protein, 221t, 223–226t
 deficiency of
 causing hypocalcemia, 709t
 in infants, 754f, 755–756
 metabolism of, 239f
 repletion of, 241–242
- Vitamin D-dependent rickets type 1A (VDDR1A), 802t
- Vitamin D-interacting repressor, 221t
- Vitamin D receptor (VDR), 57–58, 221t, 223–226t, 227, 240, 242–245, 713, 802t
 calcitriol involvement with, 241–243
 genes regulating, 243–244, 244f
 mRNA, 242
 mutations in, 758f
- Vitamin D response element (VDRE), 221t, 242–243, 802t
- Vitiligo, 885t, 887t, 892–893
- VLDLs. *See* Very low density lipoproteins (VLDLs)
- V-Mak musculoaponeurotic fibrosarcoma oncogene family, protein B, 223–226t
- VMH, 944
- Voltage-gated calcium channel α 1, 223–226t
- Voltage-gated calcium channels, 220–226
- Volume regulation, physiology of, 357–368
- Volume sensor, effector pathways and, 364–368, 367f
- Von Hippel-Lindau (VHL) disease, 493f, 516–517
- V1 receptor (V1a KO), 362
- V3 (V1b) receptor, 362
- VTA. *See* Ventral tegmental area (VTA)
- W**
- WAGR/11p deletion syndrome, 968
- Water
 deprivation test, 369, 370f
- Water (*Continued*)
 metabolism disorders
 differential diagnosis of, 368–371, 370f
 specific disorders of, 371–383
- Weaver syndrome, 345
- Webbed neck, 628f, 634f
- Weight, 426–427
 of adrenal gland, 427f
 body
 glucose production *vs.*, 904–905
 gain, dysregulation, 949
 loss, dysregulation, 948–949
 at menarche, 578f
- Whipple's triad, 927
- White Addison disease (WAD)
 phenotype, 21–22
- Whole genome/exome sequencing, 21f
- Williams-Beuren syndrome (WBS), 732, 802t
- Williams syndrome transcription factor (WSTF), 221t, 242–243, 732, 802t
- WI-NAC, 243–244
- Wingless-type mouse mammary tumor virus (MMTV) integration site
 member 1, 223–226t
 member 9, 223–226t
- WNT4 gene, 117, 141
- WNT inhibitory factor 1, 223–226t
- WNT inhibitory factor (WIF), 221t
- Wnt signaling pathway, 248
 in bone mineralization, 248–249, 249–250f
- Wolcott-Rallison syndrome, 287
 etiology of, 287
 manifestations of, 287
 prognosis of, 287
 treatment of, 287
- Wolff-Chaikoff effect, 401
- Wolfram syndrome, 292, 862–863
- Wolman disease, 446–447, 464
- Women. *See also* Female reproductive system
 adolescent, bone deposition in, 259t
 mid-follicular phase, blood production rates in, 549t
- World Health Organization (WHO), 802t, 949, 956–957
- World Health Organization (WHO) Expert Committee on Biological Standardization, 1–2
- WSTF including nucleosome assembly complex (WINAC), 221t
- WSTF including nucleotide assembly complex (WINAC), 802t
- WT1 gene, 135
- WW domain-containing oxidoreductase (WWOX), 138–139
- X**
- X chromosome inactivation (XCI), 628–629
- X chromosomes
 diagnostic tests, 631–632
 differential diagnosis, 632–633
 genes, 628–631
 genomic imprinting, 631

- X chromosomes (*Continued*)
 genotype-phenotype associations,
 628–631
 and Turner syndrome, 628–631
 Xenotropic and polytropic retrovirus
 receptor (*XPR1*), 223–226*t*
XH2 gene mutations, 135–136
 X-LAG syndrome, 343
XLas, of *GNAS*, 717
 X-linked acrogigantism (X-LAG),
 513–514
 X-linked adrenal hypoplasia congenita,
 463
 X-linked congenital, diabetes insipidus,
 381, 382*f*
 X-linked dominant hypophosphatemic
 rickets (XLHR), 759, 802*t*
- X-linked hypophosphatemic rickets
 (XHR), 221*t*
 X-linked immunodysregulation,
 polyendocrinopathy, and
 enteropathy (IPEX), 462
 X-linked lissencephaly 2, 131
 X-linked recessive hypophosphatemic
 rickets (XLRH), 802*t*
 XX disorder of sex development/
 premature ovarian failure,
 139–141, 140*t*
 46,XX disorders, 136–137
 46,XX testicular disorder of sex
 development, 139
 45,X/46,XY mosaicism,
 131
 46,XY disorders, 136–137
- Y**
 Y chromosomes, patients with,
 647–648
- Z**
 Zearalenone. *See* Mycotoxin
 Zinc finger and ring finger protein 3
 (ZNF3), 139
 Zinc finger gene regulating apoptosis and
 the cell cycle (ZAC), 282
 Zinc finger protein, multitype 2 (ZFPM2),
 139
 Zona fasciculata, 36
 Zona reticularis, 36

This page intentionally left blank

ClinicalKey®

Confidence is ClinicalKey

Evidence-based answers, continually updated

The latest answers, always at your fingertips

A subscription to ClinicalKey draws content from countless procedural videos, peer-reviewed journals, patient education materials, and books authored by the most respected names in medicine.

Your patients trust you. You can trust ClinicalKey.

Equip yourself with trusted, current content that provides you with the clinical knowledge to improve patient outcomes.



ELSEVIER

Get to know ClinicalKey at store.clinicalkey.com.